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Accuracy in Trace Analysis: Sampling, Sample Handling, Analysis

Volume I

Accuracy in Trace Analysis: Sampling, Sample Handling, Analysis — Volume I

Proceedings of the 7th Materials Research Symposium

Held at the

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Gaithersburg, Md.
October 7-11, 1974

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FOREWORD

The Analytical Chemistry Division of the NBS Institute for Materials Research, a major national laboratory for analytical chemistry is concerned with improving the accuracy and precision of analytical measurements throughout the Nation, and exercises leadership in solving important analytical problems that affect many different segments of the economy as well as the health and safety of our citizens. This laboratory at present has some 100 technical people who are engaged in the development and utilization of more than 60 different analytical techniques ranging from activation analysis to mass spectroscopy and x-ray spectroscopy. The NBS Analytical Chemistry Division is charged with conducting analytical research, preparing and analyzing Standard Reference Materials, and performing service analysis for other divisions within the NBS and the Federal establishment. One important mechanism for the Analytical Chemistry Division in exercising its leadership is in the sponsoring of special conferences and symposia and providing a congenial atmosphere in which scientists from throughout the world may exchange their views and ideas in the field of Analytical Chemistry. Experts are invited to participate in these conferences and an attempt is made to advise all scientists of these meetings and to invite them to contribute papers to these symposia.

The first Materials Research Symposium sponsored by the Institute for Materials Research was held October 3-7, 1966, shortly after NBS moved to their new laboratories in Gaithersburg, Maryland. This first Symposium was also sponsored by the Analytical Chemistry Division and was concerned with trace characterization of materials. Subsequent to that Symposium, more specialized conferences sponsored by the Analytical Chemistry Division have been held on such subjects as quantitative electron probe microanalysis, modern trends in activation analysis, and ion-selective electrodes. "Analytical Chemistry: Key to Progress on National Problems," held in June 1972 was the American Chemical Society's annual summer symposium sponsored by the ACS Division of Analytical Chemistry and the journal *Analytical Chemistry*, and co-sponsored by the NBS Analytical Chemistry Division. More recently, several workshop-type meetings sponsored or co-sponsored by the Division were held at the Gaithersburg facilities. These include a workshop on oil pollution monitoring, on aerosol measurements, on secondary ion-mass spectrometry and on ozone measurement technology. The proceedings of most of these conferences and most of the workshops are available from

the Superintendent of Documents, Government Printing Office, Washington, D.C. 20402.

This volume is the proceedings of the 7th Materials Research Symposium also organized by the Analytical Chemistry Division. The theme, Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis, attracted over 400 scientists, representing a broad spectrum of industrial, governmental, and educational institutions from the United States and from many foreign countries. The format of the Symposium consisted of invited plenary speakers, who discussed specific topics in the areas of sampling, sample handling, data interpretation, analytical methodology, and a number of contributed papers generally corresponding to these topics.

The large attendance at this Symposium as well as the lively and interesting sessions indicate that trace analytical chemistry is one of the key disciplines needed by materials scientists, environmentalists, biologists and engineers to understand and ultimately solve the increasingly complex technological problems now facing the Nation. It is timely that the Analytical Chemistry Division of the Institute for Materials Research sponsor such a symposium at this time.

John D. Hoffman, *Director*
Institute for Materials Research

PREFACE

This volume, the formal report of the proceedings of the 7th Materials Research Symposium held at the National Bureau of Standards in Gaithersburg, Maryland, October 7–11, 1974, is comprised of the invited and contributed papers given at the Symposium. The objective of the Symposium was to define the present status of accuracy and the means of obtaining accuracy in trace analytical chemistry.

Over the past several years there has been an explosion in the number of chemical analyses performed, especially in the areas of environmental quality, plant and animal tissue analyses, biological fluids, high purity materials and geological samples. Because the results of these analyses are used in making important projections and decisions both short- and long-term, it is essential that they be reliable. The various factors which contribute to obtaining accurate analytical results were explored in the Symposium. Particular emphasis was placed on the whole analysis, from taking the sample through the interpretation of the results, rather than concentrating on measurement processes only. The Symposium consisted of four and one-half days of papers. Leading authorities, with broad knowledge of the problems in the areas discussed, were invited to present the keynote lectures. Simultaneous sessions consisting of groups of papers corresponding roughly with the themes of the invited papers were presented in the afternoons.

It is hoped that this volume will provide much of the necessary background information and directions for the future to assist in establishing priority goals and a rationale for solving the difficult problems in accuracy in trace analysis in the next several years.

Identification of commercial materials and/or equipment by the authors in these proceedings in no way implies recommendation or endorsement by the National Bureau of Standards.

An undertaking of the magnitude of this Symposium and of the proceedings, would not have been possible without the cooperation and assistance of a large number of dedicated people. The enthusiastic participation of the invited speakers, the session chairmen and those who contributed papers is deeply appreciated. Many members of the staff of the Analytical Chemistry Division and of the National Bureau of Standards served on numerous committees and assisted during the Symposium in various capacities.

Special thanks are given to Ronald B. Johnson and Robert F. Martin of the Institute for Materials Research. They provided all of the fiscal management of the Symposium and assisted with the other administrative

matters associated with an operation of this magnitude. Mrs. Sara R. Torrence of the Office of Information Activities, under the direction of W. E. Small, deserves a special note of praise for her work in organizing the accommodations, social program, and coordinating the logistical aspects of the Symposium. Members of the staff of the Office of Technical Publications, under the direction of W. R. Tilley, have given invaluable assistance in the many phases of publishing these proceedings. Special thanks are due to Rebecca J. Morehouse and Miriam K. Oland of the Computer-Assisted Printing Section for producing this book using computerized photocomposition techniques.

Within the Analytical Chemistry Division special thanks are given to Mrs. Barbara Turner for her untiring effort in correspondence with the authors, and especially to Mrs. Rosemary Maddock who has provided the coordination and editorial assistance in the many phases of preparing these proceedings.

Philip D. LaFleur, *Chief*
Analytical Chemistry Division

ABSTRACT

This book is the formal report of the proceedings of the 7th Materials Research Symposium: Accuracy in Trace Analysis. This volume contains the invited and contributed papers presented at the Symposium, and which treat problems of sampling and sample handling as well as the usually-discussed analytical methodology. Many important techniques and methods are described, and extensive references are presented, to give deeper insight into the problems of obtaining accurate results in trace analytical chemistry. Accordingly, this volume should not only stimulate greater interest in research in these areas but should provide a valuable guide for everyday analytical problems.

Keywords: Accuracy; analysis; analytical chemistry; sample handling; sampling; trace analysis.

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THE NEED FOR ACCURACY IN A REGULATORY AGENCY

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A discussion of the need for accuracy in the food and drug industries is presented. Problems involved in sampling, sample handling, and methods of analysis are discussed as well as the establishing of permissible limits of contaminants and additives.

Keywords: Accuracy; drugs; foods; regulation; regulatory agency.

At the outset I should make clear my incompetence to talk about chemistry from the viewpoint of a chemist, since a chemist I am not. However, I have been asked to share some of my thoughts with you concerning how I view certain needs of a regulatory agency with respect to analytical chemistry. Usually when we speak of accuracy as such we refer to a true value in the sense that exactly that amount is present in a given medium. However, if we go back to the derivation of the word accurate from the Latin, *accuratus*, we find it means prepared with care, careful, exact in the sense of freedom from mistake or error. And I shall speak to accuracy in the sense of prepared with care and free of mistakes, for that is how I believe the word accuracy applies to the legal responsibilities of a regulatory agency involved with the detection, identification, and measurement of particular substances in various media. It also should be made clear from the outset that I will be biased toward the Food and Drug Administration and particularly with respect to analyzing food for the presence of potentially harmful substances.

Perhaps a brief overview of our general responsibilities is in order so that some of our concerns can be set into perspective. As you know we deal generally with many problems but two categories become apparent—the presence (or absence) of a substance in food resulting from

intentional addition or exposure during processing versus the presence of a substance in food from accidental contamination of the food from natural, environmental, or processing sources.

When we are dealing with intentionally present substances, we are starting with given facts and it then becomes a matter to determine what the limitations are that pertain to the facts. For example, is the substance to be added to foods pure? Purity becomes a matter for both scientific and legal definitions since the legal requirement is that food not be adulterated. But what if the substance to be added is not what it seems to be? What if the additive is adulterated—then obviously the food will become adulterated and hence illegal if an adulterated additive is added to the food. Sounds simple, doesn't it? Not quite so. How is the additive derived? Is it synthesized from intermediate compounds that in turn may have some contaminants and side reaction products? What should we look for? Here is where the scientific detective work becomes a major importance. You know better than I some of the chemical techniques that can be applied to determine the likelihood that a substance is relatively pure. For example, how sharp and symmetrical are your GLC peaks? What do those peaks represent? Do you get corroboration of the suspected identity of the compounds represented by the peaks by mass spectrophotometric analysis? And why does this become important? Simply because we are primarily concerned with safety. And what is safety? Perhaps the best answer to that question is that there is no easy answer. We may think of safety as being relative. Relative to what? Relative to a defined hazard. What is a defined hazard? My answer is that it represents a risk—hopefully at least semiquantifiable—of an adverse effect on human health resulting from a given exposure to a particular substance or agent. And our job is to assure the American people that the very best job of protecting human health against defined hazards is being done within the capabilities of our resources both physical and human, and within the scope of what we presently know about chemistry, toxicology, microbiology, pharmacology, etc. One is always faced with such limitations simply because that is the way that life is. There are a finite number of skilled chemists in the world and some of them work in regulatory agencies. Analytical techniques are constantly being improved with the advances made in basic sciences, and our worries over potential health hazards also tend to increase with increases in our ability to detect a variety of substances naturally or artificially present in various spheres of our environment, including our food, water, and air. Fortunately, as our knowledge increases, our capabilities to predict adverse effects on health increase, so our ability to protect human health increases commensurately.

So we proceed with the best care that we can, given the circumstances of the real world as we know it today. Tomorrow may be different. It usually is. And this is so particularly with reference to substances present in foods as natural ingredients or resulting from environmental contamination. Here we enter a brave new world, where previously a potato was a potato and a sweet potato is just another kind of potato with a different shape, taste, and color. Then lo and behold you chemists begin to identify naturally present substances like solanine and ipomeamarone and we then ask how have we survived eating potatoes for so many years? Some of you are intimately familiar with the world of natural plant alkaloids and others of you are involved with mycotoxins where Pandora's box has been opened much wider in the last decade. Aflatoxin—a potential cause of liver cancer—occurs in a variety of natural foods as a result of fungal growth of *Aspergillus flavus* and related strains. It has been detected in corn growing on the stalk and in peanuts, just to mention two foods. Major efforts have been underway by the FDA in the last decade to reduce human exposure to aflatoxins by various control programs involving food harvesting and food processing. Some of these controls involve FDA guidelines or action levels—*i.e.*, when these levels of a substance in particular food commodities are detected, legal action is taken to remove the food from interstate commerce—which is where FDA has the authority to act. Notice in this type of instance what is involved is not just the banning of an additive to food—it involves banning the food itself and thus reducing the available food supply. Many other fungal toxins are under investigation and further control programs may be effected in the future as indicated.

Animal organisms are extremely sensitive to changes in chemical structures. Of the 76 possible chlorinated derivatives of dibenzo-paradioxin, the toxicities range from biological effect at the picogram level to practically biological inertness. Therefore, it becomes very important to determine the chemical structure of the substance in question, and related chemical moieties of potential toxicological concern. The relationship between analytical chemistry and toxicology is inseparably close. There is no sense in requiring the toxicological testing of a substance in test animals if it is the wrong substance to be concerned about. Questions concerning the relevance of animal metabolites to human metabolism become very real.

One question always exists: Is the problem just with the ingredient in food, or is there an ingredient in the ingredient that we should be looking at more closely? Thus, when we are reviewing a food additive petition we ask many questions of fundamental importance. What is the substance in question chemically? How pure is it? Are there any contaminants occur-

ring inadvertently? Have we looked for all potential contaminants or side reaction products of potential relevance to human health? What toxicological testing has been performed according to which protocols and what was the result? At which doses did particular toxic manifestations first begin to appear? Was the next lower dose a no-effect dose or were there subtle effects that would have gone unnoticed unless these questions were asked?

Notice that the question concerning intentional human exposure to food additives or to drugs used in food-producing animals that may leave residues in those animal products used for human food: Is the substance in question safe enough to permit human exposure? With respect to substances present in food resulting from being naturally present or from environmental contamination the question becomes: Is the potential threat to human health resulting from such exposure in certain food commodities sufficiently real to warrant removal of that particular portion of the food supply from human consumption?

Suppose now we are satisfied that we have sufficient chemical and toxicological information on which to base a decision to permit the use of the substance being added to food as an additive or as a drug to be used in food-producing animals, or in the case of a natural ingredient of food and environmental contaminants in food, to permit ingestion of the food itself? Such use will be permitted at certain levels, depending upon the degree of toxicity involved and the importance of the particular food commodity to our diet; hence estimates of expected total human exposure become of essential importance in safety decision-making of a regulatory agency such as FDA. Given that we have now made a decision that a certain level in certain foods will be permitted, how do we go about enforcing that level if such is deemed necessary or desirable in order to assure human safety?

And here we re-enter the extremely important world of analytical chemistry and the need for accuracy in a regulatory agency. Some of the decisions involving whether or not a certain amount of a particular substance was or was not in a particular food commodity can make an enormous difference to consumers and to industry. If we needlessly restrict a useful technical additive, we may impair the efficiency of the food industry to deliver a certain product for human consumption at a particular cost. Thus industry's pocketbook can get hurt needlessly, along with major dislocations of physical and human resources, including food industry employees, and eventually those costs will be passed through to the consumers in the form of increased prices for particular food products. And if we take action against a particular food commodity because of the presence of a potentially toxic amount of a harmful natural ingredient or environmental contaminant, we ban the food itself—which becomes very

costly to food producers and consumers alike. On the other hand, if we leave a harmful food on the market, we needlessly expose consumers to an unexpected and unnecessary hazard. Thus, it is important to determine what levels shall be permitted under which circumstances for particular food ingredients and commodities relative to the total diet and expected human exposures from food sources. At times, in dealing with environmental contaminants we have to consider nonfood sources of exposure, such as those exposures that may occur from air, water, occupational, and therapeutic sources.

So here we are now faced with setting an action level or a tolerance which, if exceeded, will cause us to leap into action, if we spot the offender. A statistically valid sampling program becomes of major importance to monitoring programs set up to detect violations of tolerances in the case of approved additives or drugs, and potentially hazardous levels of natural toxicants or environmental contaminants. Let's assume we now have some representative samples selected for testing by the FDA. We may be looking for excessive methyl mercury in fish, aflatoxins in corn, violative residues of drugs or polybrominated biphenyls in meat or milk, lead, cadmium, or polychlorinated biphenyls in foods, *etc.* How can we ensure accuracy of analytical results to the public? I reemphasize that I am using the word accuracy in the sense of using utmost care to assure that the identity of the substance and the quantity reported have been carefully determined so that these results will be supportable in court if our action is contested legally.

The integrity of the sample has to be preserved at all times; therefore, responsible custody has to be assured. When the sample is analyzed, the aliquot has to be representative of the sample. The test has to be performed by competent chemists using acceptable standardized techniques to determine identity and report quantity detected. The test for a violative regulatory sample is usually repeated by another FDA chemist to assure that no error has been made and that the determination is verifiable.

What are the acceptable standardized techniques that we use? How do we standardize our procedures to assure that our methods are of sufficient reliability; that they are accurate and precise so that we can be assured that a reported violation is a real violation warranting legal action?

Many of you are very familiar with the Association of Official Analytical Chemists (AOAC) and the procedures involved in standardizing the chemical tests we use to assure that the methods are capable of producing reliable results. Essentially, many available methods are examined and the more promising ones tried and compared. Then one particular method is selected and put through a major trial involving several collaborating analytical laboratories to determine the degrees of experimental error

between laboratories. The approval process for complex methods may involve several trials until a satisfactory set of conditions are developed which will produce acceptable results when tested in a blind fashion on amounts known by the particular coordinator to be in the food commodity.

So in conclusion I would like to say that our needs for accuracy are not absolute in the sense of being 100 percent certain that the amount being reported is 100 percent coincidental with the true amount present. Recoveries are not usually perfect for a variety of reasons familiar to most of you. But what is of major importance to us in regulatory agencies is that there is *at least as much* of the substance in question truly present, as that amount required for legal action after careful testing. While we insist on accuracy in the sense of using utmost care to minimize the possibility of human or equipment error, we also strive for accuracy in the sense of coming as close as we can to reporting the true amount present. We are aware that our responsibility not to underestimate a potential health hazard is of paramount importance. But a not infrequent problem for us is having another laboratory not familiar with regulatory procedures and needs reporting to us higher (or lower) amounts in a given sample than we report and then finding out later that their methods did not take into account the presence of interfering substances which gave false positive (or negative) results by the test procedures used, whereas our procedures did take such sources of error into account. In closing, I do not mean to imply that FDA methods are the only good methods available, but I do believe our methods and reported results generally to be well-founded in view of the special needs of a regulatory agency.

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ACCURACY AND TRACE ORGANIC ANALYSES

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Accuracy in trace organic analysis presents a formidable problem to the residue chemist. He is confronted with the analysis of a large number and variety of compounds present in a multiplicity of substrates at levels as low as parts-per-trillion. At these levels, collection, isolation, identification and quantification are all very difficult. Sample contamination and substrate interferences can also lead to large errors. Obtaining accurate qualitative data is often more of a problem than accuracy of quantitative data. Retention times and peak height measurements from gas chromatography coupled with highly sensitive, but nonspecific, detectors are most commonly used in residue analysis. Although dual column and/or dual detector determination, partition values and chemical derivatization are often employed, lack of good reference standards, interferences and poor detector specificity frequently cast doubt on the qualitative and quantitative accuracy of data upon which regulatory decisions may be made. Mass spectrometry and Fourier transform spectrophotometry offer partial solutions to qualitative accuracy where this instrumentation is available. However, less expensive and more sensitive specific detectors for gas chromatography are most needed. Means of quantitating residues from such complex industrial mixtures as polychlorobiphenyls and toxaphenes are far from adequate. Finally, collection systems for environmental media often lack efficiency, especially for volatile organic compounds in air.

Keywords: Concentration; detectors; extraction; gas chromatography; isolation and clean-up; mass spectrometry; organic trace analysis; qualitative accuracy; quality control; quantitative accuracy; reference materials; sampling.

I. Introduction

When one assesses accuracy, he tends to think in terms of how close he has come to the bullseye, or even to the target as is often the case. In trace

organic analysis, perhaps the more important consideration is whether he aimed at the right target. In the determination of complex organic compounds present at trace or ultratrace levels in our environment, accurate qualitative results are very often more difficult to obtain than accurate quantitative measurements.

The organic analytical chemist is confronted with an almost infinite number of chemical species encompassing a broad spectrum of structure and functionality. In environmental analysis, he must be concerned with their determination at concentrations down to the part-per-trillion level in a multiplicity of media ranging from air to human adipose tissue. It has been estimated that at least 10^5 organic compounds can be expected to be present in a sample of air at a concentration level of one part-per-trillion [1]. Even greater numbers may be contained in other media. The analyst must successfully isolate, concentrate, purify and often derivatize any of these compounds before he can first identify it and finally quantify it. In both of these determinative steps, the need for accuracy is becoming increasingly important. The need for qualitative accuracy probably outweighs the need for quantitative accuracy in organic residue analyses.

A. Pesticide Residue Analysis

Work in the field of pesticide residue chemistry has done much toward the development of the sensitive methodologies required for trace organic analyses. Pesticides are probably the most important group of organic chemicals which are deliberately introduced into the environment. Over a billion pounds of organic pesticides are manufactured in the United States yearly [2]. More than 800 different and often very complex chemical compounds are in use as pesticides. They are ultimately dissipated throughout the biosphere to the extent that their residues are found in everything from the air we breathe to our own body tissues.

In the early days of pesticide residue analysis (before about 1965), the chemist depended largely on column and thin-layer chromatography and colorimetry, which were sensitive only to the microgram level. Neither quantitative nor qualitative accuracy were easy to achieve. With the impetus provided by public appeals such as that generated by Rachel Carson's *Silent Spring* [3], a great deal of effort was expended during the 1960's toward the development of more highly sensitive and specific analytical methodologies for pesticides. Building primarily on developments in gas chromatography and improving isolation and clean-up procedures for multi-class compounds, the residue chemist has realized a

millionfold increase in his ability to determine organic pesticides and related compounds. While qualitative accuracy has improved significantly, quantitative accuracy has by no means kept pace with improvements in detection.

B. GAS CHROMATOGRAPHY

Most trace organic analyses today involve gas chromatography (g.c.). Pesticide residue chemistry has played a major role in the development of that field. Many of the most valuable g.c. detectors were devised for determination of pesticides. The most widely used g.c. detectors today are flame ionization, electron capture (or affinity), thermal conductivity, flame photometric, thermionic (or alkali flame ionization) and electrolytic conductivity. The sensitivities and specificities of these detectors are given in table 1. Despite the fact that they offer little or no specificity, the flame ionization and electron capture detectors are most frequently used in trace organic analysis because of the high degree of sensitivity required. The flame photometric detector is more reliable than the thermionic detector, although less sensitive, and is heavily used for determination of organophosphorous and organosulfur compounds. In the phosphorous mode (526 nm filter), its sensitivity for organophosphate pesticides is usually adequate for most residue applications. The electrolytic conductivity detector developed by Coulson [4] has good specificity for chlorine-, sulfur- and nitrogen-containing organic compounds, but lacks the sensitivity necessary for trace analysis. A highly sensitive, specific detector for organonitrogen compounds is badly needed due to the growing

TABLE 1. *Gas chromatography detectors*

Detector	Specificity	Sensitivity (g)	Linear range[a]
Thermal conductivity	Nonspecific	10^{-8}	10^{5}
Flame ionization	Nonspecific	10^{-11}	10^{7}
Electron capture	Semispecific	10^{-13}	50 to 100
Alkali flame ionization	Specific for P, S, X, N	10^{-12} (for P)	10^{3}
Flame photometric	Specific for P, S	10^{-10} (for P)	None
Electrolytic conductivity (Coulson)	Specific for X, S, N	10^{-9}	10^{3}

[a] Reference: C. H. Hartman, Anal. Chem. **43,** 113A (1971).

markets for the many new carbamate pesticides, which may ultimately replace the more environmentally persistent organochlorine pesticides. One solution to this problem may be the new microelectrolytic conductivity detector devised by Hall [5] with which reportedly as little as 10 pg of nitrogen can be detected. Much hope is also held for its utility as a specific detector for organochlorine pesticides with a sensitivity approaching that of the electron capture detector. At present, however, only the electron capture detector, which has poor specificity, and the essentially universal flame ionization detector afford the sensitivities demanded for the determination of such organic pollutants as pesticides, polychlorobiphenyls, phthalates and vinyl chloride which must be monitored at the parts-per-billion or parts-per-trillion levels. Accuracy of qualitative data, therefore, still remains the primary challenge to the environmental chemist.

C. The Analytical Scheme

The analysis of environmental or biological samples for trace amounts of organic contaminants seldom can be performed directly because of the multitude of interfering substances present in the matrix. Therefore, rather lengthy, multi-step procedures usually are required before the determinative step can be reached. It is through this process that most quantitative errors are introduced.

A typical analytical scheme for the determination of trace organic residues follows these basic steps: 1. extraction, 2. isolation and clean-up, 3. concentration, 4. identification, and 5. measurement.

In order to remove the compound of interest (usually along with a myriad of other compounds) from the matrix in which it is present (tissue, soil, water, sampling media), initial extraction with an organic solvent usually is required. Liquid-liquid partitioning and Soxhlet extraction are employed most often [7]. Complete extraction seldom is achieved. Animal tissues and soils present a large problem for extraction of organic and organometallic compounds. Binding to or solubility in the substrate may be so great as to result in poor extraction efficiencies. Furthermore, these substrates are particularly troublesome for yielding extractable material which interferes with electron capture detection. Isolation and clean-up of the compound is, therefore, mandatory. This may involve column or gel permeation chromatography [6], both of which may result in further losses due to irreversible column adsorption.

After chromatographic clean-up, as well as after extraction, the compound being sought is contained in a relatively large amount of solvent

(and possibly in more than one fraction). This solution must be concentrated before the sample is introduced into the determinative instrument for identification and measurement. Concentration (perhaps by a factor of 10,000 or more) not only results in further loss of the compound, but increases the possibility of interferences from the solvent itself. Loss by volatilization may be severe, especially for compounds having high vapor pressures. These losses may be minimized through use of the Kuderna-Danish concentrator shown in figure 1 [7]. With this apparatus, solutions can be concentrated from several hundred milliliters to a few milliliters in one step with only small losses of even somewhat volatile compounds. This procedure has the added advantage of speed, requiring only 15-30 minutes to reduce a 200 ml volume of hexane to about 5 ml. Further concentration to 50 or 100 μl is possible using micro-Kuderna-Danish concentrators. Often the use of a "keeper," usually a heavy paraffin oil, is desirable to assure minimal losses of more volatile compounds. The keeper, however, may interfere with some analyses (*e.g.*, flame ionization

Figure 1. Kuderna-Danish concentrator with three-ball Snyder column and expansion flask.

of mass spectrometry). Some recovery data are given in table 2 for the concentration of a 100 ml hexane solution of several pesticides to 10 ml, 1 ml and 0.1 ml (the latter, with and without a keeper).

TABLE 2. *Losses of pesticides on evaporation in Kuderna-Danish concentrators*

Pesticide	Original amount in 100 ml hexane (μg)	% Recovery on concentration to[a]			
		10 ml	1 ml	0.1 ml	0.1 ml with "keeper"
Diazinon	40	102 (2.4)	85 (4.4)	71 (1.8)	83 (1.8)
Aldrin	1.0	103 (2.8)	85 (4.0)	69 (1.2)	81 (0.6)
Malathion	40	85 (2.4)	91 (5.2)	77 (3.0)	88 (0.6)
Parathion	10	93 (4.4)	84 (4.0)	70 (1.2)	82 (2.4)
Dieldrin	2.5	103 (5.6)	92 (4.0)	78 (0.6)	90 (1.2)
p,p'-DDT	5.0	96 (9.2)	91 (5.6)	78 (3.0)	90 (2.4)

[a] Averages of six determinations for each pesticide. Standard deviations given in parentheses. Gentle stream of nitrogen used to assist concentration below 10 ml.

D. IDENTIFICATION

Identification is the most difficult step of trace organic analysis. Generally, the residue chemist knows what compound or group of compounds he is seeking, and attempts identification by comparing the unknown he has isolated from the sample to a known reference standard. Since gas chromatography is most often used in the determinative step, the most frequently used method of identification is by comparison of the gas chromatographic retention times of the standard and unknown under identical conditions. This method presents many possibilities for error as tens of thousands of organic compounds may elute from a given gas chromatographic column under a single set of conditions during the normal time span required for analysis. In addition, the retention time of the compound will change with fluctuations in carrier gas flow rate, column temperature and other parameters, as well as with variations in injection techniques. To circumvent some of these pitfalls, the analyst may use relative retention times; that is, he can compare the retention times of the unknown and standard to that of the third compound, which has been added to both the sample and standard solution. He may also compare relative retention times on two columns of different polarities (see fig. 2). This dual-column confirmation can be carried out in one operation if a column "combiner" is used. With some selectivity in the isolation and

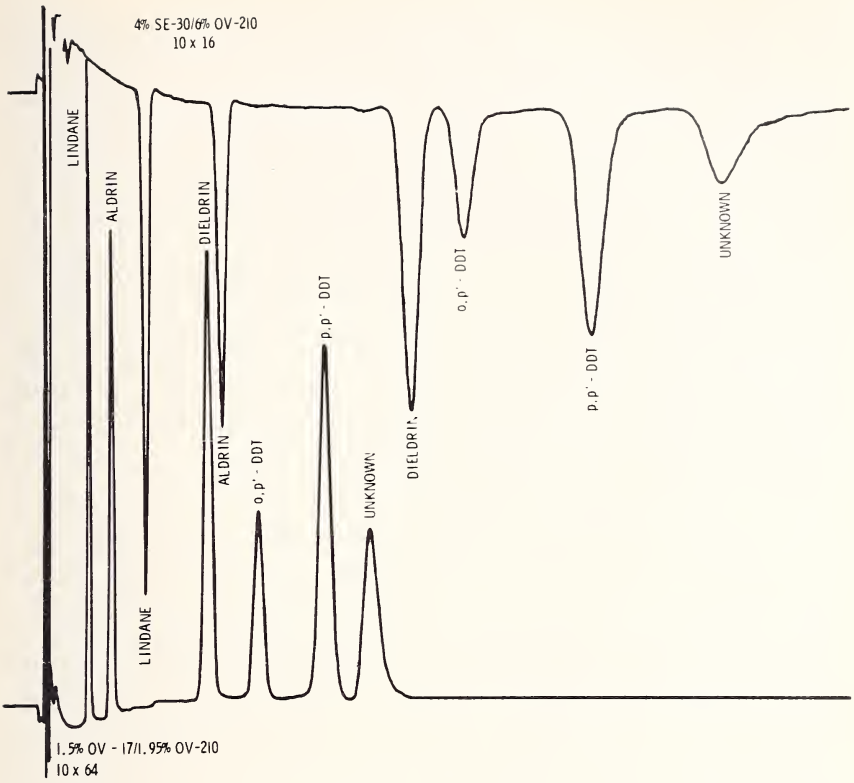

Figure 2. Dual column confirmation of pesticides by electron capture detection. Pesticides (from left): lindane, aldrin, dieldrin, o,p'-DDT, p,p'-DDT and unknown. Top chromatogram: 4% SE-30/6% OV-210 on Gas-Chrom Q. Bottom chromatogram: 1.5% OV-17/1.95% OV-210 on Gas-Chrom Q. Both columns 6.3 × 183 cm glass. Carrier gas: nitrogen, 65 ml/min Oven temperature: 200 °C. Detector: tritium, D.C., 215°. Chart speed: 1.27 cm/min.

clean-up steps (*i.e.*, fractionation of compounds by class or functionality), the electron capture detector and dual-column confirmation can probably afford 80 to 90 percent qualitative accuracy for the determination of organic compounds with high electron affinities. Most laboratories do not conduct further confirmatory studies.

When it is possible to use two gas chromatographic detectors, further confidence in qualitative accuracy can be achieved. For example, simultaneous analysis by electron capture and flame photometric gas chromatography is very useful for confirmation of organophosphorous pesticides (see fig. 3). In the case of the phosphorothioate class of insecticides (which includes malathion and parathion), simultaneous chromatograms

can be generated by monitoring both phosphorus (POH) and sulfur (S_2) emissions with a dual flame photometric detector.

If more qualitative accuracy is to be achieved, more sophisticated means of identification are required. Spectroscopic analysis usually affords the most information about an organic compound. Infrared and nuclear magnetic resonance spectroscopy are widely used by the organic chemist for structure identification. However, even with Fourier transform systems to enhance the signal to noise ratios of these spectrometers, detectabilities are still in the microgram to high nanogram range. This precludes their use for most trace analyses. Furthermore, interfacing with a gas chromatograph is difficult or impossible. Mass spectrometers, on the other hand, are readily interfaced with gas chromatographs and in many cases have sufficient sensitivity for identification at the residue level. Achievable detectabilities are in the nanogram range for total ion monitor-

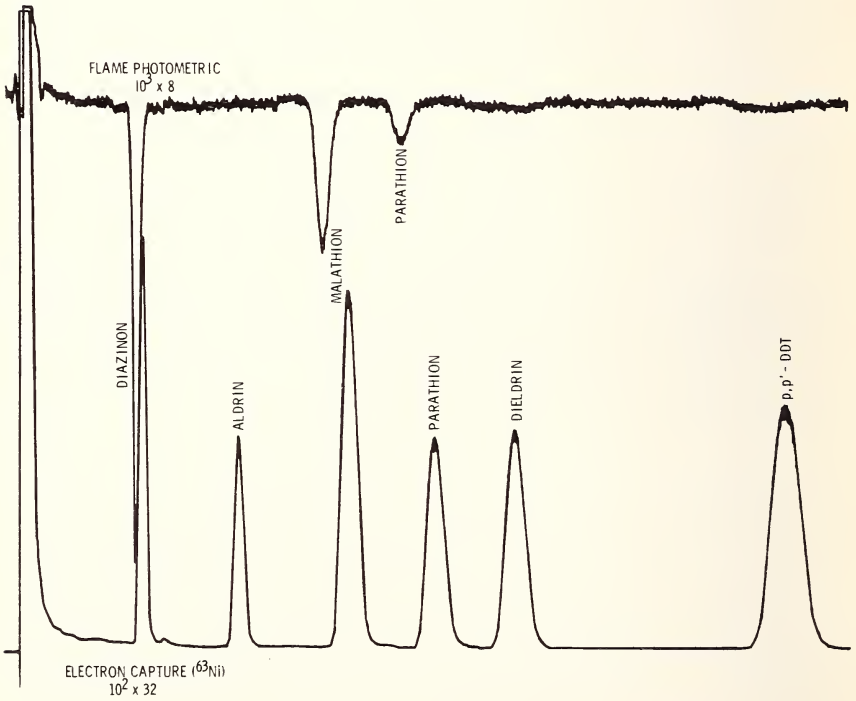

Figure 3. Simultaneous gas chromatograms of organochlorine and organophosphorus pesticides using electron capture and flame photometric detectors. Columns: 6.3 × 183 cm 4% SE-30/6% OV-210 on Gas-Chrom Q, 200°, nitrogen carrier gas; Detectors: Pulsed Nickel-63, 270°; Flame photometric detector, 526 nm filter, 200°. Chart speed: 1.27 cm/min.

ing or as low as several picograms for single ion monitoring. Although the mass spectrometer is most often used as a qualitative instrument, it can also be very useful for quantitative trace organic analysis. This use is discussed in the following section.

E. QUANTITATIVE MEASUREMENT

The first prerequisite to accurate quantitative measurement is the availability of good *primary standards*. In organic analyses, this is not always easy to assure. Because of the large numbers of different compounds required, the analyst must depend mostly on the manufacturers or chemical specialty suppliers for reference materials. A purity level of 95 to 99 percent is generally considered adequate. All too often, however, the compound stated to have 98 percent purity by the supplier is, in fact, only 75 percent pure. To be safe, the chemist should purify the chemical by recrystallization, sublimation, distillation, or other suitable means. Differential thermal analysis (DTA) often is convenient for determining the purity of organic analytical standards.

Organic compounds are subject to a wide variety of oxidation, hydrolysis, isomerization and polymerization reactions. Instability of organic standards is, therefore, often a problem. Storage conditions should be such as to retard degradative processes; *e.g.*, storage under refrigeration, inert atmospheres and protection from light. Purity also should be checked periodically by melting point, DTA or other appropriate means.

Preparation and use of secondary standards also must be given due consideration. In multiclass, multiresidue analysis, working standards are made up with a number of components in one solution in order to reduce analysis times. Care must be exercised that these mixtures do not contain compounds which are incompatible. Stabilities of standard mixtures may differ from those of pure standards or solutions of individual primary standards.

In some cases single compounds may not exist for, or be representative of, the organic pollutants of interest. The chemical may be manufactured, used and introduced into the environment as a complex mixture, the exact composition of which is unknown. A well known example of this are the polychlorobiphenyls or PCB's. There are 210 possible isomers and homologs of PCB's, 102 of which are considered probable [8] and likely to be encountered in environmental samples. A commercial PCB mixture may vary slightly in composition from batch to batch, but the residue chemist's primary problem is trying to interpret what he finds in a sample in terms of the source of contamination. The distribution patterns of

PCB's are altered in the environment because of differences between the volatilities, solubilities and chemical reactivities of the individual components of the original mixture. If a PCB mixture is introduced into an environmental or biological medium and later extracted for analysis, the pattern of peaks determined by gas chromatography may be drastically altered from that of the "standard" (*e.g.*, see fig. 4). For lack of a better way, quantification is usually accomplished by comparison of total peak heights or areas of all matching g.c. peaks in the sample with that for the standard. When the source of contamination is unknown, identification is also made by peak matching with commercial mixtures. Any similarity is likely to be purely fortuitous.

The problem of accuracy in PCB analysis is not easily soluble. Because of the complexity of commercial mixtures, identification of individual

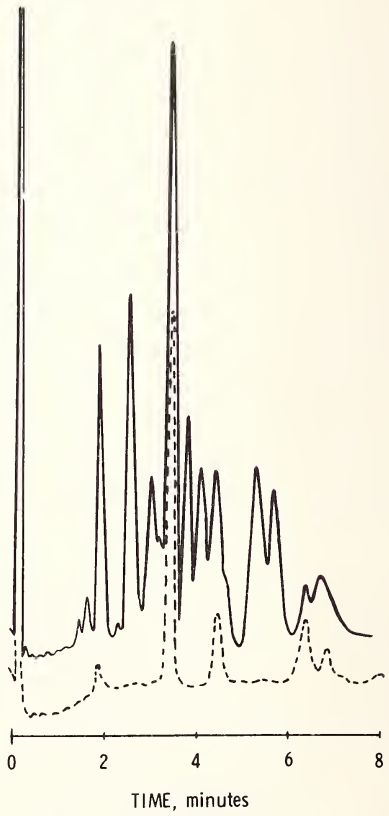

Figure 4. Alteration of polychlorobiphenyl mixture by rat. Top chromatogram: Aroclor 1016 standard. Bottom chromatogram: PCB's extracted from brain of rat dosed with Aroclor 1016 for 12 months.

isomers and homologs is not practical. Complete separation by gas chromatography is impossible and even the mass spectrometer cannot distinguish between isomers. One possible solution to the quantitative problem is to convert all of the PCB components to one entity by perchlorination [9]. The total PCB content is then measured as decachlorobiphenyl. Analytical methodology for this technique, however, has not been established completely.

It is of interest to note that before the existence of PCB's in the environment was recognized, pesticide residue chemists reported a PCB component as DDT in many samples. Doubtlessly, other PCB components have also been misidentified as organochlorine pesticides by residue chemists. The relative retention times (to aldrin) of the major components of a commercial PCB mixture are shown for comparison with those of several important pesticides in table 3. This particular mixture is no longer manufactured in the United States, but other mixtures of mostly lower molecular-weight PCB's are in use.

Similar problems associated with complex commercial mixtures exist in the pesticides field. Toxaphene, strobane, and chlordane are important examples. These mixtures are even more complex than PCB mixtures in that they consist not only of isomers and chlorine homologs of a given compound, but may also contain several structurally different com-

TABLE 3. *Relative retention times for polychlorobiphenyls and some pesticides*

Aroclor 1248	RRT_a[a]	Pesticide	RRT_a
	0.44	2,4-D (ME)	0.44
	.55	Simazine	.54
	.62	Lindane	.62
	.73		
	.80		
	.83	Heptachlor	.83
	.90	Ronnel	.90
	1.03		
	1.10		
	1.18		
	1.32	Methyl parathion	1.34
	1.50		
	1.80	*p,p'*-DDE	1.82
	1.92	Captan	1.92
	2.24		
	2.30		
	2.73	Endosulfan II	2.72
	3.12	*p,p'*-DDT	3.12

[a] RRT_a: Retention time relative to aldrin on 4 percent SE 30/6 percent QF-1 at 200°. 70 ml/min.

pounds. A gas chromatogram of technical chlordane is shown in figure 5. While only 18 different components of chlordane have been identified [10], one of these is heptachlor, a widely used insecticide. Some of the components of chlordane are readily oxidized, especially in biological systems, while others are unchanged. Identification and quantitation of chlordane in environmental samples is, therefore, nearly impossible. Metabolites such as oxychlordane [11,12] can be determined and used as an indication of chlordane exposure, but quantitative assessment of exposure or contamination is not possible.

Reference materials consisting of the compounds of interest at known concentrations in different substrates are required for determination of recoveries and limits of detection in multiresidue analysis. Preparation of accurate matrix standards may not be simple. The chemist must be able to disperse uniformly the standard compound in the matrix and remove residual solvents without loss of the compound. Even then, the nature and extent of the binding of the compound to the substrates may not be the

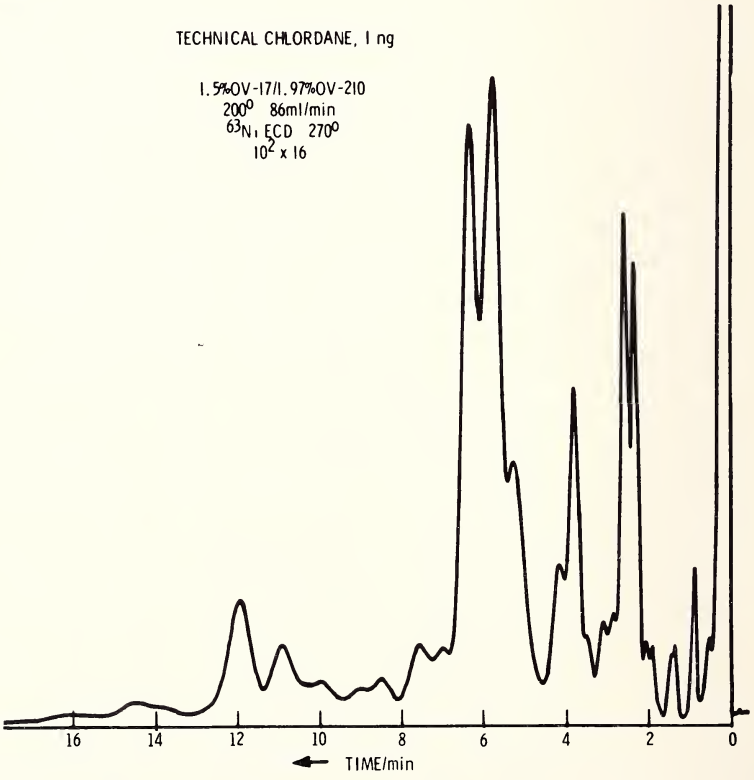

Figure 5. Gas chromatogram of technical chlordane.

same as that which would result through usual routes of environmental contamination or biological assimilation. Hence, use of recovery data in quantitative analysis still leaves an element of uncertainty. The analyst should be alerted to these potential problems and devote sufficient time to development of "spiking" techniques.

Preparation of the sample for analysis is also very important with respect to quantitative accuracy. Inefficient extraction and losses encountered in sample preparation are principal contributors to analytical errors in trace organic analysis. Steam distillation resulting from sample drying techniques can cause high losses, as can adsorption to drying agents.

Sample collection is frequently difficult to achieve with precision and accuracy. The collection of a representative sample has always been a major challenge to the environmental chemist. A great deal of study, involving population distribution, meteorology and statistics, goes into the design of monitoring operations conducted by the U.S. Environmental Protection Agency in efforts to establish accurate pollution profiles. Continuous air monitoring is probably the most difficult to accomplish accurately, especially for airborne organic pollutants. These compounds are usually present in ambient air at concentrations in the nanogram per cubic meter (ppt) range. A collection device which samples a relatively high volume of air is necessary to meet the required limits of detection. To be efficient, the sampler must entrap the atmospheric constituents quantitatively in a very short period of time and retain them against elution by the large volumes of air which subsequently or concurrently pass through the collection medium. Since organic compounds found in the atmosphere differ widely in their volatilities and chemical functionalities and may be present in gaseous, aerosol, particulate or particulate-adsorbed states, sampling efficiencies will necessarily vary considerably for any given sampler. The difficulty of collection of pesticides from air demonstrates this problem well. As a result, a satisfactory sampler for airborne pesticides has not yet been developed. In fact, a single air sampler for all pesticides never may be practical.

Calibration of air samplers is not always simple, particularly in the field, and is often neglected or improperly done. To determine sampling efficiencies, known concentrations of compounds in accurately measured air volumes must be generated then introduced into the sampler over both intermittent and continuous time sequences. This is difficult to achieve experimentally, hence, sampling errors probably out-weigh analytical errors in most cases.

After entrapment, the organic compounds must be extracted or otherwise removed from the sampling medium for analysis. In some cases it may be possible to elute them from the collection medium directly into the

analytical device by application of heat and gas flow. In most cases, however, this is not practical. If the compounds are to be retained effectively in or on the sampling medium after collection, they may not be removed easily for analysis. Since rather polar collection media are required, recovery of the compounds may be as difficult as recovery from fatty tissue and soil. Interferences from the multitude of airborne organic constituents, as well as those inherent in the collection medium, also contribute to the difficulty of qualitative and quantitative analysis.

Similar problems also apply to continuous water sampling. Batch sampling, either of water or air, increases the probability of obtaining nonrepresentative samples. In addition, adsorption of trace quantities of the compound on container walls significantly can reduce accuracy. It has been estimated that the walls of most 1-liter sample containers have an adsorption capacity of 5×10^{-5} g [1]. Filling such a container with an air sample for analysis even at the part-per-million level (1.2×10^{-6} g) could, theoretically, result in complete loss by wall adsorption. Certainly a dynamic equilibrium will exist between already present adsorbates (*e.g.*, water) and the compound of interest, but high losses are still possible. The importance of thorough flushing of the sample container is quite evident.

Many organic compounds are more susceptible to oxidation or other degradative processes when present in low concentration in a sample matrix. Delays between collection and analysis should, therefore, be minimized.

Contamination is another major enemy of quantitative and qualitative accuracy in trace organic analysis. Oils from finger tips; residue from improperly cleaned glassware; organic matter in distilled water; trace impurities in solvents, reagents, and chromatographic adsorbents; and extractables from plastics are all commonly encountered sources of contamination. Care must be exercised to avoid touching interior parts of glassware, including rims, even with gloved hands. Glassware must be meticulously cleaned and rinsed, before use, with solvents of high purity. Chromic acid cleaning is necessary for most glassware, especially for organic sample substrates. Water must be double- or triple-distilled and even then it must often be extracted prior to use. Solvents must be high purity. "Nanograde" or "pesticide" quality usually suffice, but even these should be concentrated and analyzed for possible interfering contaminants before use. Anhydrous sodium sulfate has long been known to be a source of an unknown contaminant, which can interfere with pesticide residue analysis by electron capture gas chromatography. Extraction with hexane is necessary for purification of this drying agent. Phthalates and other plasticizers often show up in organic residue determinations. Sources of these may be plastic bottle caps, plastic sample containers and

gloves. Generally only fluorocarbons, such as Teflon, Tedlar, or Kel-F can be considered free of plasticizers and other extractable additives.

Detector response reproducibility is a factor frequently overlooked by the trace analyst in the assessment of accuracy. In electron capture gas chromatography, operation of the detector outside the limits of its linearity of response is probably the greatest single source of quantitative error. The EC detector has a relatively limited range of linearity. This is especially true for the nickel-63 detector. In an electron capture detector, the standing current generated by the radioactive cathode varies considerably with the applied voltage. A response curve such as curve A shown in figure 6 should be generated by the standing current when the polarizing voltage is varied. The standing current increases with voltage until a maximum or plateau is reached. The voltage at which this plateau is reached will depend on the condition of the detector. A "dirty" detector will require a higher polarizing voltage for maximum response (see curve B, fig. 6). The most nearly linear response is obtained immediately below the plateau (in region X, fig. 6). Sensitivity is generally greatest in this region also, but it may be more desirable to operate at a slightly higher voltage than that which produces maximum sensitivity in order to avoid the peak

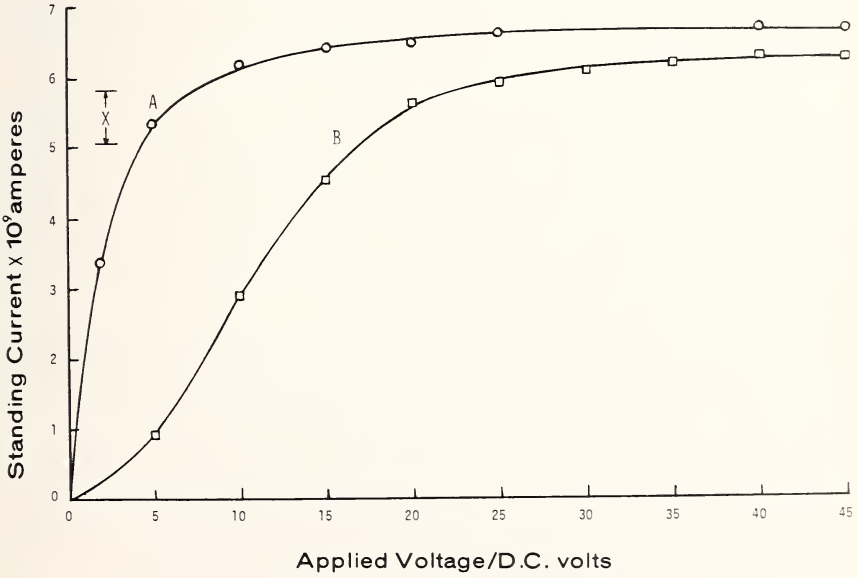

Figure 6. Standing current versus applied polarizing voltage dc for a tritium parallel-plate electron capture detector. Curve A: Clean detector (X = optimum range of operation). Curve B: Fouled detector.

"overshoot" shown in figure 7. Optimum performance is usually at 80 to 90 percent of maximum standing current. If one operates above 90 percent of the maximum response, unusual behavior can sometimes result and can lead to large and unsuspected quantitative errors. Figure 8A shows the response of an EC detector at the optimum polarizing voltage to a group of pesticides. In figure 8B, the response has changed drastically, producing elongated peak sides. This condition can be caused by in-

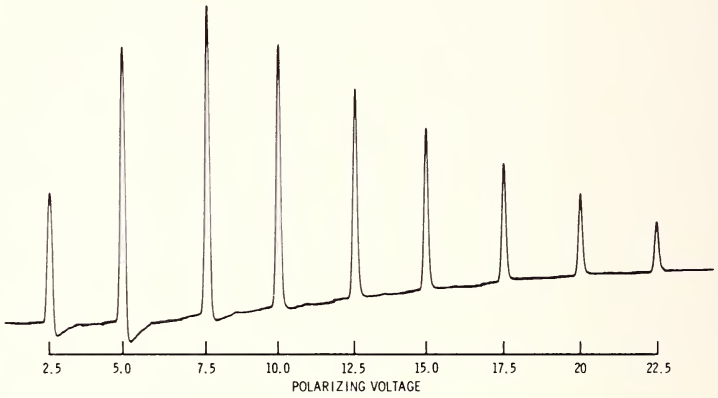

Figure 7. Electron capture response versus applied voltage dc for aldrin. Tritium parallel-plate detector. Optimum voltage is 10 volts dc.

creasing the standing current to above 90 percent maximum. Sometimes the standing current can increase due to the cleansing action of solvents or heat on the detector. This can occasionally happen during the course of a routine analytical procedure, as is illustrated by the two gas chromatograms shown in figure 9. In this case, the analyst was unaware that anything was wrong since the effect was obscured by the narrow peak profile [13].

Many of the problems discussed above can be avoided by pulsing the polarizing voltage (*i.e.*, using alternating rather than direct applied voltage). However, the linear dynamic range of the detector may be decreased in this mode.

Other gas chromatographic detectors have peculiar response behavior or linearity problems. A thorough understanding of the detector operation and close control over its operating parameters are prerequisites to attaining accurate quantitative results in gas chromatographic analysis.

Column performance must also be considered with regard to the overall precision and accuracy of gas chromatographic analyses. Poor g.c. column performance can result in inefficient resolution of compounds,

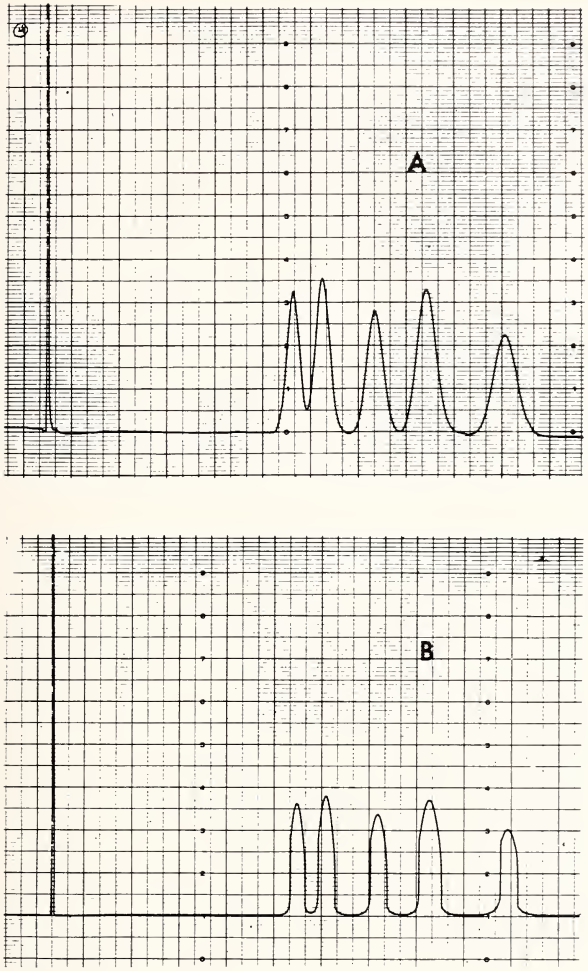

Figure 8. Trace A: Gas chromatogram of p,p′-DDE, o,p′-DDD, p,p′-DDD and p,p′-DDT; tritium electron capture detector, dc mode, optimized polarizing voltage. Trace B: Same as A except at elevated polarizing voltage (standing current above 90% of maximum).

skewed peaks from which it is difficult to obtain accurate quantitation, depressed detector sensitivity and poor recoveries.

Stationary phases for g.c. analysis must be carefully selected to satisfy the operating conditions required. When elevated temperatures (> 200°) are necessary, "column bleed," or loss of the stationary liquid phase from the column substrate, may occur and interfere with the detector response or sensitivity. This is particularly true where tritium electron capture detectors are being used. The upper limit of operation for a ^{3}H detector is set

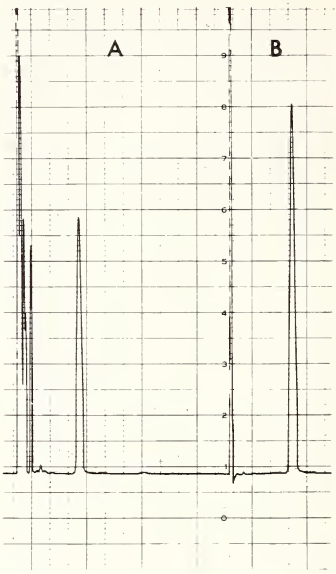

Figure 9. Trace A: Gas chromatogram of 2,4-D methyl ester using tritium electron capture detector at optimum polarizing voltage. Trace B: Same as A but with elevated polarizing voltage.

by the Atomic Energy Commission at 220°. Temperatures of 200° or more are necessary to achieve practical elution times for most complex organic compounds. With such small temperature differentials between column and detector temperatures, condensation of stationary phase in the detector can rapidly destroy its sensitivity.

Proper preconditioning, silylation and priming of a column are necessary to assure complete and uniform elution of compounds from the column and to reduce thermal degradation during chromatography. Degradation of column performance can also result from accumulation of oils and other high boiling residues. Figure 10 shows the deterioration in performance of a g.c. column with age as manifested by the dehydrochlorination of p,p'-DDT (peak 3) to p,p'-DDD (peak 4).

The efficiency of a g.c. column is also affected by the rate of flow of the carrier gas, as is shown in table 4. Once an optimum flow rate is established, care must be taken to maintain it.

Acquisition and interpretation of data are the final steps in qualitative and quantitative analyses. How this is done can in large measure affect accuracy. In gas chromatographic analysis, detector response is usually measured from recorder plots or chromatograms. The chromatographer usually employs one of three means of quantifying chromatographic

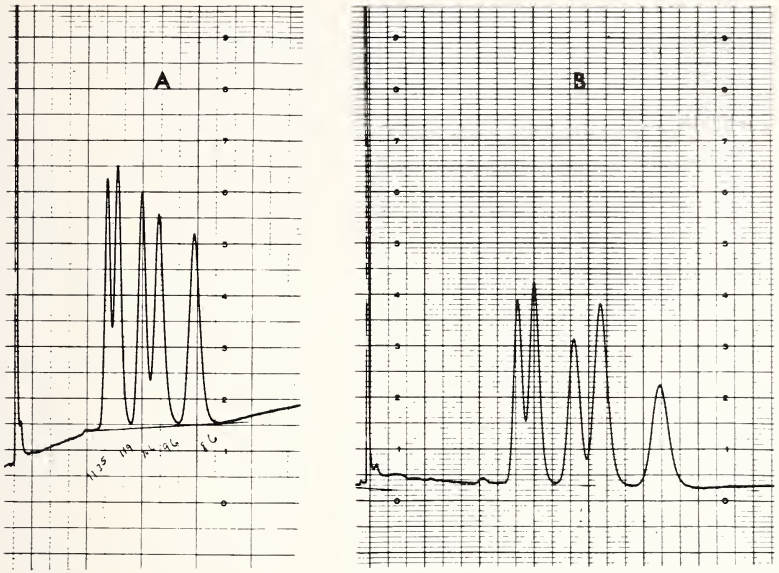

Figure 10. Gas chromatograms of p,p′-DDT, o,p′-DDD, o,p′-DDT, p,p′-DDD and p,p′-DDT on 4% SE-30/6% QF-1 at 180 °C. Trace A: New column; Trace B: Heavily used column upon which p,p′-DDT is converted to p,p′-DDD.

TABLE 4. *Effect of carrier gas flow rate on column efficiency in gas chromatography*

Flow rate (ml/min)	Theoretical plates (N)
20	1,500
30	1,970
45	2,140
60	2,050
92	1,590
145	1,310

peaks: (a) direct measurement of peak height; (b) manual estimation of peak area; or (c) electronic integration of peak area. Although most chemists tend to think of the last method as the most accurate, this is not always the case. For tall, slender peaks as type A in figure 11, peak height measurement is as accurate as any method and is the most convenient method to use. When broader peaks such as types B and C in figure 11 are involved, area measurements are generally more accurate. However, even in these cases peak heights may be used accurately if direct comparison is made to a standard of the same compound at about the same

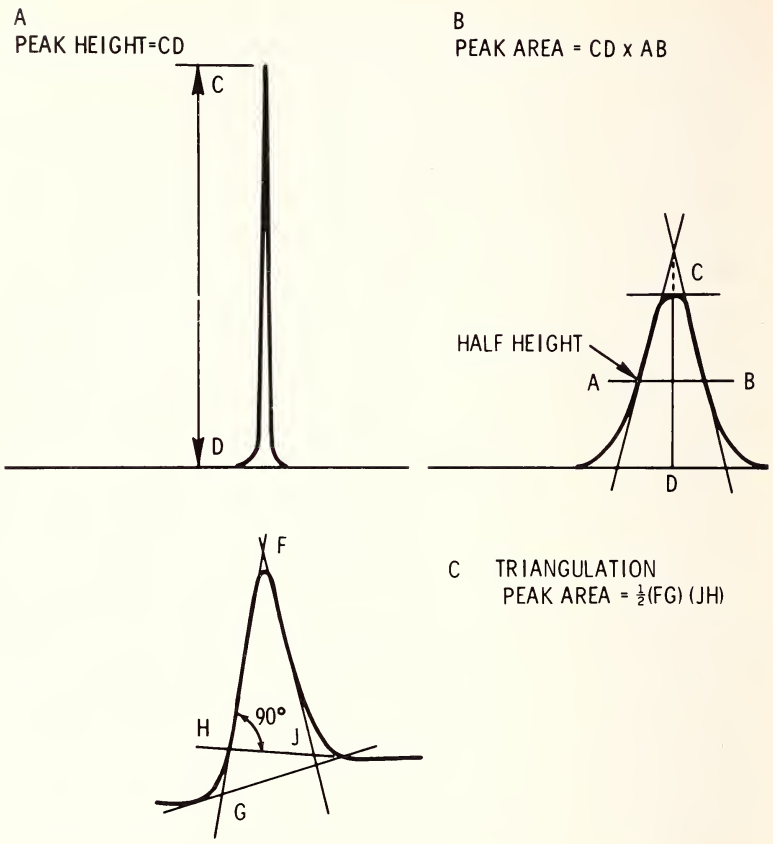

Figure 11. Three types of chromatographic peaks and appropriate methods of estimation for quantitative analysis.

concentration. It is good practice in any case to maintain standard and unknown peak heights (or areas) within 25 percent of each other. Electronic integration affords the highest precision when the peaks are well resolved and the baseline is stable. In a recent study of accuracy and precision in gas chromatography, Grant and Clark [14] found that use of peak height measurements provided comparable precision to that of an electronic integrator and was less affected by long-term variations. They also concluded that the product of peak height and width at half height method of manual area approximation (see type B, fig. 11) gave more accurate results than triangulation (see type C, fig. 11).

In comparing g.c. peaks of unknowns and standards several other precautions should be exercised. The standard and unknown should be analyzed within the shortest possible time span to avoid changes in

column conditions (temperature and flow rate). The standard should be injected immediately before and after the sample whenever practical. The same injection volume and type of solvent should be used for both sample and standard. Detector parameters (sensitivity settings) should be maintained constant throughout the analysis.

As in any analytical procedure, repeated analysis and statistical treatment of data for the rejection of outliers should improve accuracy. However, when large numbers of samples and/or long analysis times are required, this is difficult to accomplish in the available time. Automatic sampling systems with associated computerized data acquisition and reduction systems may partially relieve the burden of repetitive analysis. However, such systems seldom are capable of carrying out the most time-consuming aspects of organic analyses; *i.e.*, extraction, isolation and clean-up. Some success with automated clean-up operations utilizing such techniques as sweep co-distillation [15] and gel permeation chromatography [6] have been reported.

While *mass spectrometry* more frequently serves the trace organic analyst in qualitative analysis, it also can, and often does, satisfy his quantitative analytical needs. Both quantitative and qualitative accuracy can be increased by techniques such as mass fragmentography, isotope dilution, field desorption and chemical ionization. By these means detectabilities can be extended into the middle to low picogram range for many compounds.

In mass fragmentography [16], one or more specific ions in the mass spectrum are monitored selectively and simultaneously. This technique, also known as multiple ion detection (MID) or programmable multiple ion monitoring (PROMIM), will permit several ions from a single compound to be detected and accurately compared. Coeluting compounds from a gas chromatograph can also be simultaneously and quantitatively measured by MID. Sensitivity is increased by about two orders of magnitude over total ion monitoring. The utility of MID has been demonstrated for quantitative trace analysis of pesticides [17,18] and of drugs and drug metabolites [19-21]. Quadrupole mass spectrometers are better suited for MID than magnetic instruments because more rapid switching between ions is possible.

Double beam mass spectrometers allow separate recording of both reference and unknown compounds simultaneously, which increases the accuracy of mass measurement. Such instruments are very expensive, however, and are less popular.

Isotope dilution provides for quantification of organic compounds when complete isolation is not possible. In this procedure, the sample is "enriched" by the addition of a known quantity of the suspected compound

which has been labeled with a stable isotope (*e.g.*, ^{37}Cl). The isotopic abundances are determined from the mass spectra and the amount of compound (W) is calculated from the equation:

$$W = W_L(A/A_L - 1),$$

where W_L is the amount of labeled compound added and A and A_L are the abundances of the total and label isotope, respectively. The method suffers from poor precision (± 1 to 2%). However, by combined isotope dilution and MID techniques precision of ± 0.1 to 1 percent and accuracies of ± 0.1 to 3 percent have been reported [22].

Chemical ionization mass spectrometry [25] probably has more utility in structure elucidation than in quantitative analysis. Although a fragmentation mechanism is employed, high abundances of quasi-molecular ions are produced, which facilitates both identification and quantification. This technique has been found useful for polychlorobiphenyls [26,27] and drug analysis [28].

Special computer techniques such as signal averaging have been employed to increase signal-to-noise ratios in trace mass spectroscopic analysis for improved accuracy [29]. In signal averaging, many successive scans are made over a narrow mass range and are then added together to produce an integrated mass spectrum. By this procedure, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has been quantified in biological media at levels down to a few picograms [30].

Combined gas chromatography-mass spectroscopy has also made possible the determination of benzo(a)pyrene and benzo(e)pyrene in ambient air samples [31].

F. Quality Control

The last and one of the most important requisites for accuracy in trace organic analyses is the maintenance of a good quality control program. This is particularly important when many routine analyses are conducted and when more than one laboratory is involved in the analyses.

Quality control (QC) can be classified into two types: intralaboratory and interlaboratory. The former is concerned with establishing and maintaining high levels of precision and accuracy within the laboratory. An intralaboratory quality control program provides for optimization and standardization of analytical methodologies and instrumentation, and systematic analysis of standards and blanks for maintenance of precision and accuracy. Interlaboratory quality control is necessary when two or more laboratories are participating in the analysis of pooled or related samples.

In the latter program, a central monitoring laboratory is responsible for (a) the establishment and implementation of standard methodologies, (b) the provision of standard reference materials and collaborative check samples, and (c) the detection and correction of problems affecting the quality of output from participating laboratories.

In monitoring and regulatory agencies such as the U.S. Environmental Protection Agency and the Food and Drug Administration, the importance of effective quality control programs is obvious. The Pesticides and Toxic Substances Effects Laboratory (PTSEL) of the EPA and its predecessors has operated inter- and intra-laboratory quality control programs for nearly 7 years [32].[1] The program is designed to optimize the precision and accuracy of trace organic analysis conducted by contract laboratories and by the 10 EPA Regional Offices. Under this QC program is the National Human Monitoring Network.

The Laboratory has developed and is continually improving analytical methodologies for use by the participants. The methods are published in manual form [33]. Additionally, PTSEL operates a repository of over 500 organic chemical standards, with principal emphasis on pesticides. The Laboratory purchases under contract, pretests, and distributes gas chromatographic and column chromatographic packing materials. Electronic maintenance, calibration and trouble-shooting service for gas chromatographs and ancillary detector systems is also provided. Protocols for preventative maintenance of instrumentation (which is mostly standardized) and for acquisition and evaluation of all solvents and reagents have been established under the intralaboratory program.

Intralaboratory standards of pesticides in adipose tissue (rendered chicken fat) and human blood and interlaboratory check samples are prepared by PTSEL staff and distributed on prescheduled bases to each participating laboratory. Each participant is graded on both qualitative and quantitative accuracy, with greater weight given the former.

Over the years of operation of this quality control program considerable improvement in precision and accuracy has been achieved. Mean relative standard deviations for determination of multicomponent mixtures of pesticides in fat and blood samples have decreased from 36 to 38 percent in 1968 to 12 to 16 percent in 1974 (see table 5). Other quality control programs for organic trace analysis within EPA are administered by the Methods Development and Quality Assurance Research Laboratory and the EPA Mass Spectrometer Users Group.

Most needed in this and other quality control programs for trace organic analysis, are standard reference materials of many organics in such

[1] A training program consisting of seven courses covering the complete analytical and instrumental methodology was offered from 1966 until 1972, when operating funds were terminated.

TABLE 5. *Progress in interlaboratory precision from 1968 through 1974*
(Epidemiologic studies and human monitoring laboratories)

Interlab check sample number	Year	Number of labs	Number of compounds	Average rel std dev, %
		Fat analysis		
9	1968	21	7	38
11	1969	19	7	24
21	1972	16	7	19
24	1973	14	7	14
28	1974	10	7	12
		Blood serum analysis		
6	1968	22	6	36
10	1969	20	5	29
16	1970	22	4	21
17	1971	20	4	17
22	1972	17	4	14
23	1972	17	4	12
25	1973	18	4	13
27	1974	15	3	16

matrices as human fat and liver, soil, water, and air. Intergovernmental agency cooperation and participation by the National Bureau of Standards will be necessary if these needs are to be satisfied.

II. Summary

The trace organic analyst is faced with the formidable task of isolating, purifying, identifying and quantitatively measuring a single compound out of tens of thousands that may be present in a given sample at the trace level. Identification, or qualitative analysis, is more difficult to achieve than quantification. Qualitative errors, therefore, are common in trace organic analysis.

Quantitative accuracy is reduced by inefficient sampling procedures, poor recoveries on extraction (especially with organic matrices); by losses on isolation and clean-up; by losses on concentration; by nonlinearity or otherwise poor performance of the determinative instrument, and by lack of accurate reference standards.

Poor accuracy in organic qualitative analysis at the trace level results primarily from lack of specific detectors with the required sensitivities.

Other contributors to qualitative errors are sample contamination and chemical alteration before or during analysis.

More sensitive, specific analytical instruments are greatly needed in trace organic analysis. Mass spectrometry satisfies this need to a large extent at present.

An effective quality control program is prerequisite to both qualitative and quantitative accuracy. Good standard reference materials should also be made more available to the trace organic analytical chemist.

III. Acknowledgement

The author wishes to thank Mr. R. C. Hanisch, Mr. M. D. Jackson, Ms. K. E. MacLeod, and Mr. J. F. Thompson, all of the Chemistry Branch, Pesticides and Toxic Substances Effects Laboratory, EPA, for their contributions to this paper.

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ACCURACY AND QUALITY CONTROL IN TRACE ELEMENT ANALYSIS

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Trace element analysis, not uniquely, but to a unique degree, is affected by the problems associated with contamination of the specimen during the process of collection, as well as by the contamination of reagents and the environment during the process of analysis, which will be discussed in other presentations.

The quality control of accuracy in trace element analysis must therefore cover all phases of the analytical process, including collection. Such an extension makes it necessary to use surrogate specimen material with defined trace element(s) content beyond the laboratory, that is, in the sphere of specimen collection in the field, on a regular, protocol-directed basis. The protocol for using such surrogate specimens for accuracy control should be directed to the exposure and display of any variations in systematic bias which may occur. Such bias, or error, may be either positive or negative and may arise from defects or alterations in collection materials or in procedural errors in the collection process.

The nature of the surrogate specimens required for accuracy control depends upon both the type of specimens to be collected and analyzed and the details of the analytical process itself.

Keywords: Accuracy and precision; clinical chemistry; quality control; surrogate specimens; trace elements.

I. Introduction

The importance of accuracy and the difficulties peculiar to the attainment of analytical accuracy and precision in analysis for trace elements already have been emphasized by other speakers in this Symposium. The

consequences of inaccuracy in clinical chemistry were outlined in this Symposium last year [1] and will not be repeated here.

Quality control in trace element analysis has basic features in common with quality control as practiced in most clinical chemistry laboratories. Trace element analysis has, however, certain peculiar features which affect accuracy as well as precision to a unique degree, thus requiring special analytical precautions and applications of quality control surveillance principles which are not common in clinical chemistry.

Perhaps it would be useful to review briefly the elements of quality control as practiced in clinical chemistry laboratories and then to note the modifications and extensions called for in trace element analysis. In quality control in clinical chemistry, a number of surrogate specimens are commonly inserted in the analytical series. These surrogate specimens are intended to represent, in all essential respects, a natural specimen whose content of the desired analyte is known to a high degree of certainty. The frequency of surrogate specimen analysis depends upon the intended use of the quality control data.

If the drift of results, or within-day variability, is to be assessed a number of surrogate specimens will be analyzed; if only an estimate of between-day variability is needed, a few or one specimen each day may be used. Such control specimens may be used to assess the accuracy or the precision of the analytical process, or both.

A. Implied Assumptions

Four unstated assumptions which underlie the use of the simple quality control system outlined should be emphasized.

1. The validity (accuracy and specificity) of the analytical process for the designated analyte, in the specimen matrix, has been established.

2. The stability of the analytical process (within-day and/or run and/or between-day) has been established and is controlled to an acceptable level.

3. The validity and pertinence of the calibration process and the calibrator with respect to the designated natural specimens and the analytical process have been shown.

4. Quality control materials (surrogate specimens) of appropriate, reliably designated analyte content values, stable and free of inappropriate interferences, can be provided.

Each of these assumptions will be discussed separately.

1. Validity of the Analytical Process

The validity of any analytical process certainly can be investigated. The long experience of the National Bureau of Standards (NBS) and more recently that of the Center for Disease Control (CDC) and of many other regulatory agencies indicates that validity is an expensive commodity. It involves an exhaustive delineation and control of the analytical process and its sources of error and interferences, as applied to the designated natural specimens, the calibration process and materials, and surrogate control specimens. In the extended concept of quality control, validity could be considered the cornerstone of quality control.

2. Stability of the Analytical Process

The concepts and procedures of quality control are not normally applied to an analytical process before it has achieved a considerable degree of stability. It is difficult to separate this concept of a stable analytical process from the process of assessment of variability. In a sense, they are measured by the same quality control process and use similar surrogate specimens and control protocol(s). Unless an analytical process is under such control that the results are usefully reproducible, it is not a proper object of routine quality control. Unless quality has been achieved, it is not controllable or measurable.

3. Calibration Process and Calibrators

The calibration process and the calibrators used could have been included under the concept of analytical validity. However, since the same valid analytical process may require different calibration procedures and materials for different types of natural specimens, it is useful to discuss them separately. In trace element analysis, the calibration process needed is greatly affected by specimen matrix effects. Such efforts may alter the results by a constant amount (absolute interference) or by an amount proportional to the analyte content, or both.

4. Quality Control Materials (Surrogate Specimens)

Over many years, surrogate specimen materials have been developed, and they are available for a large number of analytes commonly measured in the clinical chemistry laboratory. Dried (lyophilized) plasma, serum,

urine, and synthetic surrogate specimens can be purchased with designated values for 60 to 80 analytes ranging from electrolytes and simple small molecules (urea, glucose) to more complex analytes, such as steroid and protein hormones and enzymes. The reliability and stability of some of the values assigned to such products may be in doubt, but they are generally very useful.

This relatively happy state of affairs does not exist for trace elements. Even for blood, blood serum, and urine, the problems of preparing and packaging as well as that of reliably characterizing the trace element content, are formidable. Added to these problems is the fact that hair, skin, and sweat, in addition to the usual fluid biological tissues, commonly are used as specimens. *Dusts* and air are objects of special attention, and each has its own peculiar matrix effects.

B. Specimen Collection and Protection

So far, we have considered the usual type of quality control that is confined to the laboratory environs. The usefulness of trace element analysis as a socio-economic tool requires a consideration of the processes involved in the collection and protection of the specimen.

The peculiarly unique feature of specimen collection and protection in trace element analysis arises from the simple fact that we are looking for "traces." Adventitious contamination easily may arise when a specimen is being obtained; for example, a specimen of blood may be contaminated with dirt on a finger during finger prick collection. Many environments are unbelievably dusty, and collection apparatus may be contaminated easily. Collection containers may contribute to contamination and, in fact, may exhibit "negative contamination" (*i.e.*, erroneously low values) under certain circumstances. The degree of such "interference" may be very large (30 to 60 times the normal values).

C. Total Process Quality Control

The first stage in quality control, as was previously mentioned, should be the demonstration of analytical validity. This should be extended to the total process; each step in the total process must be demonstrated to be valid (for example, a test tube must be shown to be free of zinc before it can be used in a zinc analysis).

The second stage in quality control is a continuing one and constitutes what is commonly termed *quality assurance*, or quality control surveil-

lance. This is the continuing assessment of each step of the analytic process, from specimen collection to the analysis of the collected data, to assure accuracy and precision from start to finish.

D. Quality Control Surveillance

Specimen Collection Control – Assuming a valid collection procedure exists, surveillance procedures appropriate to local conditions can be applied. The following approaches may be taken:

Blank Collection Vessels – with and without water, in the field.

Split Specimens – A sufficient number of specimen(s) may be collected from individuals to allow for two (or more) analyses on several (or all) of each day's collection. The split specimens may be sent to the same laboratory or to different laboratories.

Surrogate Field Specimens – Surrogate materials, simulating natural specimens, may be used in the field to test collection procedures and separate laboratories. Such specimens may also be used to test the stability of specimens in the field by storing them under ambient rather than laboratory conditions.

E. Analytical Process Control

Calibration – In trace element analysis, the stability of standard calibrating solutions is always open to question. Unless the validity of the calibration can be assured by some means, and by a surveillance procedure remains assured, the entire analytical process is useless. These approaches may be taken:

a. *Fresh solutions.* If the fresh new solutions compare well with the old, assurance is increased; if not, confusion arises.

b. *Records of response.* In a stable analytical process, the instrumental response(s) of calibrators is (are) recorded and used as a quality control measure.

c. *Known normal specimens.* Immediate analysis of a carefully drawn specimen will add assurance of quality.

d. *Linearity.* In some analytical processes, a number of calibrators sufficient to assure daily (or even each run) linearity may have to be used.

e. *Recoveries.* In some analytical processes, recoveries may have to be used to assess analyte content, even for each individual specimen.

f. *Blanks.* Blank values (or recovery intercept values) must be assessed as part of daily analysis in many procedures. Blanks are of many types, and, in the validation process, as many as practical should be explored.

II. Summary

The control of quality (that is, accuracy and precision) in trace analysis can be divided into two phases: (1) establishment of quality and (2) continual surveillance of quality.

Establishment of quality includes the initial establishment of the validity of the total analytical process from the collection of the specimen to the interpretation of the data.

Surveillance of quality must also extend to the total analytical process, including collection of the specimen.

The development and distribution of surrogate specimens appropriate for trace element studies in biologic materials is the single most effective step in both the establishment and the maintenance of quality in trace element analysis. Such well-characterized surrogate specimens will allow rapid, efficient, and effective tests for the validity of presently used procedures and for proposed new analytic procedures. Such surrogate specimens are essential for accuracy control and for continuous surveillance.

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THE ROLE OF THE NATIONAL BUREAU OF STANDARDS STANDARD REFERENCE MATERIALS IN ACCURATE TRACE ANALYSIS

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A meaningful measurement process is capable of producing numerical values of the property(ies) under test or measurement that are compatible throughout the measurement infrastructure. By this we mean that all the measurement laboratories within a given industry or technological or scientific area are capable of and, in practice do, produce measurement values for a given property on a given material that are identical and immediately comparable within some agreed on uncertainty. Such measurement compatibility results when accurate measurements are the basis upon which the work is founded. When a measurement system is accurate, then the numerical values produced are free of systematic errors and are also precise. We will show that, in practice, a certain degree of precision must be obtained before assertions of accuracy can be realistically tested experimentally.

One mode by which accuracy may be transferred to all laboratories within a measurement infrastructure is through the use of reference materials used in conjunction with reference methods. At NBS reference materials are called Standard Reference Materials (SRM's). They are well-characterized materials (in terms of accurately determined properties) useful for the calibration and/or assessment of a measurement system. When SRM's are used in conjunction with a reference method, *i.e.*, one of demonstrated accuracy, then it becomes possible to transfer accuracy throughout an entire measurement infrastructure. How this is accomplished will be discussed.

Obviously the assurance of the "built-in" accuracy of the SRM is critical. How this is done at NBS will be discussed. Finally, currently available SRM's useful in trace analysis will be considered, as well as work now in process and future plans for additional trace SRM's.

Keywords: Accuracy; accurate measurement system; precision; reference methods; standard reference material; systematic errors.

I. Introduction

The key word in the title of this paper is "accurate," and it is there because the theme of this entire Symposium is accuracy. Because there are no universally accepted definitions of accuracy or accurate analysis, it is important that these be defined in the context of this paper. We say an analysis is accurate when the numerical value of the property under measurement is free of systematic error and is also precise. In other papers [1,2], one of us (JPC) has said that numerical values free of systematic error are accurate values, consistent with traditional usage by chemists. This dual usage of the word accurate (or accuracy) to apply to either the concept of (accurate) measurement, or to the measurement numerical value itself is the cause of some misunderstanding. This misunderstanding is especially true between scientists whose work is primarily physics (or metrology) oriented, and those whose measurements are concerned principally with chemical properties, including composition. Therefore, we offer this formal definition:

An *accurate measurement system* is one that produces precise numerical values of the property or properties under test or analysis that are free of, or corrected for, all known systematic errors. Such values are also related to the "true value" of the property(ies) under test or analysis.

A full description of what constitutes a measurement system, the various kinds of errors, the interactions of accuracy and precision and other related matters, while germane to this paper, have been adequately described in the series of articles gathered together in reference [3], and will not be repeated herein.

An example, not too widely used, will make clear in a graphical way what is meant by an accurate measurement system. Let us imagine a marksman shooting at a target (fig. 1). The bull's-eye corresponds to the "true value," the number sought in most measurement systems. The marksman is to fire six shots, trying to hit the bull's-eye each time. The upper part of the figure is the result of the first set of six trials, or replicates, if you will. Since the hits (or numerical values) are not grouped together in any consistent fashion, we say immediately that there is no reproducibility in the system (or, that we are imprecise). As to whether there are errors of a systematic nature involved, is extremely difficult to say at this point. We can only guess (hopefully in an educated sense) as to some physical cause to explain the widespread scatter. We decide to eliminate the marksman (analogous to the use of more automated analytical systems), hold the rifle in a vise, and fire off six more shots with the result shown in the middle of the figure. We now have good reproducibili-

SRM's are *well-characterized* (in terms of end-use and with "quality assurance" mechanisms spelled out) and *certified materials* (by International Organizations, National Governmental Laboratories, National Standard Bodies, and Industrial or Trade Associations), produced in quantity so as to be continually available without regard to national boundaries:

- To help develop *reference methods* of analysis or tests; *i.e.*, methods of proven accuracy

AND/OR

- To *calibrate* a measurement system in order to:
  - Facilitate the *exchange of goods*
  - Institute *quality control*
  - Determine *performance characteristics*
  - Characterize at *scientific frontiers*

AND/OR

- To assure the *long-term adequacy* and *integrity* of the quality control process.

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Figure 1. Diagram showing systematic errors in an accurate measurement system.

ty (precision) and are also in the position of being able to ask intelligent questions concerning the now-evident inaccuracy in our system. We can now ask what might cause the shots to be high and to the left of the target—sight incorrectly adjusted; wind not accounted for; or bent rifle barrel? We can now design experiments to test each hypothesis, to identify

each source of error, and then we can finally either eliminate (physically) each error or correct for them in subsequent trials. Finally, we arrive at the desired state, illustrated at the bottom of the figure.

Insofar as this illustration is a fair analog of a measurement system, one can see that in practice it is necessary to achieve precision before the study of systematic error can begin in a reasonable way. We shall examine this proposition in more depth later in the paper.

At this point one could reasonably ask, "Why is accurate analysis needed?" It can be argued that many analytical methods are relative and provide numerical values that are dependent on the method used or are relative to agreed on artifacts, serving as standards. Indeed, such relative measurement systems are of the greatest practical importance. For example, the entire synthetic rubber industry's measurement process is based on relative rather than accurate or "true value" measurements. In this industry, the instruments that measure the properties of importance, *e.g.*, viscosity, tensile strength, *etc.*, are calibrated with "standard" rubbers that have been made from recipes agreed on by the industry. These standard rubbers are compounded from NBS rubber and rubber compounding SRM's according to the American Society for Testing and Materials (ASTM) methods. Thus, all subsequent measurements made throughout this industry are referable to this common base.

Problems arise, however, when measurements must be compared across different technical or scientific fields, because standard measurement practices are rarely used or transferable across different fields. For example, in the sugar industry glucose is sometimes measured by a method involving the reduction of copper. In clinical chemistry glucose in serum is often determined by an enzymatic reaction. Neither method is based on accuracy and thus results across these two fields are not directly comparable. Yet this is not a farfetched example when one considers that in a study concerning the nutritional value of certain foods or metabolic studies the glucose intake value (from sugar industry) might be compared with an output value (from clinical chemistry lab). Here relative values will not work and, indeed, may cause more confusion than understanding.

Measurements that have legal implications have less complications when the measurements are made on the basis of accuracy, since all concerned are measuring from a fixed, nonfloating base (*i.e.*, the true value).

Indeed, the greatest argument in favor of accurate measurement is that of assuring compatibility in measurement. By compatibility we mean, as the dictionary says, the capability of getting along well together. Thus, since there can be only one "true value" or accurate value for a given property in a given material, and if all the measurers within a given measure-

ment infrastructure obtain that value (within some agreed-on-beforehand uncertainty), then all are indeed compatible, *i.e.*, they get along well together since there is no reason for disagreement—as far as the measurement is concerned at any rate. In such a measurement infrastructure, *accuracy assures compatibility on an independently realizable basis.*

How does one, then, in practice, spread measurement accuracy throughout the measurement infrastructure? One of the most direct ways of achieving this goal is to provide scales—and methods, which describe the use of the scale—that are accurately calibrated. Since we are concerned at this Symposium with the accurate analysis of materials in trace quantities, our scale becomes a material or materials of accurately known composition and our methods we will call reference methods. In the United States well-characterized materials are called Standard Reference Materials (SRM's), if produced and certified by the National Bureau of Standards.

In the balance of this paper we will discuss: (1) what an SRM is; (2) how NBS builds accuracy into its SRM's; (3) how an SRM serves as a transfer mechanism for helping to bring about or to assure accurate measurement; (4) currently available and planned SRM's useful in trace analysis.

II. Standard Reference Materials

The formal definition used at NBS to describe an SRM is shown in figure 2. Note that the definition is cast primarily in terms of the end-uses of SRM's. There is now serious consideration, at least on the international scene to drop the "Standard" from the phrase Standard Reference Materials and to call the entire hierarchy of materials that are used for one or more of the purposes described in NBS definition by the generic term, "Reference Materials." The various classes would then be appropriately modified, *e.g.*, Certified Reference Materials for those reference materials whose properties have been guaranteed or certified by recognized standards bodies. Other classes of reference materials now in existence but not yet uniformly named might include: *e.g.*, Secondary Reference Materials—materials whose properties have been checked by or against Certified Reference Materials; Commercial Reference Materials—materials produced commercially for reference purposes, but whose guarantee rests solely with the producer. Other possible examples come readily to mind.

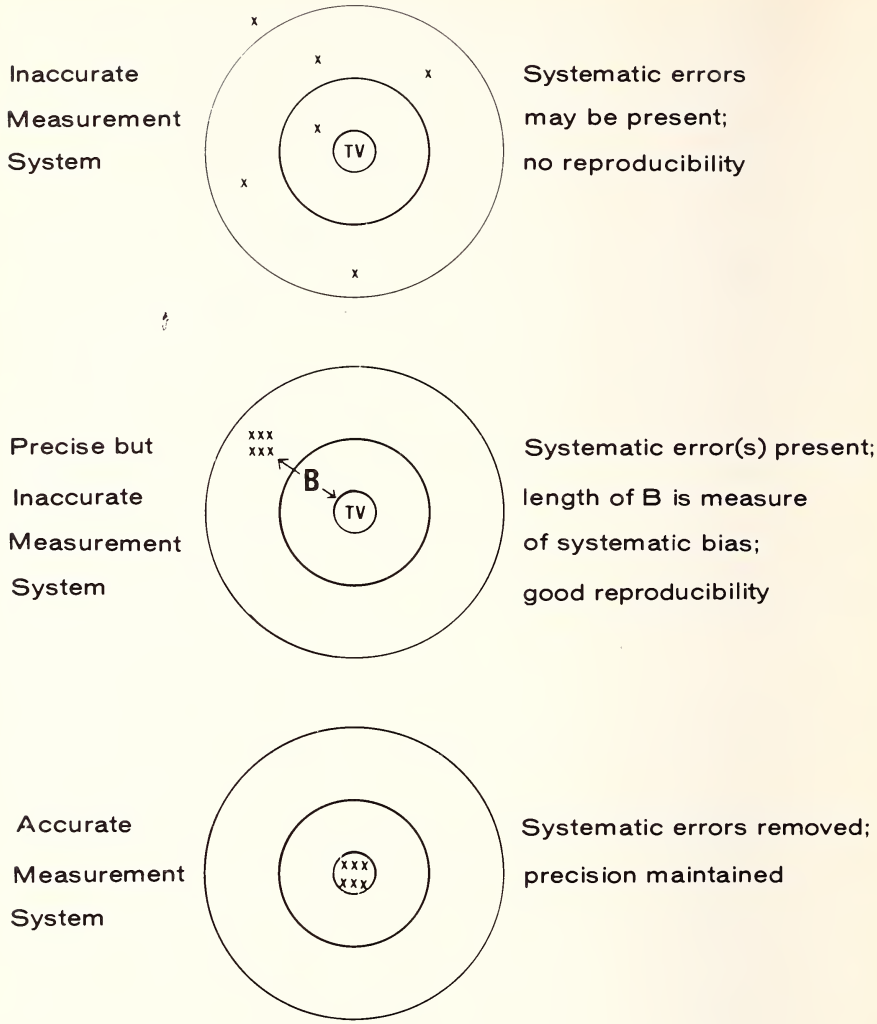

Figure 2. Formal definition of a Standard Reference Material.

It should be noted that the International Union of Pure and Applied Chemistry Commission I.4 on Physicochemical Measurements and Standards will in all likelihood recommend to the entire body the use of "Reference Materials" as the generic class name with three sub-classes: (1) Pure Substances as simple chemical entities that are used to realize the International System of Units (SI) base units or other important scales, *e.g.*, the International Practical Temperature Scale; (2) Calibration and Test Reference Materials (CTRM's) to include all reference materials not

in the class of Pure Substances, and also not certified; and (3) Certified Reference Materials – those CTRM's certified as described in the NBS definition.

III. Building Accuracy into SRM's

If, as we will show later, the SRM is to serve as the mechanism for transferring or spreading accuracy throughout a measurement infrastructure, then the numerical values of the properties measured and certified for an SRM must first of all be in themselves accurately known. Thus, the certifying laboratory must have formal mechanisms established to insure this result. At NBS three modes of measurement are used to assure that the values of the SRM property(ies) are accurate. These are: (a) definitive methods, (b) reference methods, (c) two or more independent and reliable methods.

A. Definitive Methods

In the first mode, measurement of the property is done using a method of analysis resting on "first principles." In clinical chemistry such a method has been given the name "definitive method," and will be the name used in this paper. A definitive method is one in which all major significant parameters have been related by direct or a solid chain of evidence to the base or derived units of the SI. Thus, if mass, time, and temperature are critical parameters in the measurement process then in the exposition of the written method, the scientist will say how each of these has been controlled, how traceability to the base or derived units has been accomplished, how stability with respect to these has been assured, *etc.* Further, he will be able to give with a high degree of confidence bounds to the limits of uncertainty (*i.e.*, the limits to the systematic errors).

Although not describing an SRM for trace analysis, the paper by Armstrong *et al.* [4] outlining the calorimetric method used for the measurement and certification of the Benzoic Acid SRM 39i is an example par excellence of the definitive method.

The article by Moore *et al.* [5] outlining a definitive method, the determination of calcium in serum by isotope-dilution mass spectrometry (IDMS), is an example in the trace field. Calcium in serum is found at the 2 to 3 millimoles per liter level.

At NBS, ID-MS has been used extensively as a definitive method for the accurate measurement of inorganic constituents at the trace levels. The equation which relates concentration to the analytical parameters is:

$$\text{Concentration of sample (wt)} = \frac{W_{sp}C[A_{sp} - RB_{sp}]}{BR - A} \cdot \frac{M}{W_s}$$

W_{sp} = Weight of spike solution, grams
C = Concentration of spike, μmoles/gram of solution
A_{sp} = Atomic fraction of isotope A in spike
B_{sp} = Atomic fraction of isotope B in spike
A = Atomic fraction of isotope A in sample
B = Atomic fraction of isotope B in sample
R = Experimentally measured ratio of A/B in the spiked sample
M = Atomic weight of analyte
W_s = Weight of sample, grams

The power of the ID-MS method rests on two aspects. First, all chemical manipulations are done on a weight basis and involve straightforward stoichiometric separations, precipitations, *etc.*, to determine W_{sp}, C, and W_s. Second, the mass spectrometric determinations involve only ratios and not the absolute determinations of the isotopes involved. Therefore, no instrumental corrections or errors are involved. This, of course, is an oversimplification of the experimental situation and readers are urged to examine the reference given above.

In table 1 are given the actual analytical data for the uranium content of the NBS Trace Elements in Glass SRM 614-15, as determined by ID-MS at NBS.

Further evidence of the power of this technique and mode of operation is shown when one examines the final certified numbers for all four concentration levels [6].

TABLE 1. *Uranium in glass by ID-MS (definitive method)*

Replicate number	Concentration (ppm)
1	0.824
2	.823
3	.822
4	.823
5	.823
$\bar{x} = 0.823$;	$\sigma = \pm 0.0007$

The certified value is 0.823 ± 0.002 ppm (wt). The expressed uncertainty includes method imprecision, an allowance for estimated, unknown systematic error, and includes heterogeneity of the entire SRM lot of material.

Over a period of time the measurements of the uranium content were made on all four of the trace element glasses at four different concentration levels. In addition, each measurement was made on different portions of the material so that the final uncertainty quoted includes the material heterogeneity, the method imprecision, and an allowance for unknown systematic error. See table 2.

A brief examination of the data in table 2 shows that the relative uncertainty of about 0.25 percent is constant over a wide range of concentrations. This indicates strongly that the method is unbiased insofar as concentration effects are concerned. At the lowest concentration level, the uncertainty becomes larger as might be expected when one begins to approach the region where blank corrections start to become significant. Other measurements by non-NBS laboratories using this and other techniques have confirmed the accuracy of this method.

Obviously, limitations of time, money, technical skills and resources preclude the widespread use of the definitive method. Further, most analytical methods cannot ever be classified as definitive methods, usually because there is no straightforward theory that relates all the experimental variables to the final result. It would be difficult, if not impossible, *e.g.*, to conceive of a definitive method based on emission spectroscopy simply because the theory that relates the energy (or light intensity) in a given spectral line to the concentration of the excited species is much too complex for direct laboratory validation.

TABLE 2. *Trace uranium in glass*

SRM number	Certified value	Uncertainty	Percent relative uncertainty
610–611	461.5	1.1	0.24
612–613	37.38	0.08	.21
614–615	0.823	.002	.24
616–617	.0721	.0013	1.8

B. REFERENCE METHODS

As defined here, a reference method is a method of proven accuracy. Here the accuracy of the method rests on or is demonstrated usually by (but not always), a definitive method. One may immediately ask why not use the definitive method itself as the reference method. The answer is primarily economic, secondarily, technical. Definitive methods are, as

was indicated above, expensive, time-consuming and often require special apparatus and highly skilled scientists. Reference methods are generally arrived at by consensus. That is, fairly extensive testing of the accuracy claims are made by a number of laboratories before its status is accepted by the measurement laboratories that will be using the method. One of the largest compilers of such methods is the American Society of Testing and Materials. Issued annually the latest edition of the Annual Book of ASTM Standards consists of 32,000 pages and includes over 4,700 ASTM standards, a substantial number of which could be called reference methods [7]. In technical areas where ASTM methods have been widely used and accepted, such methods are often used by NBS for the measurement of a candidate SRM. When SRM 1261, Low Alloy Steel, was in the preparation stage, it was decided to use a spectrophotometric ASTM reference method for antimony, even though the method was in the verification stages for inclusion in ASTM Methods E-350. This could be done because the accuracy requirement for antimony at the 0.004 percent level is not very stringent. Representative data from two laboratories are shown in table 3.

Because of the extremely important role reference methods play in assuring accurate measurement, we detour at this point away from the main theme to show how the accuracy of the reference method itself is proved or demonstrated.

TABLE 3. *Antimony in steel (SRM 1261)*

Reference Method—ASTM E-350 Brilliant Green

Replicate number	Antimony (wt %)	
	Lab 1	Lab 2
1	0.0045	0.00462
2	.0039	.00463
3	.0039	.00456
4	.0041	.00461
5	.0036	.00459
6	.0035	
Mean and standard deviation	0.0039 ± 0.0004	0.0046 ± 0.00003

Certified Value 0.004_2 percent.

1. Demonstrating the Accuracy of a Reference Method

To reemphasize, a reference method is a method of test or analysis that will provide accurate numerical values of the property under consideration. The critical question is how does one demonstrate or prove that a particular analytical method produces accurate results. One could proceed from a first principle approach as mentioned earlier. Alternatively, one can test the method with a material(s) whose property(ies) have been accurately determined, *e.g.*, an SRM. It is this latter approach that we wish to examine in some detail.

There are five major steps in the process. These are:

Step 1. Establish the accuracy goal, or (put another way), the degree or limit of inaccuracy to be allowed from the "true value." The accuracy goal should always be set in terms of the end-use requirements. Some scientists say that as a matter of principle one should always aim for the highest possible degree of accuracy. If there were no pragmatic considerations of time, effort, economic cost, *etc.*, we would agree. However, many, if not most, analyses are performed in response to economic, or legal, or technological needs and are therefore under serious time or cost constraints. For these reasons we say that the accuracy goal should be set just high enough to meet these practical considerations, with, however, an extra degree of accuracy built in to allow for advances in the state of the art. At NBS this latter factor is often set at about a factor of three higher than the pragmatic end-use goal.

Step 2. Choose the candidate method, which when demonstrated to produce accurate results becomes then the reference method. The candidate method should, if possible, be an already well-studied method, especially with regard to its precision and systematic error content. As will be shown shortly, it is important that the precision be considerably or at least potentially better than the accuracy goal set.

Step 3. Determine the "true value" of the property(ies) under consideration in a homogeneous, stable lot or batch of material similar in characteristics to the material to be analyzed or tested by the reference method. This may be done through the use of a properly chosen definitive method. Alternatively, an SRM may be used for this purpose. The second choice is preferred especially in terms of time saved and economic cost.

Step 4. Use the candidate method protocol (*i.e.*, the step-by-step procedure), determine in a sample of the material from Step 3, the numerical value of the property under study. The deviation of the mean ($\bar{x}$) from the true value (TV) is a measure of the systematic bias of the candidate method. If this bias is less than the limit of inaccuracy required of the

reference method, then the candidate method is (or has become, if you will) the reference method.

Step 5. If the found systematic bias is greater than the established limit of inaccuracy, then the candidate method must be further studied to identify sources of systematic error still inherent in the candidate method. As these systematic errors are identified, the candidate method protocol is revised as necessary to remove or eliminate (by correction) sufficient systematic errors so the condition stated in Step 4 is finally reached.

These five steps are shown graphically in figure 3. The circled numbers 1 through 5 correspond to steps 1 through 5, above. The following should be noted:

(a) limits of inaccuracy, or alternatively, accuracy goals are usually set symetrically around the "true value." The figure shows only the negative bias side. A mirror image of the figure to the right of the true value line exists but is not shown.

(b) in this figure, the accuracy goal would be stated as "the accuracy goal of this reference method, when demonstrated, shall be such that the numerical value of the mean (for the property under analysis or test) shall be within plus or minus two (2) percent of the "true value."

(c) at 2 on the figure, we have shown a typical normal distribution for the x's of a large number of replicate determinations using the candidate method. The precision of the candidate method must (as we will show shortly) be several times "better" than the width of the limit of inaccuracy.

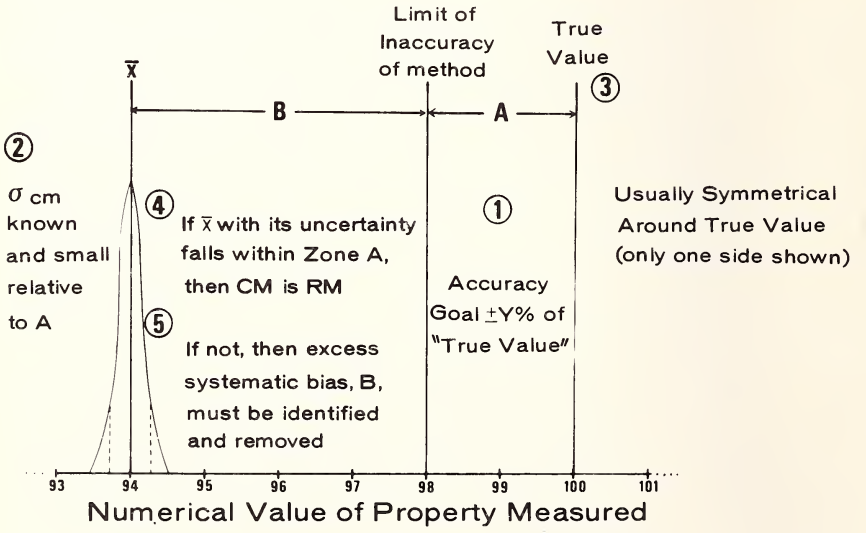

Figure 3. Demonstrating accuracy of reference method.

(d) the "true value" point, 100 in this figure, is determined independently using a definitive method or embodied (*i.e.*, known and certified) in an SRM.

(e) conditions 4 and 5 are those set forth in steps 4 and 5, above. CM is "candidate method" and RM is "reference method."

2. *The Role of Precision in Demonstrating Accurate Measurement*

Is it possible to have an accurate measurement system that is imprecise? In principle, it would seem the answer is yes, because precision, or repeatibility, has no connection with deviations from "true values." We have examined this question from a more pragmatic point of view. Let us scrutinize the following assertion made about a particular method of analysis: "Measurement method A is accurate, but in its present state of development, it is rather imprecise." We now ask the assertor to give his best estimate in terms of the accuracy and precision of method A. He answers: "I would estimate that method A is capable of providing answers that are within plus or minus one-half percent ($\pm 0.5\%$) of the 'true value.'" The standard deviation of imprecision is assessed to be 2 percent. We now ask can this assertion be reasonably tested experimentally?

In figure 4 we have plotted for 20 replications (circled points) random numbers drawn from a normal distribution where the "true value" mean is taken to be zero. These are shown as deviations from the "true value" mean = 0. When the proper calculations have been performed for this population we find:

$\bar{x}$ (population mean) = 0.26
n (replications) = 20
s (standard deviation) = 2.12
t (Student's t) = 2.09

From this we calculate the 95 percent confidence interval for the mean to be -0.73 to 1.26, shown as dotted lines in the figure. The 95 percent confidence interval for the mean is equal to $ts/\sqrt{n}$. Under these conditions, as set forth above, it is impossible to test the assertion that the inaccuracy of the method is ± 0.5 percent (deviation from the true value) as was claimed. For the assertion to be testable the confidence band must be well within the accuracy zone. In such cases as this there are two alternatives. One can make further replications to reduce the confidence interval so that it falls within the zone of accuracy. In this instance, n would have to be increased to more than 60, obviously an unhappy choice if costs and

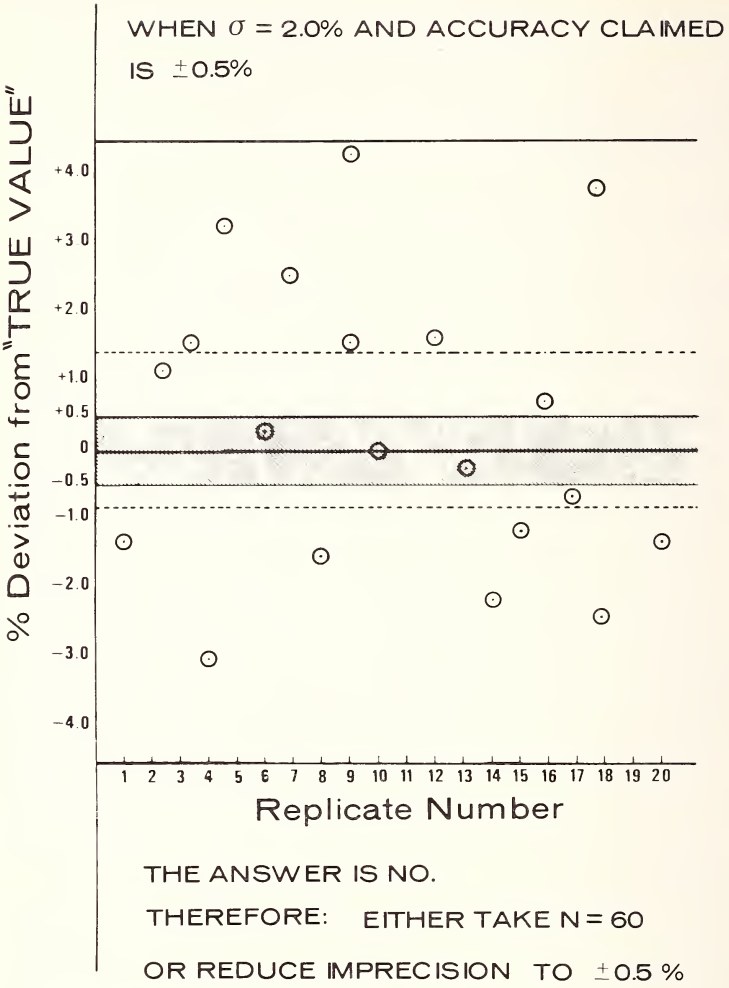

Figure 4. The role of precision in demonstrating accuracy.

time are involved, as is usually the case in actual laboratory trials. The alternative is to increase the precision markedly.

In figure 5, we examine the same situation as in figure 4, except that the precision has now been improved, the standard deviation now being T = 0.5. The data for a sample of 20 from this population yielded the following:

$\bar{x} = 0.06$
$n = 20$
$s = 0.48$
$t = 2.09$

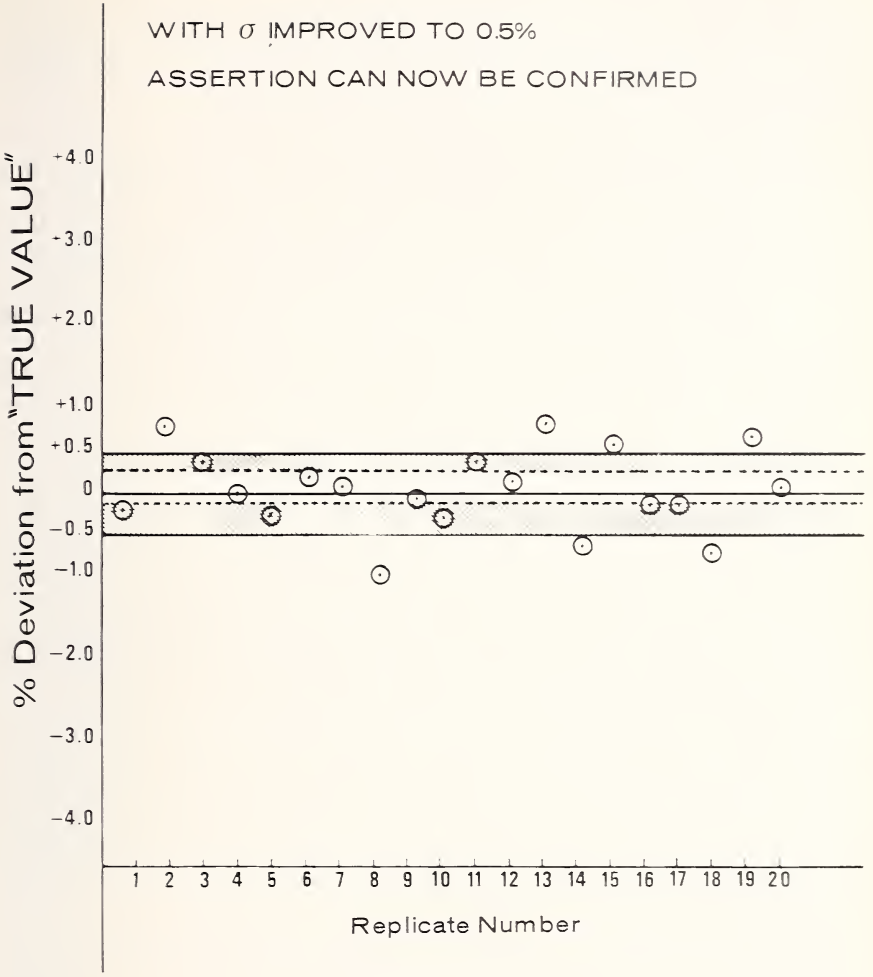

Figure 5. The role of precision in demonstrating accuracy (improved precision).

The 95 percent confidence interval for the mean now extends from −0.16 to 0.28, which is well within the zone of accuracy, and we now conclude that the assertion can readily be tested.

Thus, we may say that while a claim for an accurate but imprecise method may be made, such a claim can be tested experimentally only when a very large number of replications can be performed. Practically, it will usually be more expeditious to work on improving the precision of the method.

Cali *et al.*, in writing about the development of a reference method for the determination of calcium in serum [8,9], have discussed in great detail the necessary steps required to establish the validity of reference methods in general.

C. Two or More Independent and Reliable Methods

It often happens that for a particular SRM, especially those in new or advanced state of the art areas, definitive or reference methods simply do not exist. In these instances, at NBS, we then require that the property under measurement be made by at least two independent and reliable methods. By independent we mean that the basic principles used for the analysis must be entirely different. A copper, *e.g.*, determined by atomic absorption spectrometry and a spectrophotometry would qualify; copper determined by two variations of a titrimetric procedure would not. By reliable we mean that the candidate method must have been successfully used in similar analytical situations (same concentration range, similar interferences, *etc.*) as that pertaining to the SRM system. Further, the method must be one where the investigator can make a confident statement, based on experience, as to the estimated systematic errors in the method. These errors must be small relative to the accuracy required for the property of the SRM under test.

This approach is based on the rationale that the likelihood of two independent methods being biased by the same amount and in the same direction is small. Therefore, when the analytical results agree, then we can state with some assurance that they are likely to be accurate results. Of course, agreement by three methods (or more) essentially guarantees such a conclusion.

In practice at NBS many SRM's are certified for trace elements using this mode, because there are a wide variety of techniques and methods available. A recent example of the use of this mode is the NBS SRM—Mercury in Water (SRM 1642). Three strikingly different analytical techniques were used: isotope dilution spark-source mass spectrometry (SSMS), atomic absorption spectrometry (AAS), and neutron activation analysis (NAA). The results of 10 replicate determinations by each of the three techniques are given in table 4.

At the nanogram concentration level for this SRM, these results must be considered to support the certified value with a large degree of confidence.

The interlaboratory comparison mode, or "round-robin" has been widely used both at NBS and in other national laboratories for the certifi-

TABLE 4. *Analytical data for mercury in water, SRM 1642 (ng/ml)*

Replicate number	(SSMS)	(AAS)	(NAA)
1	1.187	1.16	1.12
2	1.172	1.16	1.15
3	1.204	1.15	1.01
4	1.185	1.16	1.13
5	1.216	1.15	1.29
6	1.168	1.21	1.08
7	1.186	1.18	1.13
8	1.178	1.20	1.20
9	1.148	1.12	1.21
10	1.225	1.21	1.07
Mean = and s_x	1.187 ± 0.023	1.17 ± 0.029	1.14 ± 0.080

Certified value: 1.18 ± 0.04 ng/ml.

cation of SRM's. This mode is a variation of the two or more independent and reliable method approaches we have been describing. It is a mode that must be used with the greatest restraint and under very carefully prescribed and controlled conditions. At NBS, this approach is used only when these circumstances apply: (1) the SRM under study is in a technical area that is well-established and one where many good, reliable methods exist; (2) each of the laboratories in the network are of very high quality and are known to produce very reliable results; (3) each laboratory agrees to the conditions set forth by NBS; (4) NBS controls the experimental design and evaluation of data; (5) a previously issued SRM (either the predecessor, if a renewal SRM, or else one having similar properties to the SRM candidate) is used by each laboratory as an internal quality control check during the course of the work. When these conditions are met and maintained this mode may be used with assurance to produce SRM's of high accuracy and integrity. Alternatively, when operating controls are lax or missing, the results of such exercises are often misleading and confusing and should never be used to certify SRM's.

IV. The SRM as a Mechanism for Transferring Accuracy

Obviously not every laboratory within a given measurement infrastructure has the capability or interest or economic incentive to make accurate measurements from first principles. By this we mean the development, testing, and utilization of an analytical method starting from a theoretical

basis, through the experimentation necessary to prove the method *(e.g.,* specificity, sensitivity, allowable limits to ambient variability, *etc.),* the elucidation of sources of systematic error, the removal of or correction of identified systematic errors—all in a sense, starting from scratch. It is principally to bypass this long, time-consuming, expensive process that there has come into being standard, or umpire, or recognized methods of analysis that hopefully have been put to the test and which any qualified scientist can now use with confidence. The SRM is an important and integral part of this process for it provides a material with a known answer. If the SRM is run through the measurement process and the certified answer is obtained, then one may with some assurance expect that the measurement process will yield the correct value for the unknown material (of similar nature to the SRM).

Let us imagine a laboratory within a measurement infrastructure faced with the problem of becoming compatible with sister laboratories. We further suppose that the measurement problem is the determination of trace amounts of copper in a botanical matrix. Further, we know an SRM, Orchard Leaves, is available in which copper at the level of interest has been measured and certified. Finally, our analytical method is neutron activation analysis.

Now the theory that relates all the pertinent parameters to the copper content of the sample is well-established, and we could therefore proceed from a first principles approach. First we would determine experimentally the value of each of the parameters in our equation, and calculate the answer. Then we would evaluate the magnitude of the systematic errors of each of the parameters and combine these appropriately to give an estimate of the inaccuracy of our result. The equation of interest states that the number, N, of copper atoms in a unit weight of sample is:

$$N = f(i,\phi,\sigma,A_t,t,T)$$

where i = isotopic abundance of the copper nuclide activated by the nuclear reaction induced; ϕ = neutron flux to which sample is exposed; σ = nuclear cross section for the reaction that produced the copper radionuclide; A_t = radioactivity induced in the copper radionuclide determined at time t; t = time elapsed from end of irradiation to counting; and T = duration of irradiation.

Now Cali and coworkers [10] have shown that radioactivation analysis has inherent within it potentially large uncertainties when the first principles approach is used. Uncertainties of 50 percent or more are not uncommon in several of the factors appearing in the equation. Experienced investigators will not use this approach if alternatives are available.

Having discarded the above approach, our laboratory scientist might decide to test his copper procedure by using the Orchard Leaves SRM to see if he could obtain the certified values of the copper content (12 ± 1 μg/g).

This would be done by irradiating simultaneously a small, but accurately known weight of pure copper and a known weight of the SRM. Conditions during irradiation and during determination of the two induced copper radioactivities would be identical (or with very small differences) and thus all the factors would cancel in the two equations except the N's and the A's leaving:

$$N_{SRM} = N_{Cu} (A_{SRM}/A_{Cu})$$

As was shown earlier, the analyst must know or determine the method precision and it must be considerably smaller than the overall uncertainty (in this case $\sim 8\%$) if he is to know whether his method gives results that fall within the accuracy range of the certified value.

Now if the property(ies) of the SRM have been *accurately* measured and certified, then the SRM when used as just described is capable of transferring its accuracy in turn to the laboratory using it. If the accurate answer is obtained for the SRM, then, again with some confidence, the unknown will yield an accurate answer. Deviations from the correct answer, making, of course, allowances for the imprecision of the method, are known immediately to be the result of systematic errors. These, if present, can be investigated, found, and removed, so that the corrected measurement process used is on the basis of accuracy. The laboratory will thus achieve compatibility, independently realized, with all other laboratories within the measurement infrastructure that are applying the same control and validation procedures, *i.e.*, using the same SRM, and correcting for systematic errors if necessary.

V. Currently Available and Planned NBS-SRM's Useful in Trace Analysis

The list of currently available NBS-SRM's that the analyst will find useful in helping to bring about the more accurate measurement of trace constituents is shown in table 5. Space does not permit a complete listing of all the trace constituents measured and certified. This information is contained in the NBS-SRM Catalog [11].

There is no argument that it would be most desirable to have an SRM for every conceivable matrix, because matrix interference effects are one

TABLE 5. *NBS-SRM's useful in trace analysis currently available*

SRM No.	Name	Certified for:
1571	Orchard Leaves	14 trace elements, 5 major and minor elements
1577	Bovine Liver	9 trace elements, 3 major and minor elements
608–619	Trace Element Glasses	35 trace elements (not all certified)
1632	Trace Elements in Coal	14 trace elements including Pb, Hg, Cd
1633	Trace Elements in Fly Ash	12 trace elements
1630	Mercury in Coal	Hg 0.03 ppm
1610–1613	Hydrocarbons in Air	Methane in air 0.0001 to 0.1 mol/percent
1604–1608	Oxygen in Nitrogen	Oxygen 1.5 ppm to 978 ppm
1601–1603	Carbon Dioxide in Nitrogen	CO_2 300–400 ppm
1677–1681	Carbon Monoxide in Nitrogen	Carbon monoxide 10–1000 ppm
1665–1669	Propane in Air	Propane 2.8–475 ppm
1625–1627	SO_2 Permeation Tubes	Permeation rate of SO_2 @.56–3 μg/min
607	Potassium Feldspar	Rb, Sr content, Sr isotopic ratio
685	High Purity Gold	Cu, Fe, In
680–681	High Purity Platinum	12 trace elements
682–683	High Purity Zinc	6 trace elements
726	Intermediate Purity Selenium	24 trace elements

of the largest sources of systematic bias, especially true in trace analysis. Indeed, NBS in past years has tried to provide SRM's with the exact composition as the samples to be analyzed. Thus, the NBS metal SRM's (over 350 types) were made in adherence to this principle. However, with a greatly increased demand and need for SRM's in areas of environmental and health-related measurement the capability of NBS to continue along this path does not exist. Thus, we have produced a botanical SRM (Orchard Leaves) that has wide applicability over a wide range of matrices—as long as these are botanical in nature. Similarly for our biological matrix SRM (Bovine Liver) and our glass matrix SRM's (Trace Elements in Glasses). That these SRM's are meeting a widespread need is evidenced by substantial sales and by their being referenced quite extensively in the recent analytical chemical literature.

On the other hand the lack of a standard is sometimes more easily realized. For example, the importance of chromium in biological systems has been well documented [12]. In May of 1974, a workshop on the problems of chromium analysis was held at the University of Missouri Trace Substances Research Center. The consensus of attending scientists was that the many worldwide projects relating chromium deficiencies to

a variety of diseases are severely limited by analytical difficulties and lack of agreement on analytical data. Values reported at this workshop for the chromium content in the NBS-Bovine Liver SRM varied from 50 to 700 ppb. The discrepancies are apparently caused by the inconsistent behavior of volatile metallo-organic chromium compound, known as the Glucose Tolerance Factor (GTF). This factor has been found to be extremely important in the treatment of diabetes and is possibly important in other medical and health problems including aging. At present this factor cannot be quantitatively determined due to the lack of a definitive chromium standard containing this compound. It has been proposed that NBS prepare a Brewers Yeast Standard which contains chromium predominately as GTF. This proposed work is currently being evaluated.

In table 6 is given a list of trace element SRM's that are currently in the R&D or production stages. Note the heavy emphasis on environmental SRM's.

Finally in table 7 are listed some of the candidate SRM's. We emphasize that this is a representative, but not a complete or final listing.

TABLE 6. *SRM's currently being prepared and of significant value in trace analysis*

Name	To be Certified for:
Mercury in Water	Hg at 2 constituent levels 1 ppm 1 ppb
Lead in Gasoline	Pb at 4 constituent levels
Citrus Leaves, Pine Needles, Tomato Leaves, Alfalfa	Similar to Orchard Leaves
NO_2 Permeation Tubes	Permeation Rate of NO_2
Copper Benchmark Standards	Approximately 23 trace element impurities
River Sediments Standards for Radioactivity	Especially environmentally important radionuclides

TABLE 7. *SRM Candidate materials under consideration*

Methane and Non-methane Hydrocarbons in Air
Vinyl Chloride in Air
Trace Elements in Oyster Meats
Spinach—Trace Elements, Nutrient Elements
Grain—Trace Elements, Nutrient Elements
Brewers Yeast—Especially Chromium
Industrial Hygiene Standards—Toxic Constituents
River Sediment—Industrial Pollutants
Calcium in Steel
Trace Elements in High Temperature Alloys—Especially Bi, Pb
Rare Earth Elements in TEG's (Glass)
Trace Elements in Aqueous Solutions—Water pollution
. . . and many others yet to be fully identified and whose needs are not yet fully justified.

At NBS the assessment of needs and priority evaluation for production scheduling is a continual process, and items are being constantly added, and sometimes deleted.

VI. Conclusion

SRM's play an important role in trace analysis. Because the properties are measured and certified on the basis of accuracy, then, any laboratory through the use of such SRM's as are appropriate to its problem, may place its analytical results on an accuracy basis. It has been asserted, and we believe it is true, that when the laboratories in any given infrastructure have moved onto a basis of accuracy then all such laboratories' results are compatible. By compatibility we mean that the results, being related to the "true value," now agree within the uncertainties that have been stated by each laboratory. In this day of confusion about the validity and "correctness" of analytical data, upon which widespread and far reaching social, political, and economic decisions are made concerning environmental or health affairs, such measurement compatibility is greatly to be desired and indeed required if rational decisions are to be reached.

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INTERPRETATION OF ACCURACY OF TRACE ELEMENT RESULTS IN BIOLOGICAL MATERIALS

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With the recent realization that trace elements have a very important role, either beneficial or harmful, in man, trace element analysis has become an increasingly important field of research in clinical medicine, biology, nutrition, and environmental studies. Many researchers have investigated the amounts of various elements in man, animals, plants, and types of tissues.

The interpretation of trace analytical results for biological materials is discussed from the viewpoint of their accuracy. Interpretation of results of an analysis of an unknown sample depends to a great extent on prior evaluation of the analytical method employed. The accuracy of the method is best determined by the analysis of standard samples. In the absence of standard samples of a similar nature to the unknown samples, the results of the method are compared with those obtained by other methods of analysis. Both of these approaches are discussed. An evaluation of published results for the determination of the 15 trace constituents in the standard reference material Orchard Leaves is presented from the viewpoint of accuracy.

A case history is presented to illustrate the problems associated in the development of a method for a biological sample and the evaluation of the accuracy of the method.

Keywords: Accuracy; biological materials; data interpretation; standard materials; trace elements.

I. Introduction

During the past few years there has been an increasing realization of trace element chemistry in biological systems. This awareness has been stimulated by the rising concern in industrial nations of the impact of man on his environment and its biological effect on him. Of primary sig-

nificance is the role played by trace elements and whether or not they are beneficial to the biochemistry of man.

It has been known for several years that a number of trace elements are of biological significance to man, but only recently has it been shown that these substances are required nutrients [1-3]. The essential trace elements are Co, Cr, Cu, F, Fe, I, Mn, Mo, Se and Zn with Ni, Sn and V possibly essential. They have specific metabolic functions, and deficiencies of them result in syndromes that in certain cases have been observed in man. Certain trace elements such as Li, Be, Ba, Ni, Ag, Cd, Hg, As, Sb, Bi, Pb, and Br can also be correlated with adverse effects in man. See figure 1.

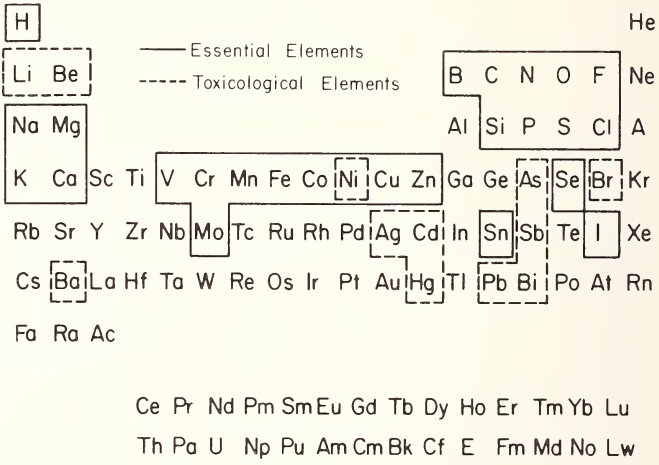

Figure 1. Important elements in biological material.

With the realization that trace elements have a very important role, either beneficial or harmful, in man, trace element analysis has become an increasingly important field of research in clinical medicine, biology, nutrition, and environmental studies [4]. Various researchers have investigated the amounts of various elements in man, animals, plants, and types of tissues [2,5,6]. Many different techniques have been used for these measurements such as flame emission and atomic absorption spectrometry, neutron activation analysis, emission spectroscopy, spark source mass spectrometry, x-ray fluorescence, and electroanalytical and chemical analysis. There is no dearth of methods for trace element analysis so that the study of biological samples would not appear to present any problems. Certainly the analytical literature has been continuously bombarded with review articles comparing the relative merits of different techniques in a general qualitative fashion. Just how good are these dif-

ferent trace methods? The goal of this paper is to discuss the interpretation of analytical results for biological materials from the viewpoint of their accuracy.

II. Discussion

The ultimate goal in quantitative analysis is accuracy, *i.e.*, the ability to approach the "true" value. Errors affecting an experimental result may be classified as either systematic or random. The former, occasionally referred to as determinate errors, are due to causes over which the analyst has control, and their undesirable effects on the accuracy of the result can be avoided or corrected. Random errors are not subject to control and are manifested even in the absence of systematic errors, by variations in the result. Although the magnitude of random variations can be reduced by carefully keeping all operations identical, they are never eliminated entirely. The precision of the results thus depends on the random errors. In this situation the distinction between precision and accuracy becomes less apparent, that is the *precision defines the accuracy that can be achieved in the absence of systematic errors* [7].

To develop a highly accurate method requires considerable time and effort; however, not all biological analyses require the ultimate in accuracy. The degree of accuracy required in a trace element determination in biological materials depends on the nature of the study. At one extreme, the analyst may be requested to determine small changes in concentration on the order of a few percent, thereby requiring methods of the highest accuracy and precision. Often, survey analyses of large numbers of samples for large numbers of trace elements are requested where lesser accuracy is permissible.

As mentioned earlier a variety of techniques have been used to analyze biological samples. These techniques can be classified according to their ability to provide either single-element or multielement information in a given analysis. The single element techniques which include flame emission and atomic absorption spectrometry, neutron activation analysis, isotope-dilution mass spectrometry, electrochemical and chemical analysis, involve optimization of conditions to provide maximum accuracy for the determination of the element of interest. Although some of these techniques can provide simultaneous information on a few elements, the only really comprehensive techniques at the present time are emission spectroscopy, spark source mass spectrometry, x-ray fluorescence, and instrumental neutron activation analysis or activation analysis with radio-

chemical group separations followed by high resolution gamma spectrometry. These serve as valuable survey methods; however, they involve a compromise in experimental conditions to produce an average optimum condition for the simultaneous determination of many elements. As a consequence, these methods result in unequal accuracy for the various elements present in biological samples. The judicious use of techniques for the type of information desired is implied.

Now that we have recognized that some methods of measurement may be inherently more accurate than others, the problem still remains to interpret the results of an analysis in terms of their accuracy and consistent with the use to which the numbers are to be put.

Interpretation of the results of an analysis of an unknown sample depends to a great extent on prior evaluation of the analytical method employed. The accuracy of the method is best determined by the analysis of standard samples. In the absence of standard samples of a similar nature to the unknown samples, the results of the method are compared with those obtained by other methods of analysis.

A. Use of Standard Samples

Until recently there did not exist certified standards for trace element determination in biological matrices. In 1971 the National Bureau of Standards announced the availability of the first of a series of botanical standard reference materials analyzed for chemical elements [8]. This material, identified as SRM 1571, Orchard Leaves, is presently certified for 15 trace constituents, two minor constituents, and three major constituents. Values for nine additional elements are provided but not certified. In 1972 the first animal tissue, SRM 1577, Bovine Liver, was made available which is certified for 11 trace elements [9].

Prior to the availability of these SRM's, a number of uncertified standard biological materials were made available by various laboratories to permit the standardization of laboratory analyses over an extended period of time, as well as to allow an intercomparison with the work of other laboratories. These include dry plant materials [10-15], animal tissue [15], blood sera [16] and clinical standards. The material for which most analytical data exists is Bowen's Kale sample. These materials should not be confused with the certified standard reference materials which have been carefully prepared and analyzed by the NBS over a period of time using methods with high reliability.

In the 3 years that the Orchard Leaves standard reference material has been available, only a limited number of trace analytical studies have been

reported in the literature involving their use. It is interesting, however, to examine these results in order to evaluate the effectiveness of the various methods in approaching the "true" values, *i.e.*, the certified values. Table 1 summarizes these trace element values and the methods employed along with the NBS certified values arranged in decreasing order of concentration. The indicated limits on the concentration are equal to the entire range of NBS observed results, or two standard deviations (commonly referred to as the "95 percent confidence limit"), whichever is larger. The respective laboratories have not been identified to preserve anonymity.

Since only 7 of the 15 certified trace elements have 4 or more reported values with a maximum number of 8 for Zn, a rigorous statistical treatment is impossible. In addition to the need for larger numbers of values, it would require calculating weighted averages for each component, making due allowance for the respective variances of all the methods used. Instead, we are forced to examine the spread of values for any given element, bearing in mind the various factors contributing to the spread.

In the case of Fe, of the 7 reported values only 2 RNAA values fall within the NBS limits, while the highest and lowest outliers with 14 percent and 35 percent deviations, respectively, were reported using SSMS. Three values fall within the limits for Mn, with SSMS once again producing the highest and lowest outliers. Two of the 3 reported values for Na fall within the limits while SSMS resulted in a 48 percent deviation. It should be noted that Na is a particularly difficult element to quantitate by SSMS because of thermal ionization in addition to spark excitation. The maximum number of values is reported for Zn and interestingly enough most of the values reported are acceptable. Similarly good results are reported for As. Three of the five values for Cu lie outside the NBS limits with SSMS producing the furthest outlier. All three reported results for Rb are acceptable. Mercury values show two outliers, both produced by RNAA. The three results for Cd are all within the range. Finally, only one of the six reported values for Se falls outside the limit and it was produced by RNAA.

Of the 56 reported values for these certified trace elements in Orchard Leaves, by far the largest number were obtained by RNAA. Twenty of these 25 RNAA values fell within the limits prescribed by NBS, indicating that these methods are capable of good accuracy for this type of material. The second largest number of values were obtained by SSMS. Of the 10 reported only 2 fell within the acceptable limits and all but 1 outlier were produced by 1 laboratory. SSMS is a multielement technique where, as mentioned earlier, poorer accuracy can be expected; however, the results reported here indicate that the problem may rest with the

TABLE 1. *Results of determination of certified trace elements in SRM 1571 orchard leaves*

Element	NBS certified value[a] (μg/g)	Reported values[b] (μg/g)	Technique[c]	Deviation (%)
Fe	300 ± 20			
		195	SSMS	35
		246	CL	18
		271	AF	10
		276	XRF	8
		290	RNAA	3.3
		300	RNAA	0
		343	SSMS	14
Mn	91 ± 4			
		76	SSMS	16
		86	INAA	5.5
		86	INAA	5.5
		87	RNAA	4.4
		88	AF	3.3
		89	XRF	22
		107	SSMS	18
Na	82 ± 6			
		76	RNAA	7.3
		77	RNAA	6.1
		121	SSMS	48
Pb	45 ± 3			
		19	SSMS	58
		45	XRF	0
B	33 ± 3	—	—	—
Zn	25 ± 3			
		24	XRF	4
		25	SSMS	0
		25	RNAA	0
		25	AA	0
		26	RNAA	4
		26	RNAA	4
		29	RNAA	16
		30	AF	20
As	11 ± 2			
		8.7	RNAA	21
		8.9	RNAA	19
		10	RNAA	9.1
		10.6	XRF	3.6
Cu	12 ± 1			
		7.9	RNAA	34
		10	RNAA	17
		12.6	XRF	5
		13.1	SSMS	9.2
		14	INAA	17
		29	SSMS	140

TABLE 1. *Results of determination of certified trace elements in SRM 1571 orchard leaves*—Continued

Element	NBS certified value[a] (μg/g)	Reported values[b] (μg/g)	Technique[c]	Deviation (%)
Rb	12 ± 1			
		11	RNAA	8.3
		11	XRF	8.3
		12	RNAA	0
Ni	1.3± 0.2			
		1.3	XRF	0
		6.8	SSMS	420
Hg	0.155 ± 0.015			
		0.091	RNAA	41
		.14	INAA	9.7
		.15	RNAA	3.2
		.16	AA	3.2
		.20	RNAA	29
Cd	0.11 ± 0.02			
		0.11	RNAA	0
		.12	RNAA	9.1
		.12	RNAA	9.1
Se	0.08 ± 0.01			
		0.055	RNAA	31
		.077	SF	3.8
		.08	SF	0
		.08	RNAA	0
		.087	SF	8.8
		.088	RNAA	10
U	0.029 ± 0.005	—	—	—

[a] Indicated limits are equal to the entire range of NBS observed results, or two standard deviations, whichever is larger.

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[c] Techniques include atomic absorption (AA), atomic fluorescence (AF), chemiluminescence (CL), instrumental neutron activation analysis (INAA), neutron activation analysis with radiochemical separations (RNAA), spectrophotometry-fluorometry (SF), spark source mass spectrometry (SSMS), and X-ray fluorescence (XRF).

laboratory reporting the results. The two SSMS values that fell within the limits were performed by another laboratory. The third most used technique was XRF and all seven reported values fell within the acceptable limits. Similarly, only one of the four reported INAA values fell within the limits, indicating the poorer accuracy of these methods. The three reported values by SF and the two reported values by AA were all within the limits.

It is dangerous to generalize from such a small amount of data, but the pattern that emerges resembles a similar study reported by the author [17] for the evaluation of trace elemental data in the analysis of lunar samples. Namely, that single element techniques are capable of higher accuracy, but perhaps more important, the skill of the laboratory is the dominant factor in achieving good results.

It should be mentioned that all of the laboratories that reported results had the advantage of knowing in advance the certified values. Presumably, they rejected their poorer values and polished their methods to eliminate or minimize their systematic errors so as to come closer to the certified values. But that is one of the main objectives in using SRM's. Previous round robins on biological samples with no certified values have shown a much larger spread of values [18]. Once a laboratory has developed a satisfactory trace method and established its accuracy through the use of certified standards, it can be assumed that comparable accuracy will be obtained in the analysis of unknown samples of a similar nature provided the procedure is followed in an identical fashion.

B. Use of Independently Analyzed Samples

In the absence of available certified standards for methods of complex biological materials, evaluation by independent analytical methods is often resorted to. Several determinations should be performed by each method, so that good estimates of the precision of each method may be obtained. If there is a significant difference between the two means obtained, systematic error is probably present in one or both methods. Although the magnitude and the sign of the discrepancy between the two means may indicate the nature of the error, further work must usually be done to discover its source. If there is no significant difference between the two means, there is no reason to suspect the presence of appreciable systematic error in either method. However, this does not exclude the possibility of systematic errors in some instances.

The success of this approach depends upon the reliability of the comparative method, since it too must have been properly calibrated. In the

trace element analysis of biological materials, the use of neutron activation analysis as an independent method is particularly helpful. Since neutron activation analysis is based on the nuclear properties of the constituents of a sample, it is not dependent upon their chemical form, so that synthetic standards may be employed for calibration. Similarly, wet chemical methods and flame spectroscopy can be used when limited numbers of elements are involved, since they can often be calibrated with synthetic standards. If large numbers of biological samples of a similar nature are to be analyzed, it may be more practical to choose a comparable sample as an "in-house" standard and analyze it for the species of interest by a variety of independent methods where applicable. This standard can then be used for future analyses of this type of sample.

To best illustrate the problems associated with the development of a method for a biological sample and the evaluation of the accuracy of the method, I have chosen to present a case history based on work of my laboratory. The method, which involves the rapid simultaneous determination of Na, K, and Ca in blood serum using our recently developed vidicon flame spectrometer, illustrates the use of analyzed bovine serum samples as calibration standards and independent methods of analysis of human serum samples to determine the accuracy of the method [19].

The simultaneous analysis is accomplished by direct injection of 200 μl of serum into a nitrous oxide-acetylene flame. The transient signals produced by flame emission are dispersed using a grating monochromator and detected using a silicon intensified target vidicon tube. The vidicon spectrometer allows simultaneous monitoring of the spectral region 807 nm to 847 nm, the information being acquired in 500 electronic channels.

The analytical curves for Na, K, and Ca were prepared simultaneously by injecting 200 μl of bovine serum at three concentration levels for these elements into the flame with a microsyringe. These "standards" or reference materials in surrogate matrix were supplied by the Center for Disease Control (CDC), Atlanta, Ga. [20]. Streptomycin, penicillin and fungizone were added to the serum samples at the CDC to prevent any bacteriological action that might lead to analytical errors. The concentrations of each element in each bovine serum standard are given in table 2.

An important aspect of this method is the use of serum standards rather than aqueous synthetic calibration standards. Use of aqueous standards results in erroneously high values for the determination of Na, K, and Ca in a control serum which is commercially available (Lab-Trol, Dade Reagents, Miami, Fla.). The increase in signal using serum is probably due not only to changes in flame conditions caused by the introduction of proteins into the flame, but also to changes in the solution nebulization

caused by differences in surface tension and viscosity. By analyzing samples of known concentration we were able to determine systematic errors which were then eliminated.

To evaluate the method under actual clinical conditions, human serum samples from hospital patients were analyzed. Table 3 shows the results of the simultaneous determination of Na, K, and Ca in 10 serum samples using the vidicon flame spectrometer method. Actual values for the identical serum samples obtained by the clinical laboratory at the hospital are shown for comparison. For Na and K they employed an Instrumentation Laboratory model 143 flame photometer where the serum samples are automatically diluted (1:200) using a peristaltic continuous-flow dilutor. A Li internal standard is automatically added to the samples when they are diluted. Since the instrument is a continuous-flow device, the amount of serum consumed depends on how long the sample is aspirated. Sodium

TABLE 2. *Analytical data on the CDC reference sera*

Standard	Sodium[a] (meq/l)	Potassium[b] (meq/l)	Calcium[a] (mg/dl)
1	124	2.74	5.16
3	138	4.66	8.97
5	154	6.71	13.0

[a] CDC Reference laboratory values.
[b] NBS isotope dilution mass spectrometry values.

TABLE 3. *Analysis of hospital serum samples*

Patient	Sodium (meq/l)		Potassium (meq/l)		Calcium (mg/dl)		
	Vidicon	Clinical	Vidicon	Clinical	Vidicon	Referee	Clinical
1	143	142	3.80	3.7	9.56	9.37	9.4
2	139	137	4.00	4.1	9.69	9.76	9.6
3	140	136	4.45	4.6	9.83	10.4	9.6
4	144	143	4.02	4.0	9.44	9.23	9.1
5	145	145	4.25	4.4	9.67	9.75	9.6
6	131	134	4.33	4.6	8.07	8.06	8.0
7	141	144	3.93	4.1	9.67	9.48	9.2
8	142	142	4.46	4.8	9.79	9.47	9.6
9	142	142	4.06	4.1	9.58	9.65	8.9
10	145	143	3.97	3.9	9.76	9.81	9.2

and K were determined simultaneously with this system using an air-propane flame. The hospital values for Ca were obtained using the cresolphthalein complexone colorimetric method [21]. This method requires 100 μl of sample and a 10-minute incubation time. 8-Hydroxyquinoline is used to retard the Mg interference.

Let us now compare the results obtained by the new vidicon method and the independent clinical methods used by the hospital.

1. Potassium

The standard deviation of a single determination of K was estimated from five successive injections of control serum to be 0.05 meq/l using the vidicon flame spectrometer. This gives a relative standard deviation of 1.19 percent for the determination of K, which compares well with the reported standard deviation [22] for the hospital system of 1.2 to 2.0 percent for K. The average deviation of the vidicon flame spectrometer values from the clinically obtained values shown in table 3 is 3.22 percent. A standard t-test for paired variance indicates that at the 95 percent confidence level, the two sets of data are equivalent. As shown previously, the vidicon flame method has an accuracy of 2.2 percent for the determination of K in a serum standard.

2. Sodium

The standard deviation of a single determination of Na was estimated from five successive injections of control serum to be 0.50 meq/l using the vidicon flame spectrometer. This gives a relative standard deviation of 0.36 percent for the determination of Na using this method which compares well with the reported standard deviation [22] for the hospital system of 0.9 percent for Na. The average deviation of the vidicon flame spectrometer values from the clinically obtained values shown in table 3 is 1.18 percent. A standard t-test for paired variance indicates that even for a confidence level of only 55 percent there is no significant difference between the two sets of data. As shown previously, the vidicon flame method has an accuracy of 1.4 percent for the determination of Na in a serum standard.

3. Calcium

The standard deviation of a single determination of Ca was estimated from five successive injections of control serum to be 0.17 mg/100 ml using the vidicon flame spectrometer. This gives a relative standard deviation of 1.77 percent for the determination of Ca using this method. The

average deviation of the vidicon flame spectrometer values from the clinically obtained values is 3.12 percent. A standard t-test for paired variance indicates that at a confidence level of greater than 99 percent there is a significant difference between the two sets of data. For this reason the Ca determinations were also run on the same samples using an optimized single-element flame emission procedure. A 50 μl sample of each serum was added to a 10 milliliter volumetric flask containing 0.4 milliliters of butanol as an antifoaming agent, and diluted to volume. Samples of the three CDC serum standards were diluted in the same manner. A calibration curve was made by aspirating the diluted CDC serum standards into the nitrous oxide acetylene flame in the conventional manner. The Ca 422.7 nm line was determined using the vidicon flame spectrometer with a slit width of 20 μm. No filters were employed. The results of these determinations are listed in table 3 as referee values for Ca. The average deviation between the simultaneous multielement vidicon results and the single-element flame emission Ca determinations is 1.81 percent. A standard t-test for paired variance indicates that even for a confidence level of only 10 percent, no significant difference exists between these sets of data. Considering the good agreement obtained in the analysis of control serum and the good agreement obtained with the referee method, the vidicon results are considered to be more reliable than the calcium determinations made using the cresolphthalein complexone method.

Thus, by comparing the results of analysis of real samples with those obtained using samples of known concentration as well as with those obtained by independent methods of analysis, it was possible to establish that the new vidicon flame method was capable of an accuracy and precision of 2 percent or better. Provided the method is applied in an identical fashion, comparable accuracy and precision will be obtained on subsequent serum samples. Periodic analysis of control samples should be performed to insure continued high accuracy.

III. Summary

In summary, interpretation of the accuracy of results for biological materials, or for that matter any material, depends to a considerable extent on the prior evaluation of the analytical method used. This is best done by the analysis of standard samples of similar nature to the unknown samples. Lacking these standard samples, comparison of the results of the method with those obtained by independent methods of analysis are mandatory. With the increasing interest in trace element chemistry in biologi-

cal systems, one may hopefully look forward to the greater availability of SRM's.

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PRECISION AND ACCURACY IN SILICATE ANALYSIS

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The precision and accuracy of analytical results in geological publications range widely. High-quality analyses are typical of the research in geochronology and in other areas of isotopic investigations, and this work has resulted in substantial improvement in the data that are now evolving in more routine geological investigations. The trace elements Rb, Sr, Ba, and the rare earth elements are widely used in petrological investigations, and accurate results are obtained routinely by isotope-dilution techniques and mass spectrometric measurements. It is now commonplace for the results from different laboratories to agree within 1 to 2 percent of the amount present. In contrast, the results from atomic absorption, x-ray fluorescence, neutron activation, and optical spectrographic methods are much more variable and are less reliable. The increasing availability of reference samples is easing some of the problems of the instrumental analyst, but the reference samples have some problems of their own. The lack of well-characterized reference samples is a major barrier to better quality instrumental analyses.

Keywords: Accuracy; analytical bias; reference samples; silicate analysis.

I. Introduction

The precision and accuracy of analytical results in geological publications range widely. In part, this results from the great variability in composition of rocks and minerals, and references to problems arising from matrix effects in instrumental analyses of geological materials are common. This variability in chemical composition as well as unusual combinations of elements has been equally frustrating to the conventional analyst. Methods that work well for some rocks fail for others. For example, the determinations of SiO_2, Al_2O_3, TiO_2, total Fe, MgO, and CaO, the main portion, in ordinary basalts are no problem, but the same elements in alkali-rich basalt characterized by high TiO_2 (3-4%) and P_2O_5 (1-3%) are a

problem. The chemist, unaware of the potential pitfalls, may deliver a bad analysis, or recognizing the problems, may be obliged to expend considerably more time and effort than normal on the analysis.

II. Laboratory Bias

Geologists have long recognized that the results from different chemical laboratories cannot be freely compared and used interchangeably. Rock series diagrams and mineral variation plots based on chemical data from a single laboratory generally show less scatter of data points than similar diagrams plotted from data from several laboratories. Analytical laboratories with a volume of work develop a degree of precision or reproducibility of results. In some laboratories remarkably good precision is obtained. Commonly the results from a laboratory will vary from accepted standards in a predictable manner. If the laboratory bias can be determined, the quality of the results may be improved by applying suitable corrections.

Rb-Sr radiometric age measurements from a large number of laboratories from all parts of the world can be compared with a good degree of confidence. The measurements involve determinations of the atomic ratio $^{87}Sr/^{86}Sr$ and the concentrations of Rb and Sr. Isotopic ratios can be determined precisely (1-2 parts in 7,000) with a well-constructed mass spectrometer, but the value for a pure strontium salt rarely is the same when determined in different laboratories or even in the same laboratory using different instruments. The ratio, of course, is a constant, and the different answers are caused by instrumental or procedural differences. The various factors that might cause differences in the measured ratio are not of primary concern to this discussion, and it suffices to say that the different ratios obtained for a given strontium salt result from instrumental or laboratory bias.

From early developmental days, cooperation among the geochronology laboratories has accelerated progress, as for example, in the free interchange of analyzed samples and of methods. To compensate for instrumental bias a procedure was introduced of normalizing the observed atomic ratio $^{86}Sr/^{87}Sr$ to a value of 0.1194 [1] and making a corresponding correction in the observed value for $^{87}Sr/^{86}Sr$. Most laboratories soon found that the normalizing procedure was not applicable for all data, but only within a limited range of variation for the ratio $^{86}Sr/^{88}Sr$. Further progress was made when a number of laboratories began using a strontium salt as a laboratory reference sample to monitor the mass spectromet-

ric procedures. An Eimer and Amend $SrCO_3$ that was widely used became known as the MIT standard. More recently, Standard Reference Materials became available from the U.S. National Bureau of Standards, and these have eased considerably the work of the isotope geochemist:

SRM 984 Rubidium chloride
SRM 987 Strontium carbonate
SRM 988 Strontium-84 spike assay and isotopic solution standard
SRM 607 Potassium feldspar, trace rubidium and strontium.

Blattner [2] has cited selected Rb data on the U.S. Geological Survey rock standards to illustrate relative laboratory analytical bias. These data are given in table 1 with some modifications. One set of optical spectrographic data included by Blattner has been deleted, and atomic absorption and mass spectrometric data have been added. Laboratory A refers to data published by myself and colleagues [3]. No advantage is gained by identifying other analysts.

TABLE 1. *Published rubidium data on rock standards*[a]

Lab	G-2	GSP-1	AGV-1	BCR-1	Method[b]
A	161	244	62	44	FP
B	175	260	70	50	XRF
C	185	272	71	53	NAA
D	203	337	73	50	OS
E	165	246	63	43	AA
E	170	248	64	47	AA
F	166	240	65	45	XRF
Average[c]	169 (6)	253 (5)	67 (3)	47 (6)	MS

[a] Results are given in ppm.
[b] FP = flame photometry
XRF = x-ray fluorescence
NAA = neutron activation analysis
OS = optical spectroscopy
AA = atomic absorption
MS = mass spectrometry
[c] Number of laboratories is given in ( ).

III. Accuracy

The mass spectrometric data (table 1) permit a quantitative assessment and a comparison of the accuracy of the Rb data, and in table 2 the results from the laboratories A-F are compared with the average mass spectrometric values. It is seen that my results (Lab. A) have a negative bias,

but more important that they are wrong and are from 4 to 7 percent too low. It might be said that for some geological investigations the caliber of the data is adequate, but if significant progress in geological research is to be made, better data are needed. For samples to be used as standards, the results are simply unacceptable.

TABLE 2. *Ratio of other results for rubidium compared to mass spectrometric average values*[a]

Lab	G-2	GSP-1	AGV-1	BCR-1	Method[b]
A	0.95	0.96	0.93	0.94	FP
B	1.04	1.03	1.04	1.06	XRF
C	1.09	1.08	1.06	1.13	NAA
D	1.20	1.33	1.09	1.06	OS
E	0.98	0.97	0.94	0.91	AA
E	1.01	0.98	0.96	1.00	AA
F	0.98	0.95	0.97	0.97	XRF

[a] For individual values see table 1.
[b] FP = flame photometry
XRF = x-ray fluorescence
NAA = neutron activation analysis
OS = optical spectroscopy
AA = atomic absorption.

The data for Rb are shown graphically in figure 1, and comparable data for Sr are shown in figure 2. I am fully aware that a number of laboratories today produce x-ray fluorescence results for Rb and Sr that are much better than those shown in figures 1 and 2, and some of the x-ray fluorescence data are fully as good or better than mass spectrometric results. These laboratories, however, are regrettably few in number.

Data for SiO_2 on the U.S. Geological Survey rock standards are shown in figure 3. Again the laboratories include the selections made by Blattner [2] with the addition of three laboratories, A, B, and M. The SiO_2 results are compared to Flanagan's [4] "1972 values," and it can be seen (fig. 3) that only the gravimetric and the photometric (J) results come close to the accepted figures. Some laboratories now supplement instrumental analyses of silicate rocks and minerals with gravimetric determinations of SiO_2. An accuracy of 0.2 percent of the amount present is fully reasonable for gravimetric determinations and is needed for petrological and mineralogical research. Instrumental analyses have failed in this regard, and in figure 3 it will be noted that the low-silica, high-magnesia peridotite (PCC-1) and dunite (DTS-1) were particularly troublesome.

Comparative data for CaO from the same laboratories used for SiO_2 are shown in figure 4. Flanagan's [4] "1972 values" were used for reference

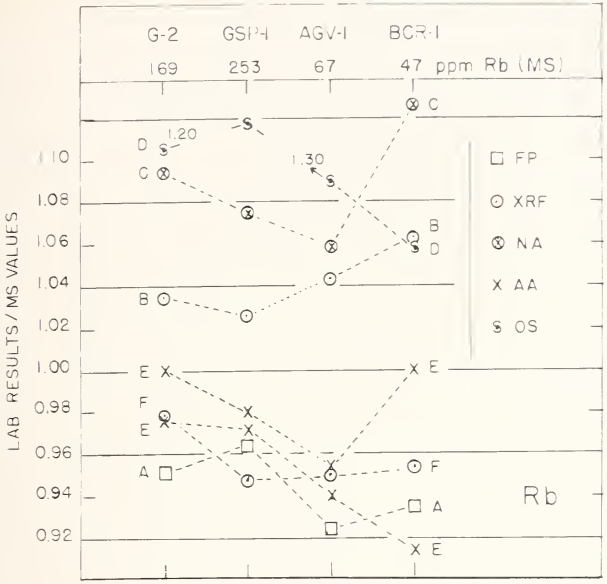

Figure 1. Selected Rb data on rock standards compared with mass spectrometric (MS) average values. FP, flame photometric; XRF, x-ray fluorescence; NA, neutron activation; AA, atomic absorption; OS, optical spectrographic.

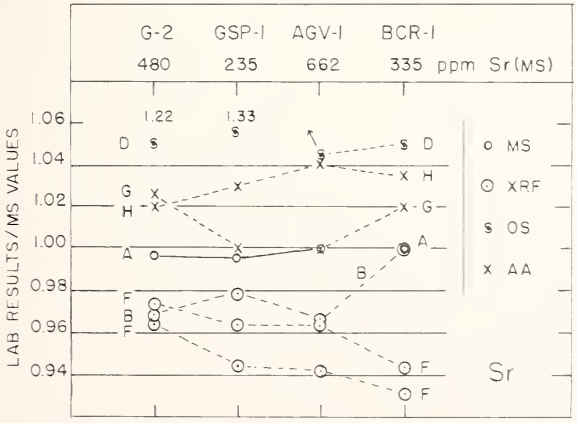

Figure 2. Selected Sr data on rock standards compared with mass spectrometric average values. Abbreviations as in figure 1.

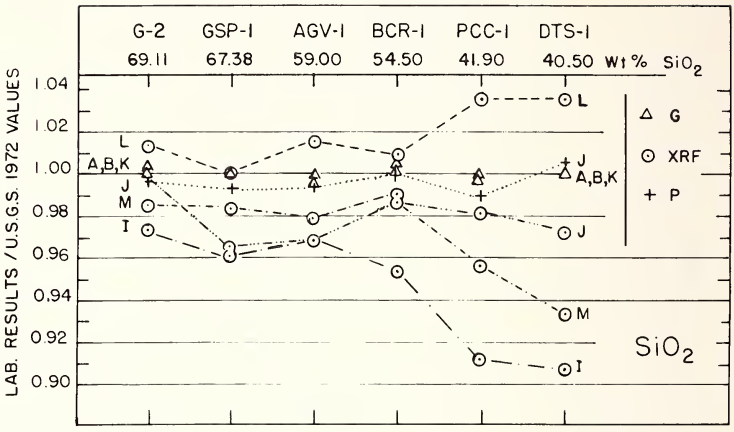

Figure 3. Selected SiO_2 data on rock standards compared with 1972 values [4]. G, gravimetric; XRF, x-ray fluorescence; P, photometric.

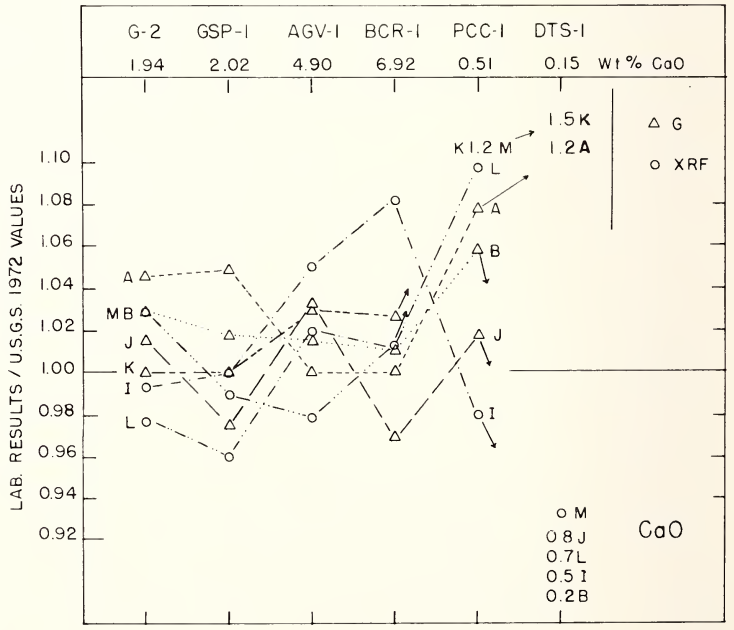

Figure 4. Selected CaO data on rock standards compared with 1972 values [4].

and give an unusual pattern which reflects some serious problems in the determination of CaO by conventional as well as instrumental methods. Again, PCC-1 and DTS-1 with low contents of CaO gave serious trouble, and the ratios for most laboratories for DTS-1 are so large that they cannot be shown in the diagram. Equally disturbing are the results for AGV-1 in which the data for five laboratories are positively biased. Of the six samples, Flanagan [4] gives recommended values for only three and averaged values for G-2, AGV-1, and PCC-1. This very obvious difficulty with the available silicate rock reference materials is well known to chemists and needs no special comment.

The determination of the rare earth elements (REE) within recent years has taken on special significance in studies pertaining to the generation of magmas in mantle and crustal regions as well as later changes in magmas attributed to differentiation by fractional crystallization, contamination, and so forth. The geological aspects of REE research as well as some of the procedures are reviewed by G. N. Hanson (this volume). Most of the present-day data are obtained by neutron activation or by mass spectrometric methods, but geologists have long used emission spectrographic data for some of the rare earth elements, and at this time I wish to compare some recent data for lanthanum and ytterbium. The rock samples are from an alkalic, silica-undersaturated rock series from Ross Island and vicinity, Antarctica.

As seen in figure 5, the emission spectrographic data for La show a good correlation with Na. One sample (No. 29) plots separately, but the adherence of the other samples to the line suggests that sample 29 is somehow abnormal, and a geological rather than analytical cause is involved. Thirteen of the samples were also analyzed by isotope-dilution mass spectrometry. The emission spectrographic results on these samples may be compared with the mass spectrometric results in figure 6. The slopes of the lines are somewhat different, but the trends are similar. Sample 29 is definitely aberrant and is not a normal member of the rock series.

Emission spectrographic data for Yb in the Antarctica samples are plotted against Na in figure 7. In a general way Yb increases with Na, but the correlation is poor. Thirteen of the samples were analyzed by isotope dilution and the mass spectrometric and emission spectrographic results are compared in figure 8. The mass spectrometric data clearly show the coherence between Yb and Na in the alkali olivine basalts and trachybasalts, but suggest a more complicated relationship in the phonolitic rocks. Sample 29 is shown to be aberrant by both emission spectrographic and mass spectrometric analyses.

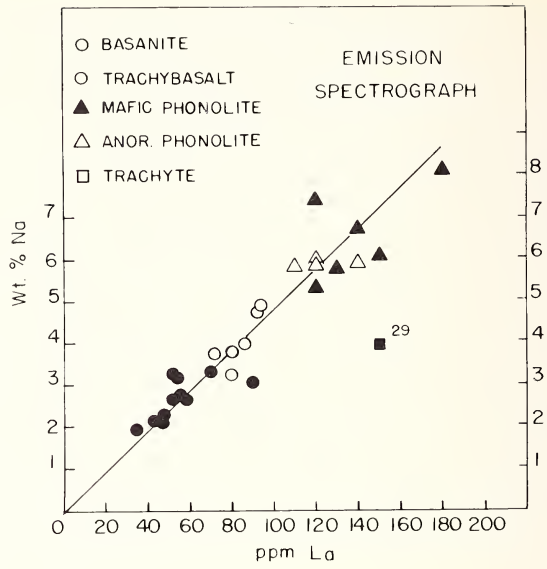

Figure 5. Variations of La (OS) with Na (FP). Volcanic rock series, Ross Island and vicinity, Antarctica.

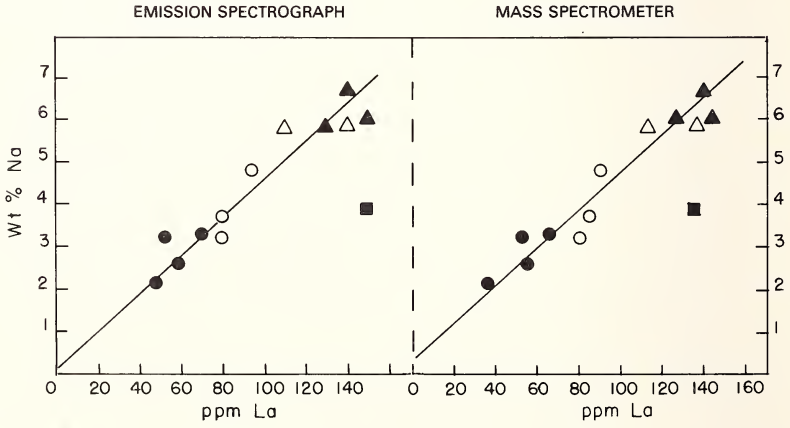

Figure 6. Comparison of emission spectrographic and mass spectrometric results for La. Volcanic rocks series, Ross Island and vicinity, Antarctica.

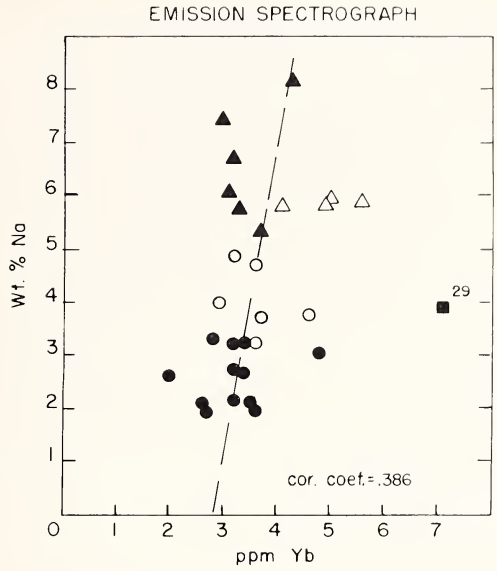

Figure 7. Variations of Yb (OS) with Na (FP). Volcanic rock series, Ross Island and vicinity, Antarctica. Symbols as in figure 5.

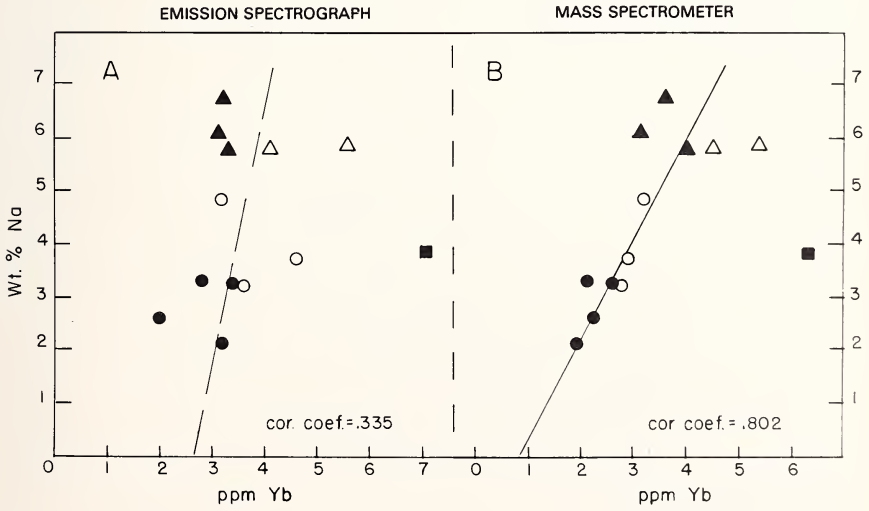

Figure 8. Comparison of emission spectrographic and mass spectrometric results for Yb. Volcanic rock series, Ross Island and vicinity, Antarctica.

IV. Concluding Remarks

The question may be properly raised in view of the examples given, what is the future of silicate analysis? Few laboratories can now afford to do silicate analyses by conventional methods. Instrumental analyses are here, and it behooves us to make the best possible use of them and to upgrade their quality. Unfortunately, in my opinion, geologists now seem obsessed with quantity rather than with quality of their chemical data. In many cases statistical devices and computer programs appear to be used largely to contrive an aura of scientific respectability. Blattner [2] suggested that "... all quantitative rock and mineral analyses be reported together with results obtained for rock and mineral standards, analyzed frequently enough to define a concentration scale within certain limits." I hope that the few examples given here have demonstrated the futility of this recommendation which would only swell our mushrooming literature with a mass of bad to useless data.

Flanagan [5] suggested that rock analysts might use standardized analytical methods and procedures similar to ASTM practices so that the results might be more comparable. There is merit in the suggestion of some standardization, but this is better achieved through education of analysts, better reference samples, and research on the limitations as well as on the best areas of application of the available instruments. Some instruments, as for example, flame and atomic absorption spectrophotometers, have such great sensitivity that the analyst must resort to large dilutions or use very small samples. The small sample has plagued emission spectrographic results for years, but geologists have shown a remarkable persistence in ignoring the limitation of rock or mineral sample size.

Many, if not most geologists have highly erroneous ideas about the conventional rock analysis, commonly referred to as "classical." Many apparently good determinations are accidental and the result of compensating errors. Consider CaO, for example, which may be low if some passes through the oxalate precipitations and is not recovered from the magnesium. It may be high owing to coprecipitation of Mg, Sr, and other cations. Some assume that all the Sr will be weighed with the Ca, but in my experience about 60 percent is carried down with the calcium oxalate if double precipitations are made. In the "classical" J. Lawrence Smith method for the determination of the alkalies similar problems are encountered, and, in addition, there is the problem of correcting for the reagent blank. Al_2O_3 in a conventional analysis is

$$R_2O_3 - (Fe_2O_3 + TiO_2 + P_2O_5 + SiO_2 + \text{others}) = Al_2O_3.$$

Clearly there are some advantages in using instrumental methods wherein constituents as important as CaO or Al_2O_3 can be determined directly.

Probably a fewer number but well-characterized or certified standard samples would better serve the needs of most chemists today. We need to know exactly how much CaO there is in the reference material, not how much one might expect to find with a well-described procedure or with a specified instrument. In this regard I feel that special reference should be made to mass spectrometry which affords a remarkable advantage over other approaches in that the isotope-dilution spiking technique permits clean separations without the necessity of 100 percent recoveries. Because of this a variety of chemical and physical methods can be used for the removal of interfering elements.

In conclusion, I should like to say that although the goal may not be immediately attainable, we should strive for an accuracy of approximately 1 percent of the amount present, except for some major constituents, such as SiO_2 in silicates, where an accuracy of 0.1 percent of the amount present is needed. Obviously the methods, equipment, and the facilities will require adjustments to the concentration levels. At low concentrations airborne contamination becomes an overriding consideration, and clean-room facilities become necessary.

V. Acknowledgements

I thank G. N. Hanson and S. S. Sun, State University of New York at Stony Brook for permission to use their mass spectrometric La and Yb data. I am indebted to my colleagues, Mineral Constitution Laboratories, Pennsylvania State University and to numerous individuals who have made available to me their unpublished Rb and Sr results on the U.S.G.S. rock standards. Much of the data used in this paper was obtained in investigations supported by the National Science Foundation (Geochemistry and Polar Programs).

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USE AND INTERPRETATION OF WATER QUALITY DATA

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Water quality data are collected by State and Federal pollution control agencies to characterize baseline quality, identify trends, detect and document violations in quality standards and discharge permit conditions, develop mathematical models for determining present and future pollution control requirements, and characterize the movement, fate, and effects of specific pollutants entering the water environment. This information is used in formulating and implementing water quality management goals, strategies, and plans and in evaluating the effectiveness of resulting local, State, and Federal pollution abatement efforts. The kinds of interpretations to be made from water quality data, the interpretive methods to be used, and the level of reliability desired of the results must all be decided before an effective sampling program can be designed. These considerations dictate the number and spatial arrangement of stations, the parameters to be evaluated, sampling frequencies, and the duration of the sampling program. Because of supportive relationships among parameters, data on a given parameter are seldom evaluated independently of the data on the other parameters measured. Results are grouped in various combinations and displayed in any of a number of ways for interpretation, depending on the objectives in mind. Statistical procedures are an important tool in the interpretive process, but should never be used as a substitute for judgment. The economic and other impacts of management/legal decisions based on the data require that proper consideration be given to data reliability measures and the confidence levels of the results obtained, in addition to the results themselves.

Keywords: Accuracy; data interpretation; monitoring; water; water quality.

I. Introduction

The scope of this paper is limited to the pollution control aspects of water quality data use and interpretation. No attempt has been made to address the numerous other potential uses of water quality data. The paper is intended to provide an overview of a topic requiring a multi-volumed treatise if covered on a parameter-by-parameter basis.

Interpretation of data on quality requires several other kinds of water data. Generally, supplemental hydraulic, hydrographic, hydrological, and meteorological data are needed to identify the factors that produced the quality conditions found. In addition, information on land use practices, types of commercial and industrial activity, population size, and similar factors usually are needed.

If water quality data could be acquired cheaply, investigators would not have to give much consideration to the kinds of data interpretations to be made until after all of the data that could possibly be required were collected. In reality, however, water quality and related data are expensive to acquire. Thus, the number of sampling locations, the parameters to be analyzed, and the sampling frequency must nearly always be set at some minimal level. This makes it imperative to design the sampling program to achieve specifically those objectives of the study. Accordingly, the data interpretation phase can be seen to be tied very closely to the study design phase. The specific objectives of a water quality study have perhaps the greatest single bearing on selection of the number and spatial distribution of stations, parameters, sampling frequencies, and study duration.

II. Monitoring Objectives and Data Uses

There are seven basic objectives that cover essentially all water quality monitoring conducted in support of pollution control programs. These are:

- Characterization of existing quality (short-term)
- Long-term trend assessment
- Detection of instances of standards and permit noncompliance
- Documentation of the nature of standards and permit noncompliance
- Development and validation of mathematical models for short and long range water quality management planning
- Characterization of the movement, fate, and effects of pollutants
- Assessment of treatment process and control technique effectiveness

Monitoring activities designed to meet the above objectives, as a whole, provide the information necessary to identify existing and emerging environmental problems, to plan and carry out effective pollution control strategies, and to assess the combined effectiveness of local, State, and Federal pollution abatement efforts. Primary uses of the data gathered in each of the above seven kinds of monitoring activities are described below.

A. Characterization of Existing Quality

Short-term data on water quality are used to identify the uses for which various water bodies are suitable and the nature and relative magnitude of existing water quality problems. This information is required in the formulation of water quality goals, design of effective pollution control programs, allocation of resources commensurate with the relative magnitude of the problems, development of realistic water quality and effluent standards, and evaluation of the degree of compliance with existing standards and effluent permit requirements (at the time of the survey). The initial characterization of a given water body also may provide the baseline data for future water quality trend assessments.

B. Long-Term Trend Assessment

Information on trends in quality over a period of time provides the only true measure of the overall effectiveness of pollution abatement efforts by local, State, and Federal agencies, and the private sector. This information can also serve to identify emerging problems in time to implement appropriate preventative measures. True water quality management lies in preventing problems, not in attacking problems once they have been allowed to occur. Accordingly, trend monitoring should receive greater emphasis as the existing water quality problems throughout the nation are solved.

C. Detection of Standards and Permit Noncompliance

Data that show instances of noncompliance with water quality standards and effluent permit requirements are used to signal the regulatory arms of pollution control programs into action. Information compiled annually on the number of instances of noncompliance is also useful as an indirect measure of progress in the achievement of water quality goals.

Most of the monitoring performed by permittees on their effluents is of this nature. They use the information to signal when adjustments in their treatment or control processes are needed to prevent violations from actually occurring. Regulatory agencies use the data collected by the permittees to assess the degree of compliance with permit requirements.

D. Documentation of Standards and Permit Noncompliance

Once an apparent violation in standards or permit requirements has been detected at a given location, monitoring generally must be intensified to adequately document the violation in a manner suitable for successful legal action. This information must clearly delineate the nature of the violation and those responsible for it. Where the violation has a significant impact on water quality or uses (*e.g.*, a fish kill), this too should be documented adequately.

E. Mathematical Modeling

Mathematical models depicting cause and effect relationships among waste sources, ambient water quality, and various environmental influencing factors (*e.g.*, hydrological, hydrographic, and meteorological conditions) are developed from data collected through rather exhaustive surveys carried out under steady-state or nearly steady-state conditions. That is, the characterization of all waste contributions to the reach of interest, the resulting water quality within that reach, and associated pertinent environmental factors must all be gathered during a period when these interdependent variables are at equilibrium. These data are then used to derive the model. Once developed and validated, the model simulates water quality conditions that would result from various alternative waste treatment and control schemes under a variety of hydrological conditions.

These models are used in identifying the most cost-effective solution to existing problems and in developing plans for meeting pollution control needs 5 to 50 years in the future, based on economic and demographic projections covering these periods. Mathematical models have been particularly valuable in determining waste treatment requirements necessary to meet water quality standards in streams.

F. POLLUTANT CHARACTERIZATION

Detailed information is needed on the origin and behavior of each significant pollutant that enters the water environment. It is important to characterize each pollutant's typical patterns of movement through the environment, its transformation into other compounds, its ultimate fate, and any ecological or other effects on the environment at each stage of its journey. This information permits pollution control authorities to assess where and in what form the pollutant poses its greatest threat. This knowledge, in turn, provides a basis for setting standards for the pollutant and a means to identify the most effective point for interception and control of the pollutant.

G. ASSESSMENT OF CONTROL SYSTEM EFFECTIVENESS

Data also are needed on the effectiveness of various experimental and conventional waste treatment processes and other pollution control techniques under a variety of operating conditions. This information is used by pollution control authorities to stipulate effluent limitations in discharge permits, develop effluent standards, and to assess the adequacy of the state-of-the-art treatment and control systems for meeting future pollution control requirements.

The wastewater treatment system operator needs data on influent and effluent quality on a continuing basis to operate effectively the treatment system and to ensure against possible violations of permit conditions and applicable standards.

III. Data Interpretation Considerations

A. SURVEY DESIGN

As already indicated, the nature of the data interpretation to be made should be determined before the first sample is collected and, in fact, greatly influences when, where, how often, and over what time period the samples are to be taken and what parameters are to be measured. A key factor influencing the selection of monitoring stations and the number of samples to be collected over the course of the survey is the data confidence level desired for the survey results. In general, the higher the desired confidence level, the greater the number of monitoring stations

and samples per station required. There is always a trade-off, therefore, between cost of the monitoring effort and the minimum acceptable level of confidence.

The amount of data to be generated in the survey and the best approach to be followed in its analysis and interpretation must also be given careful consideration in the survey planning stage. If the volume of data to be generated is large, arrangements for use of a computer should be made.

B. Parameters for Describing Water Quality

There are in the order of 100 parameters used more or less routinely in the evaluation of water quality (table 1). Literally thousands of other constituents also may be of interest from time to time. These parameters are used either to assess the suitability of a water body for various uses or to assess the impact of some factor (*e.g.*, wastewater discharge or alteration of flow regime) on water quality. In either case, interest focuses on those general characteristics of a water body that make it suitable or unsuitable for the uses desired: 1. aesthetics, 2. nuisances and hazards, 3. physiological effects on man, other animals, and plants, and 4. economic effects. Minimum quality requirements stipulated in State-Federal water quality standards were derived from judgments based on these considerations.

C. Aesthetics

First, water should be pleasant to the senses of man and capable of supporting life forms of aesthetic value. Visible pollution such as unnatural color, sludge banks, floating solids, foam, oil films, algal blooms, and excessive aquatic weed growths should be absent. There should also be an absence of constituents in amounts sufficient to produce undesirable tastes or odors, alone or in combination with other constituents present or added in the treatment process. Parameters used to assess or define the aesthetic quality of water are listed in table 2.

D. Nuisances and Hazards

In addition to being aesthetically appealing, water should not pose a nuisance or hazard (*i.e.*, potential to injure) to man or to desirable aquatic and other life forms. For example, the concentration of suspended or settleable solids should not be so great as to: 1. prevent others from sighting a submerged or unconscious swimmer, 2. impair the growth of desirable

TABLE 1. *Parameters used frequently in evaluation of water quality*

Physical	Chemical, Inorganic	Chemical, Organic	Microbiological
Temperature	Dissolved oxygen	BOD (Immediate, 5-day, long-term)	Coliform, total & fecal
Specific conductance	Dissolved carbon dioxide	COD	Fecal streptococci
Turbidity	Hydrogen Sulfide	Chlorine demand	Total plate count
Light penetration	Minerals:	Total organic carbon	Salmonella
Color	Acidity	Methylene blue active substances	Shigella
Odor	Alkalinity	Carbon chloroform extracts	Viruses:
pH	Calcium	Carbon alcohol extracts	Coxsackie A & B
Total solids (volatile & fixed)	Magnesium	Cyanide	Polio
Suspended solids (volatile & fixed)	Bicarbonate	Pesticides	Adenovirus
Floating solids	Carbonate	Oil & grease	ECHO
Sediment	Hydroxide	Phenolics	
Concentration	Hardness		**BIOLOGICAL**
Particle size	Bromide	**NUTRIENTS**	
Bed load	Chloride		Plankton
Radioactivity	Fluoride	Organic-N	Periphyton
	Sulfate	NH_3-N	Benthos
HYDROLOGICAL & METEOROLOGICAL	Dissolved solids	NO_2-N	Fish
	Trace elements:	NO_3-N	Waterfowl
Volumetric flow rate	Aluminum Lead	Total-P	Wildlife
Velocity	Arsenic Manganese	Soluble-P	Vascular plants
Time of flow	Barium Mercury	Organic-P	Chlorophyll
Depth	Beryllium Nickel	Orthophosphate	
Tide variation	Boron Potassium	Polyphosphates	
Wind speed & direction	Cadmium Selenium		
Solar radiation intensity	Chromium Silver		
Air temperature & humidity	Copper Sodium		
	Iron Zinc		
	Radiochemical		

TABLE 2. *Key parameters used to assess aesthetic quality of water*

TEMPERATURE	TASTE AND ODOR
FLOATING SOLIDS	TURBIDTY
SETTLEABLE SOLIDS	COLOR
OIL AND GREASE	NUTRIENTS
SURFACTANTS	BIOLOGICAL MATTER
DISSOLVED OXYGEN	

TABLE 3. *Key parameters used to assess nuisance and hazard characteristics of water quality*

TURBIDITY	BOD
TOTAL RESIDUE	COD
FILTRABLE RESIDUE	HARDNESS
NONFILTRABLE RESIDUE	IRON
SETTLEABLE SOLIDS	MANGANESE
BOTTOM DEPOSITS	PHENOLICS
DISSOLVED OXYGEN	OTHER FISH FLESH TAINTING SUBSTANCE

bottom organisms, or 3. form sediment banks that could hinder navigation. Biodegradable organic matter should not be present in amounts that could produce anaerobic conditions at ordinary low stream flows. Constituents that taint fish flesh, stain bathroom fixtures, or produce other deposits in plumbing should not be present. Parameters used in assessing nuisances and hazards are listed in table 3.

E. PHYSIOLOGICAL EFFECTS

Waters to be used for: 1. a potable supply for humans, livestock, and other animals, 2. irrigation supply for crops, 3. recreation, or 4. maintenance of a suitable fishery and wildlife habitat, should not contain toxicants, disease producing organisms, or other constituents at levels sufficient to produce adverse physiological effects on the animals or plants in question. Parameters of particular interest from a physiological standpoint are listed in table 4.

F. ECONOMIC EFFECTS

Waters should not contain substances at concentrations that unduly increase the cost of treatment required to render the waters suitable for potable, industrial process, cooling, or other withdrawal uses (*i.e.*, uses requiring removal of the water from the water body). In addition, in-place uses such as commercial fishing, shellfish harvesting, shipping, and recreation should not be adversely affected by water quality. Table 5 lists key parameters used to assess the economics of the water treatment required to render the water suitable for its intended use. Parameters utilized to evaluate the impact of water quality on the intended in-place uses are those applied to assess aesthetics, hazards, and physiological effects.

G. WATER QUALITY REQUIREMENTS

State-Federal water quality standards contain two key elements: 1. the classification of all waters covered by the standards according to their designated uses and 2. a stipulation of the quality requirements for each classified water use. These water quality requirements are based on considerations of aesthetics, nuisances, hazards, physiological effects on man, aquatic life, livestock, wildlife, and agriculture, and economic effects. Table 6 lists key parameters used in quality criteria for each major water use classification. Table 7 gives typical limits found in State-Federal standards for some of the parameters associated with selected water use classifications.

H. POLLUTANT BEHAVIOR

When evaluating water quality data for pollution control purposes, materials balances should be made between source and ambient waters

TABLE 4. *Key parameters used to assess physiological effects on water quality*

TEMPERATURE	CALCIUM	MERCURY
pH	MAGNESIUM	NICKEL
ALKALINITY	POTASSIUM	SELENIUM
DISSOLVED OXYGEN	SODIUM	SILVER
SPECIFIC CONDUCTANCE	LITHIUM	ZINC
FILTRABLE RESIDUE	SODIUM ADSORPTION RATIO	CYANIDE
CHLORIDE	ARSENIC	SULFIDES
BROMIDE	BARIUM	PESTICIDES
FLUORIDE	BERYLLIUM	BIOASSAY
AMMONIA	CADMIUM	RADIOACTIVITY
NITRATE	COPPER	RADIONUCLIDES
ALUMINUM	CHROMIUM	BACTERIA
BORON	LEAD	VIRUSES

TABLE 5. *Key parameters used to assess economic effects of water quality*

TEMPERATURE	MANGANESE
ALKALINITY	SILICON
pH	OTHER TRACE ELEMENTS
HARDNESS	TRACE ORGANICS
FILTRABLE RESIDUE	COLOR
IRON	

TABLE 6. *Key parameters used to define water quality requirements for various water uses*

Public water supply	Industrial water supply	Agricultural water supply	Aquatic life and wildlife maintenance	Recreation and aesthetics
Coliform bacteria	Process (Except Foods)	Farmstead	Temperature	Recreation
Turbidity	pH	(Same as for Public Supply)	Dissolved oxygen	Coliform
Color	Turbidity	Livestock	pH	Turbidity
Taste–Odor	Color	(Similar to that for Public Supply)	Alkalinity/Acidity	Color
Trace metals	Hardness	Irrigation	Dissolved solids	pH
Dissolved solids	Alkalinity/Acidity	Dissolved solids	Salinity	Odor
Trace organics	Dissolved solids	Specific Conductance	Carbon dioxide	Floating materials
Chloride	Suspended solids	Sodium	Turbidity	Settleable materials
Fluoride	Trace metals	Calcium	Color	Nutrients
Sulfate	Trace organics	Magnesium	Settleable materials	Temperature
Nitrate	Cooling	Potassium	Floating materials	Aesthetics
Cyanide	pH	Boron	Tainting substances	Turbidity
Radioactivity	Temperature	Chloride	Toxic substances	Color
	Silica	Trace metals	Nutrients	Odor
	Aluminum			Floating materials
	Iron			Settleable materials
	Manganese			Nutrients
	Hardness			Temperature
	Alkalinity/Acidity			Substances adversely affecting wildlife
	Sulfate			
	Dissolved solids			
	Suspended solids			
	Sanitary			
	(Same as for Public Supply)			

TABLE 7. *Typical permissible ringes of selected parameters associated with various water uses*[a]

Index	Surface water for public supply	Irrigation	Fisheries maintenance	Primary contact recreation
Temperature (°F)	85	55–85	5° Change	85
pH	6.0–8.5	—	6.5–9.0	6.5–8.3
Alkalinity ($CaCO_3$)	250	—	20 mg/1 Change	—
Dissolved Oxygen	≥4.0	—	≥7.0	≥5.0
Turbidity (JTU)	200	—	25	25
Color (Std. Units)	75	—	50	50
Dissolved Solids	500	500	240	500 (Fresh water)
Chloride	250	100	250	—
Sulfate	250	200	—	—
Ammonia (as N)	0.5	—	0.02	—
$NO_2 + NO_3$ (as N)	10	—	—	—
Boron	1.0	0.75	—	—
Chromium	0.5	.1	.05	1.0
Copper	1.0	.2	.02	0.2
Cyanide	0.2	—	.005	.01
DDT	.042 μg/1	4.2 μg/l	.006 μg/1	.006 μg/l
Dieldrin	.017 μg/1	1.7 μg/l	.003 μg/1	.003 μg/l
Coliform	5000/100 ml	5000/100 ml	5000/100 ml	1000/100 ml
Fecal Coliform	1000/100 ml	1000/100 ml	1000/100 ml	200/100 ml
Gross Beta radioactivity	1000 pc/l	1000 pc/l	1000 pc/l	100 pc/l

[a] Values are to be considered as maximum levels that are desirable, unless indicated otherwise. Units are mg/l unless stated otherwise.

for each significant pollutant. This provides a very good mechanism for determining whether or not all significant sources of a given pollutant have been identified. It also gives an indication of the overall precision of the survey. Any large imbalance between quantities of pollutants found in effluent discharges and in the receiving waters that cannot be otherwise accounted for casts a doubt over the entire survey results.

When interpreting water quality data, it is also necessary to be aware of, and be able to account for, pollutant transformations that may occur in the receiving waters. This is important for the materials balance and for the assessment of the environmental impact of a given pollutant or pollutant combination. Thus, a knowledge of the normal behavior and ultimate fate of a pollutant once it enters the receiving water is of immense value in the interpretation of results. This knowledge is, of course, equally valuable in designing the survey.

From the standpoint of behavior, pollutants may be divided into two categories, conservative and nonconservative. Conservative pollutants are those that undergo no significant change after entering the receiving water and remain within the aquatic environment (*e.g.*, chloride). Often, the only factors influencing concentrations of conservative pollutants once they reach the receiving waters are dilution and settling. Nonconservative pollutants, on the other hand, are those like biodegradable organics and bacteria that do undergo considerable change after entering the receiving water.

A knowledge of the physical, chemical, and biological processes that govern nonconservative pollutant behavior is necessary for full interpretation of the data. This knowledge is useful in two ways. First, it helps identify which chemical forms of a given pollutant are likely to be present. Second, it helps determine if all significant factors that are influencing water quality in the survey area have been taken into consideration. For example, if the actual results are consistent with those anticipated, it tends to verify that the data collected are, in fact, sufficient to meet survey objectives. If, on the other hand, actual and anticipated results differ, either all significant factors have not been considered or at least some of the data are inaccurate.

A number of pollutants are altered through biological activity, and conversely, biological activity is affected by a number of pollutants (at concentrations often encountered). A knowledge of these interrelated behaviors among pollutants and between pollutants and biota is used for mutual corroboration of the data collected. Very seldom, therefore, are data for one parameter evaluated independently of the data for other parameters.

I. Physical Influences

It is also imperative that various physical factors having an influence on water quality be recognized and taken into account. The velocity of water flow, for example, strongly influences the formation (or scouring) of sludge deposits, the rate of reaeration, and the degree of mixing between effluent plumes and receiving waters. Water depth strongly influences reaeration rates and the degree of stratification of water layers of different quality. The kind of materials composing the stream bottom can greatly influence the benthic community. The presence of fine shifting sand, for example, can prevent the survival of bottom organisms, producing the same net effect as toxic materials. Other pertinent factors include the operation of dams, the manner of impoundment releases, and dredging operations.

IV. Statistical Considerations

A. Data Reliability

Whenever environmental measurements are made, errors will be encountered. The sources of these errors are diverse, but may be grouped according to the scheme depicted in figure 1. Site selection error (σ_g) is the error due to nonrepresentativeness of monitoring sites. A few samples collected at a single location in a lake or river, for example, cannot be expected to be truly representative of values over the water body as a whole. Similarly, the depth at which the measurement is made affects the values observed. Another type of error, sampling error (σ_s), arises from the limited number of samples that usually are available. At any given location, one sample conceivably could be taken each hour of the year, giving 8,760 samples (365 days $\times$ 24 hours). In practice, we usually must be satisfied with a dozen or fewer samples at the site and often these are collected over a short period. If the samples were distributed randomly over the year, we could use the central limit theorem to calculate the error for the annual average value; without true randomness, however, additional sampling error problems arise. There also are errors inherent in all measurement methodologies. This measurement error (σ_m) arises from imperfections in the measurement technique itself and from inadequate quality control in applying the measurement technique. In addition, most measurement methods have minimum detectable limits near which considera-

ble error can occur. Another error, reference sample error (σ_r), results from the reference material used in the calibration process. Although reference materials are certified to have specified values, these materials usually deviate somewhat from the stated value. Often, this error can be estimated from data available from the supplier. Finally, errors may arise in the data processing and handling phase of the monitoring effort. The most common data handling error (σ_d) results from keypunch error, from failure to consider minimum detectable limits, and from clerical mistakes.

The final monitoring data are subject to the error from all five of these sources. Some of the error presents itself in the form of "bias"—values that are consistently high or low for a given laboratory—and is referred to as "systematic error." The remainder of the error, the "nonsystematic error," is randomly distributed from sample to sample, showing little uniform pattern. The data analysis and interpretation phase should recognize that these errors exist and should make a conscious effort to estimate the magnitude of these errors for each parameter that is reported. The confidence interval is the usual means by which a probability is assigned to the magnitude of these errors.

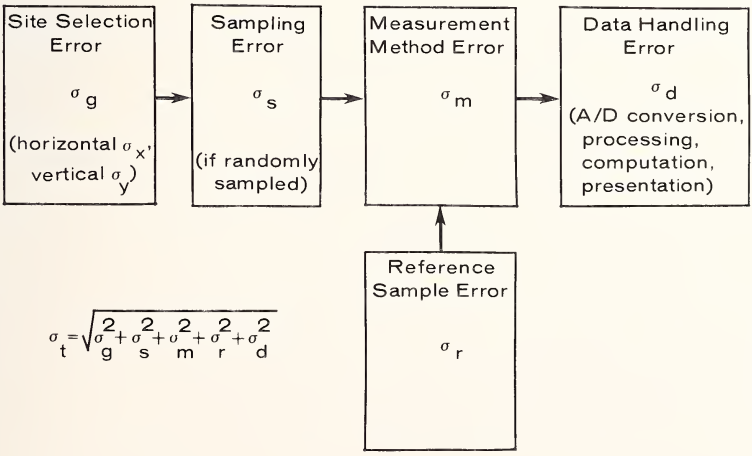

Figure 1. Sources of error in the measurement of environmental quality. (The notation in the figure refers to nonsystematic error and not to "bias," or systematic error. A similar figure could be constructed for both types of error.)

B. Trend Analysis

A most important purpose of monitoring data is to assess environmental trends. In short, these analyses attempt to answer the following questions: Has the environmental parameter changed over time? Has its value gone up or down? Is the change real or due to extraneous factors? Because different environmental parameters are often interrelated, and because parameters sometimes exhibit much randomness, these questions are not always easy to answer, even when sufficient good data are available. As a result, sophisticated statistical techniques increasingly are coming into use in the field of monitoring data analysis. These techniques include stochastic time series analysis, spectral time series analysis, multivariate regression analysis, and other approaches designed to probe the data in depth, extracting information on any changes that are real and are not just the result of random variations, man-made intervention, or seasonal and meteorological or hydrological factors.

C. Standard and Permit Violation Analysis

Another very important purpose of monitoring data is to document violations of water quality standards and permit requirements. If a violation is alleged to have occurred, the data must show beyond a reasonable doubt that it did occur. In the past, enforcement actions were concentrated in areas of gross pollution, where convincing proof was easy to provide. Increasingly, however, as the pollution control systems are installed, violations are becoming more sporadic in nature. In these situations, it is much more difficult to identify and to adequately document violations. Assume, for example, that samples were collected hourly from an effluent over a given 24-h period and the value of a given toxic constituent exceeded the limitation stipulated in the permit in only one sample, while the other samples were well below the limitation. The question immediately arises whether this one high sample was a true violation or merely the result of chance—a product of one or more of the errors in the monitoring process. With just one high value, little confidence could be placed in the premise that the observed value exceeded the legal limit. Statistically, the confidence level calculated for this situation might turn out to be 50 percent, meaning that the investigator is just 50 percent certain that the legal limit was exceeded. Clearly, this would be unacceptable in any legal proceeding. Even with additional data showing an elevated level of the same constituent in the receiving water and a recent fish kill caused by that constituent, the outcome of a legal action would be questionable. The

key to providing convincing data in this situation lies in the ability to obtain enough samples indicating a violation to be able to state with a reasonable level of confidence (*e.g.*, 95 or 99% assurance) that the average of the observed values exceeded the legal limit. Supporting documentation of any effects on the receiving waters and associated aquatic life would further substantiate the allegation and improve the chances of a favorable outcome.

V. Summary

In summary, water quality data are used by State and Federal pollution control agencies in formulating water quality goals, planning and implementing programs for their achievement, and in evaluating the effectiveness of the resulting local, State, and Federal pollution abatement efforts. The interpretations to be made from water quality data, the interpretive methods to be used, and the level of reliability desired of the results must all be decided upon before an effective program can be designed. Known relationships among parameters should be considered in the data interpretation process to aid in verification of the results. Statistical procedures are an important tool in the interpretive process and they provide an important aid for judgment. Actions taken based on the data can have major impact, economic and otherwise; therefore, proper consideration should be given to data reliability of measurements and the confidence levels of results obtained.

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INTERPRETATION OF CLINICAL LABORATORY DATA

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The central role of the clinical laboratory in the diagnosis of some diseases is discussed as well as some of the potential consequences of misinterpretation. Examples of the influence of both *in-vivo* and *in-vitro* factors on the interpretation of results are presented.

The genetic background and biological variability of the individual influences the data, even when he is healthy. The disease process may have a greater or lesser effect on laboratory data. In general, physicians usually request those tests to be performed that they anticipate being abnormal, so that the influence of disease on the test performed in the clinical laboratory is usually quite large. Other *in-vivo* influences include the mode of collection of specimens for analysis. Outside the patient the preparation of the specimen for analysis may affect the analytical results. Finally, the accuracy, precision and specificity of the analytical procedures in the clinical laboratory affect the data. It is only this group of factors that the clinical chemist is able to control and yet knowledge of both laboratory and patient factors is essential for the correct interpretation of data.

Keywords: Accuracy, precision and specificity of analytical methods; biological and genetic variability; clinical laboratory data; specimen collection; specimen handling.

I. Introduction

Correct interpretation of data from the clinical laboratory is perhaps more difficult and critical than the interpretation of data from any other type of laboratory. Often neither the physician in charge of a patient nor the laboratory scientist has available to him all the information that is needed for a correct interpretation. The knowledge that the clinical

chemist has of the accuracy, specificity, sensitivity and precision of the analytical methods is not enough for correct interpretation. It is also necessary to have a detailed understanding of the patient from whom the samples were obtained. This involves not only knowledge of the patient's demographic background but also of his disease and its treatment, and the conditions under which the specimens were obtained for analysis. This is information that the physician alone may possess.

In many situations the laboratory scientist is aware of some of the information needed for correct interpretation. So is the patient's physician, but neither is aware of all the critical factors. The size of the potential problem or lack of all essential information needed for correct interpretation of clinical laboratory data can be gauged from the knowledge that approximately two billion clinical laboratory tests are performed annually in the United States [1]. While a laboratory scientist may assist in the interpretation of data, this ultimate responsibility for interpretation rests with the physician caring for the patient from whom the specimens were withdrawn for analysis.

Although the typical hospital clinical laboratory performs as many as 300 different tests a small proportion accounts for the majority of the workload. Most of the frequently requested determinations are performed on mechanized analyzers. The size and capabilities of these instruments have become progressively greater with time. It is now possible for 20 different tests to be performed in the same instrument at the same time. One of the most widely used instruments in large hospitals performs 12 tests at one time on each sample (thus making 720 analyses in 1 hour). The introduction of one of the predecessors of this instrument into clinical laboratories stimulated the concept of laboratory screening whereby 12 determinations are made on a single specimen of blood when a patient is first seen by his physician.

The 12 tests that are performed are dictated, in part, by the frequency with which they are usually requested, but also by the ease with which the analytical methods could be mechanized. Some of the tests have such diagnostic specificity that abnormalities are strongly suggestive of certain diseases. Other tests are affected by many different diseases and their role is less for diagnosis than for monitoring the disease process and its treatment. Correct interpretation of results requires knowledge of the factors affecting each test but also of the relationship of one test to another.

II. General Considerations

The factors that may influence the concentration of constituents in body fluids are summarized in table 1. These will be considered in more detail under each heading.

Under the heading of genetic influences are considered influences that do not alter with time, *e.g.*, an individual's sex and blood group. Under the long-term physiological influences are considered the effects of aging, physical fitness, geographical location and typical diet of the individual. The short-term physiological influences include the immediate impact of meals or activity and posture as well as the time of day at which the specimen was drawn. Disease tends to affect both the long- and short-term physiological influences. Various factors such as the site of collection of the specimen and the use, or absence, of a tourniquet in the collection of the specimen comprise the sampling related influences. Many blood constituents are labile and can be affected by handling procedures. Finally the analytical procedures used in the clinical laboratory may have great influence on the reported data. Drug therapy may affect disease processes by altering the abnormal pathophysiological state. Drugs may also appear to affect a disease by possible interference with analytical procedures in the clinical laboratory.

TABLE 1. *Factors influencing concentration of constituents in body fluids*

Genetic
Long-term Physiological
Short-term Physiological
Specimen Collection
Specimen Handling
Analytical

For diagnostic purposes the clinician wishes to know if a laboratory result is normal or abnormal. Unfortunately, many physicians use finite numbers to define a range of values within which data are labeled normal, implying the healthy state. Outside these reference values data may be interpreted as abnormal. The normal range may be derived differently from center to center, but usually involves the analysis of specimens from the readily available healthy staff of the clinical laboratory and medical students and nurses. This is generally a very fit and active group of young

adults considerably different from the population at greatest risk of admission to hospital. Nevertheless, by statistical manipulation of the results obtained on these individuals normal ranges are derived. Physicians often tend to accept published normal ranges as having greater validity than should be ascribed to them and in this way unwarranted significance may be assigned to certain laboratory data. The biological variability of individuals may be ignored, so that allowance is not made for deviation from a statistically derived "normal population."

In this paper it seems pertinent to review, in some detail one of the tests that is most commonly involved in health screening and to attempt to identify the factors—both clinical and laboratory—that cause alterations in the values of the test. The test that I have chosen to illustrate the problem of data interpretation is serum uric acid. Many other tests are influenced by as many factors which underscores the magnitude of the problem of correct interpretation. When several tests are ordered at the same time the problem is compounded.

III. In Vivo Influences on Serum Uric Acid

Uric acid is derived from the metabolism of nucleic acids and from the purines ingested in the diet. It is eliminated from the body mainly through the kidneys. Three different processes are involved in the kidney. Glomerular filtration is followed by tubular secretion and reabsorption. The concentration of urate in the serum reflects the balance within the body of its formation and excretion. Reabsorption of urate from the renal tubules is virtually complete in the normal situation and the urate that actually appears in the urine is derived from tubular secretion. The processes of reabsorption, and especially secretion, are influenced by many drugs.

The concentration of serum uric acid may be measured to determine the presence of gout. The concentration of uric acid is also affected by the increased destruction of nucleoprotein that occurs with leukemia, polycythemia or hemolytic anemia. Most examinations are made to determine if the concentration of the uric acid is increased but in some situations it is reduced, for example, when drugs that have been administered inhibit the reabsorption of uric acid from the urine.

In most healthy men the concentration of uric acid in serum is between 3.5 and 8.5 mg/100 ml (0.21-0.51 mmol/l). In women the range of values is from 3.0 to 7.5 mg/100 ml (0.18-0.45 mmol/l) [2].

The serum uric acid is generally higher in relatives of patients with gout

than in the general population although these people may not present with symptoms. In certain population groups, *e.g.*, American Indians such as the Pima and Blackfoot, or in Polynesian groups the serum uric acid may be higher than the typical American Caucasian group. The Aborigine has a much higher concentration of uric acid in serum than Australian Caucasians [3]. While Fessel [4] has found that the serum uric acid in black women is as much as 0.5 mg/100 ml (0.03 mmol/l) higher than in Caucasian women of the same 60 to 69 year age group, other investigators have found no black/white racial influences on uric acid concentration [5]. In various studies, serum uric acid has been reported to be higher in individuals with blood group B, and the lower in groups A and AB than in the respective control groups [6].

In men, the average serum uric acid concentration rises steadily to reach its maximum between ages 20 and 24 years, it then falls slightly to age 30 years but thereafter remains fairly constant. In women, the maximum is reached between ages 15 and 19 but then the concentration declines to about age 40. The curve peaks between ages 50 and 54 but thereafter declines slightly [7]. This second peak in women is probably associated with the hormonal changes of the menopause. In pregnant women the mean concentration is approximately 0.8 mg/100 ml less than in nonpregnant women of the same age [7]. The time of year affects the concentration of serum uric acid. In one recent study [8] it was shown in 12 healthy men that the average serum uric acid rose from a low of about 5 mg/100 ml in February to about 7 mg/100 ml in July. The mechanism for this effect is uncertain but is more likely to be related to the effect of sunshine than a change in diet, as the summer diet generally is lower in purines than in the winter diet. There is a good correlation between the serum uric acid concentration and the altitude at which individuals live. Increasing altitude stimulates increased production of red blood cells, the turnover of which adds to the serum uric acid. There is usually a good correlation between the serum uric acid concentration and the hematocrit and blood hemoglobin concentration [9]. Positive correlations have also been reported between uric acid and serum cholesterol and stated alcohol intake [10].

The serum uric acid concentration tends to be higher in men of higher socio-economic class. Gout is also more prevalent in the higher social classes [11]. This may partly be due to the higher standard of living that these people can embrace. However, the families of men who demonstrated significant occupationally-related uric acid values did not show the same differences implying that standard of living was not the sole determinant of differences in uric acid [12]. The concentration of uric acid in

serum does seem to be related in some way to intelligence. Studies in school children have indicated that those with the best grades, or about to go to college, had a higher serum concentration than those not planning to attend college [13]. Other studies have confirmed the influence of personality, especially driving force, on serum uric acid. In blind individuals the serum uric acid tends to be as much as 1.4 mg/100 ml higher (on average) than age and sex matched controls [14].

Body build is correlated with the serum uric acid concentration. Obese individuals exhibit a higher concentration of uric acid in their serum than lean individuals [15]. With slimming the serum uric acid concentration of the obese individuals climbs even higher due to the increased cellular catabolism. With starvation β-hydroxybutyrate is increased in the plasma and competes with uric acid for excretion in the kidney. Even in children there is a good correlation between body weight and serum uric acid. Physical training causes a reduction in the serum uric acid in the typical individual [16]. Surprisingly, the uric acid of smokers tends to be lower than that of nonsmokers, possibly reflecting a dietary influence as many heavy smokers are relatively small eaters. The average concentration is less in smokers who inhale than in noninhalers [17]. Stress also influences the concentration of serum uric acid in serum. Individuals presented with stimulating challenges in their work were shown to respond with an increase in the serum uric acid concentration. When bored or in periods of anxiety the serum uric acid concentration was less [18].

A diurnal variation for uric acid exists. The serum concentration is highest in the afternoon and least during the night [19]. To this diurnal rhythmicity must be added the influence of meals. Meals rich in nucleic acids cause an increase in the serum uric acid within 1 hour. The increase may persist for as long as 4 to 8 hours. The influence of 20 g nucleic acid ingested in one meal may be observed in the urine for 2 to 4 days [20]. The ingestion of yeast has a similar effect. In young adults ingestion of a typical meal may produce a decrease in serum uric acid within 2 hours. In older adults the change is not significant [21]. Violent exercise may cause an increase of the serum uric acid by as much as 2.5 mg/100 ml. This is presumably due to increased cell turnover. The effect is manifested in the urine by an increased excretion for as long as 2 days [22]. Diet has an influence on the serum uric acid concentration although it has been reported that as much as 1 pound of meat per day does not affect the serum uric acid concentration [23]. However, there is a direct correlation between protein intake by individuals and excretion of uric acid in their urine. Our studies [24], in which healthy volunteers ingested

a diet of pure chemical adequate in all essential elements, showed that removal of purines from the diet produced a rapid and sustained fall in the serum urate. Alcohol may produce a marked increase in the concentration of uric acid in serum. This is probably due to the accumulation of lactic acid which competitively inhibits the excretion of uric acid [25].

The procedures used in the collection of the blood specimen have little effect on the concentration of uric acid except that poor technique, which may produce hemolysis, may cause erroneous results when a nonspecific analytical method is used. No preservative is normally required for blood samples but the pH dependency of the solubility of uric acid in urine requires that no acid is added if meaningful results are to be obtained. The concentration of uric acid appears to be less (by as much as 0.17 mg/100 ml) in plasma than in serum [26].

Not only does the concentration of uric acid increase in diseases in which there is obviously increased turnover of cells but in many other diseases the concentration of uric acid is also affected. The concentration may be increased in various endocrine disorders such as hypothyroidism, hyperparathyroidism and congenital vasopressin resistant diabetes insipidus. In lead and beryllium poisoning, and in various congenital disorders such as Von Gierke's disease and the Lesch-Nyhan syndrome, the uric acid concentration in serum may be increased. In sarcoidosis and in hypertension with arteriosclerosis the concentration of the urate may also be increased above normal levels. In renal failure reduced ability to excrete uric acid causes an increase in the serum concentration.

Many drugs affect the concentration of uric acid in serum. Some of these are administered to patients with the specific intent of lowering the concentration of urate. Others are administered for other reasons and have an incidental action on the urate concentration.

The drug most widely prescribed to reduce the serum uric acid concentration is allopurinol which is a potent inhibitor of the enzyme xanthine oxidase which converts hypoxanthine to xanthine and the latter to uric acid. There is also a reduction in the quantity of urate excreted in the urine at the same time. Other agents such as probenecide and cinchophen promote increased excretion of uric acid in the urine so its concentration in the urine is actually increased. This is often associated, but not always so, with a reduction in the plasma concentration. This is true of those situations in which there is augmented production of uric acid. Salicylate (aspirin) may have a similar uricosuric action, *i.e.*, it may cause an increased excretion of uric acid which may at the same time reduce the concentration in blood. This group of uricosuric agents acts by inhibiting the normal mechanism for reabsorption of uric acid from the urine in the tu-

bules of the kidney. With low doses of salicylate therapy the concentration of uric acid in serum may actually be increased but when the daily dose is as large as 5 grams the uricosuric action is observed. These paradoxical actions may be explained partly by the fact that uric acid is both secreted and absorbed in different parts of the renal tubule.

Few drugs other than allopurinol, are given deliberately nowadays to reduce the serum uric acid concentration. However, many drugs administered for other purposes also have the same effect. Many other drugs that incidentally cause an increased excretion of uric acid in the urine and which, at the same time, may cause a reduction of the serum concentration are listed in table 2. Several of these drugs have the same action as aspirin—causing an increased concentration in the serum at one dose and decrease at another level. It is noteworthy that the drugs included are administered for a variety of different purposes. Thus they include anticoagulants, *e.g.*, bishydroxycoumarin and phenindione; radiographic contrast media such as diatrizoate, iopanoic acid and ipodate. Hormone therapy with cortisone, corticotropin, and estrogen therapy in men, have the same effect. Diuretic drugs such as chlorothiazide and ethacrynic acid, when given in large doses intravenously, have a uricosuric action. Drugs that produce a diuresis, *e.g.*, mannitol or glucose may also reduce the concentration of uric acid in serum.

Other drugs, illustrated in table 3, cause an increase in the concentration of uric acid in the serum. These range from various anabolic hormones which act by augmenting nitrogen balance and increasing muscle mass to many diuretic drugs which impair urate clearance at low concentrations and drugs such as cytarabine, mercaptopurine or methotrexate which cause massive destruction of cells. Many anti-hypertensive agents, *e.g.*, mecamylamine, by decreasing the blood pressure and reducing the blood flow to the kidneys, cause an increase in the serum urate. Several antibiotics are nephrotoxic with high doses and prolonged therapeutic regimes. Nephrotoxicity is manifested, amongst other things, by an increase in the concentration of end-products of nitrogen metabolism, including uric acid, in the serum. Blood transfusions augment the available nucleoprotein for catabolism and increase the serum concentration of uric acid.

The administration of drugs to healthy individuals may not produce the same type of response as in ill individuals. Further, we have considered the administration of drugs as if they were given separately. This is rarely the case. Indeed, concomitant administration of as many as 10 or more drugs is not unusual. Such therapeutic regimes usually include several drugs specifically intended to counteract the principal disease process.

However, to these there frequently are added vitamins, iron and sedatives, all of which can be expected to influence metabolic processes and laboratory data.

TABLE 2. *Drugs reported to cause a decrease in serum urate*

Acetohexamide	Ethinylestradiol
Aspirin	Glyceryl Guiacholate
Amiloride	Griseofulvin
Azathioprine	Halofenate
Azauridine	Indomethacin
Biscoumacetate	Lithium
Bishydroxycoumarin	Mefenamic acid
Chlorothiazide	Mersalyl
Chlorpromazine	Methotrexate
Chlorprothixene	Phenylbutazone
Cinchophen	Phenindione
Clofibrate	Probenecid
Corticosteroids	Phenolsulfonethalein
Corticotropin	Radiographic contrast media
Cortisone	Stilbestrol
Estrogens	Sulfinpyrazone
Ethacrynic acid	Vinblastine

TABLE 3. *Drugs reported to cause an increase in serum urate*

Acetazolamide	Halothane
Aluminum Nicotinate	Hydrochlorothiazide
Amiloride	Hydroxyurea
Anabolic Steroids	Ibufenac
Androgens	Mecamylamine
Angiotensin	Mechlorethamine
Antineoplastic agents	Meralluride
Azathioprine	6-Mercaptopurine
Azathymine	Methicillin
Busulfan	Methotrexate
Capreomycin	Methoxyflurane
Chlorothiazide	Nicotinic acid
Chlorthalidone	Phenothiazines
Clorexolone	Phenylbutazone
Cyclothiazide	Prednisone
Cytarabine	Propylthiouracil
Diazoxide	Rifampin
Epinephrine	Spironolactone
Ethacrynic acid	Thioguanine
Ethambutol	Thiotepa
Ethoxazolamide	Vincristine
Furosemide	Xylitol
Gentamicin	

IV. In Vitro Influences on Uric Acid

To all the *in vivo* changes in uric acid concentration that present problems in interpretation of data must be added laboratory induced changes. While laboratory staff may be quite familiar with these influences on laboratory data few physicians are aware of the potential problem because they are often unfamiliar with the nonspecificity inherent in most procedures used to determine the uric acid concentration in the laboratories that they use. Most of the procedures that are in widespread use to measure uric acid involve removal of protein, either by acid, as in most manual procedures, or by dialysis of small molecules (including uric acid) away from the protein containing solution. The property of uric acid to reduce alkaline phosphotungstate is then most widely used to quantify the uric acid. Other oxidants have been used and many different alkalies have also been used. The many variations of the same analytical procedure were primarily employed in an attempt to reduce turbidity, which may produce false answers, and to improve the specificity of the reaction.

Use of the enzyme uricase which converts uric acid to allantoin obviates many of the problems of nonspecificity of the phosphotungstate procedure. However, the requirement of measuring the change in absorbance at 292 nm necessitates a good-quality spectrophotometer and renders the procedure impractical for the mass-production approach to analysis now used in the clinical laboratory. Early use of uricase involved measurement of the concentration of uric acid with related chromogens in the serum by phosphotungstate reduction followed by incubation with uricase to destroy the uric acid quantitatively and a repeat measurement of the non-urate chromogens. The inherent errors in this approach have been summarized by Henry [27]. However, the uricase procedure can be adapted well to the mechanized laboratory by coupling the reaction, under controlled conditions of pH, with peroxidase and an indamine dye and using the AutoAnalyzer [28]. This combines the advantages of specificity of analysis with efficiency.

Procedures requiring acid precipitation of protein may induce errors in any method for the measurement of uric acid. Urate may be coprecipitated with acids if the pH of the filtrate is reduced below 3. Although heat coagulation is free from such theoretical objections it is cumbersome and time consuming. Dialysis in mechanized analyzers circumvents the problems involved with protein precipitation. While theoretically it is unnecessary to use a standard with the spectrophotometric uricase procedure this is required for all other procedures.

Uric acid must be dissolved in lithium carbonate and a preservative must be added if it is not to deteriorate fairly rapidly even when refrigerated. Formaldehyde, which has been widely used as a preservative, retards dialysis in mechanized procedures and also may inhibit uricase in specific methods. Specimens for analysis are only stable for 3 to 5 days at 4 °C but up to 6 months if frozen. The concentration of uric acid in serum is much greater than that of the erythrocytes so that theoretically hemolysis should have little effect on the measured concentration. However, erythrocytes contain large quantities of other reducing substances which are released into the serum with hemolysis so that measurement by a nonspecific reduction technique will produce erroneously high results. Hemoglobin, itself, can be measured as if it were uric acid when it is present in large quantities, *i.e.*, if the specimen is badly hemolyzed. Bilirubin, always present to an extent of about 1 mg/100 ml, except when a patient is jaundiced when its concentration may be much greater, may also react like uric acid when its concentration exceeds 7 mg/100 ml.

None of the drugs listed in table 4 that affect the phosphotungstate procedure have been reported to influence the uricase procedures. While all these compounds are claimed to affect the procedures there is relatively little evidence to support the suggestion that they would significantly affect the determined serum concentration of urate at the concentrations at which the drugs are normally present in serum. The concentration of many drugs tends to be higher in urine than in plasma, as will the concentration of their metabolites, so that an interference that has been reported for urine may not exist for plasma or serum.

TABLE 4. *Compounds reported to affect non-specific analytical procedures for uric acid*

Acetaminophen	Glutathione
Acetophenetidin	Hemoglobin
Acetylaminophenol	Hydralazine
Acetylsalicylic acid	Hypoxanthine
Amminophenol	Levodopa
Aminophylline	6-Mercaptopurine
Ampicillin	Methicillin
Ascorbic acid	Methyldopa
Caffeine	Penicillin
Carbenicillin	Phenelzine
Chloral	Propylthiouracil
Cysteine	Resorcinol
Cystine	Salicylic acid
Dextran	Theophylline
Epinephrine	Tryptophan
Ergothionine	Tyrosine
Formaldehyde	Tyramine
Gentisic acid	Xanthines
Glucose	

The *in vivo* effects of drugs are not exclusively related to their chemical structure, whereas the *in vitro* effects on a laboratory method are very much dependent on the chemical structure of the drug. This results in situations in which drugs may apparently have synergistic or antagonist effects on the concentration of a constituent. For example, methicillin may cause nephrotoxicity through a direct action on the renal tubules while at the same time it may be measured by a nonspecific laboratory procedure to produce a falsely high uric acid value. This type of situation is especially dangerous when other measures of renal function also suffer from the combination of a physiological and methodological effect. Also, large doses of aspirin produce a lowering of the serum concentration of uric acid yet this may not be apparent as it acts as a reducing agent with the nonspecific analytical procedures, and will cause an artefactually high urate value.

Barnett [29] has identified clinical decision thresholds and has attempted to define acceptable errors in analytical precision. At the National Institutes of Health we have also attempted to determine the intraindividual variability of uric acid on a day to day basis [30]. These values (which were 0.5 mg/100 ml in both studies) indicate that a fairly large imprecision in uric acid measurements can be tolerated because of the wide day to day variability exhibited by most patients. For other analytes much greater precision in analysis is required because of the close hormonal control exerted *in vivo* that greatly reduces the physiological variability.

Laboratory staff should remember their obligation to use the best analytical procedures and also should be prepared to provide as much assistance as possible to the practicing physician so that he can interpret his data correctly. Uric acid has only been used as an example to illustrate the many factors that affect clinical laboratory data. Many of the same, but also additional, influences affect other constituents. It is important to put the correct interpretation of laboratory data into the proper perspective because most clinicians and laboratory scientists are unaware of the magnitude of the difficulties of doing this. It requires a large-scale cooperative effort so that all patients will ultimately reap the benefits.

V. Summary

Factors influencing the concentration of uric acid in serum are used to illustrate the problems posed for physicians in the correct interpretation of clinical laboratory data. These factors include not only the underlying

disease state of the patient but his demographic background and living habits, as well as the influence of drug therapy. The importance of the use of specific analytical procedures in the clinical laboratory is also discussed.

VI. References

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Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis.
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THE ANALYST AND ACCURACY

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It sometimes happens that an analytical chemist, in evaluating the accuracy of his analysis, subconsciously obtains highly precise and accurate analyses, when in fact, the method and techniques he uses cannot possibly yield such results. This can happen even when he uses standard reference materials or carries out a series of repetitive analyses. Examples of these are discussed along with examples of other human foibles that may leave the analyst with a false impression of his accuracy and precision.

Keywords: Accuracy; analytical chemistry; standard reference material; statistics; syndrome.

One of the most important factors in any analysis is the analytical chemist and his interaction with his analytical problems. Unless he is careful he can and will fool himself easily and completely. This can happen with his use of Standard Reference Materials (SRM). Oftentimes he may use them as a security blanket. In other words he will cite results on the SRM as an iron-clad guarantee that his values are accurate.

Over the years I have noticed that reported values tend to agree with SRM certified values more frequently than would be expected even though a range of values is involved. An example of this can be seen in table 1 for tin and molybdenum. Of the nine results cited four were unusually close to the certified value.

Another example of this security blanket is referred in the analyses of coal for selenium. These values are in table 2. It will be noted that the evolution values do not agree very well with the colorimetric values even though the value found on NBS-SRM 1632 was reasonably close to the recommended value.

TABLE 1. *Comparison of certified concentrations to data from recent literature*

Metal	SRM	Found	Certificate approved	Certificate range
	Steel 20e	0.158 .155	0.013	0.011–0.014
	Brass 37d	.966 .989	.97	.96 – .97
Sn	Al-Si 87	.060 .064	.63	.05 – .077
	Steel 101C	.0127 .0126	.008	0.077– .009
	Ounce Metal 124b	4.905 4.912	4.93	(Provisional)
	Si Steel 125	0.0067 .0067	0.007	.005–0.008
	BCS 320	.19	.22	.21 – .22
Mo	BCS 322	.042	0.045	.040– .050
	BCS 325	.16	.16	.15 – .16

TABLE 2. *Analysis for selenium in coal (Se in ppm)*

Sample/Method	Evolution AA	Colorimetric	X ray
A	0.52	2.7	
B	.95	2.8	
C		3.6	5.7
D		2.7	2.5
NBS–SRM 1632 (2.7)	2.0	3.7, 5.7	
NBS–SRM 1633 (9.3)	3.2	9.7	

Another syndrome associated with the analytical chemist is that of replicate analysis, that is, a fairly large number of replicates are analyzed. An example of this can be seen in table 3, where out of 11 determinations, 10 agree to 3 significant figures. When this analyst was given a separate group of samples of about the same carbon content but with differences in sample weights and with the carbon values unknown to him, his repeatability as seen in table 4 was reduced by a factor of about 5.

TABLE 3. *Repetitive analysis of NBS-SRM 169 for carbon*

CO_2 Net Wt mg	C Wt %
2.2	0.0441
2.2	.0441
2.2	.0441
2.2	.0441
2.2	.0441
2.2	.0441
2.2	.0481
2.2	.0441
2.2	.0441
2.2	.0441
2.2	.0441
2.2	.0441
Average 0.0444.	
Estimated repeatability 0.0021. Sample size 1.3645 g.	

[a] Certified value 0.043, range 0.037 to 0.050.

TABLE 4. *Analysis of blind samples for carbon (in wt %)*

NBS-SRM 169	19 Cr–35 Ni[a]
0.0409	0.0460
.0437	.0390
.0464	.0499
.0464	.0465
.0437	
Average 0.0442	Average 0.0454
Repeatability 0.0062	Repeatability 0.012

[a] Carbon concentration 0.042 wt percent.

This leads to another blind side sometimes seen in the analyst, namely that of extending an analytical method beyond its useful limits. In the case of the gravimetric carbon analysis just cited, a 0.1 mg balance error was used. As in any gravimetric determination four weighings are involved – two for the blank and two for the sample. In table 5 we have calculated the error involved for carbon with balance errors of .05, 0.1, and 0.2 mg for different sample sizes. In comparing the precision of the analyst just mentioned with the values of table 5 it can be seen that his 1 g sample and its reproducibility reflects a balance error of 0.1 mg. Similar considerations of extending a method beyond its limits apply to almost any analytical method.

TABLE 5. *Evaluation of weighing error*

Sample size g	Balance errors					
	0.2 mg		0.1 mg		0.05 mg	
	σ	R	σ	R	σ	R
5	0.0022	0.0061	0.0011	0.0031	0.0005	0.0014
3	.0036	.0102	.0018	.0051	.0009	.0026
1	.0109	.0307	.0055	.0153	.0027	.0076

[a] Data in percent carbon.

Another peculiarity of the analytical chemist is his "smugness syndrome." For example, in one method involving the determination of iron in niobium with orthophenanthroline, fuming with sulfuric acid to remove the fluoride used to dissolve the sample is employed (fluoride interferes). But on a calibration curve prepared using fuming, a slope of 3.77×10^{-3} was obtained as compared to a slope of 3.98×10^{-3} obtained when the fluoride is complexed with tartaric and boric acids.

Another type of smugness is involved in a method for antimony in steel. The original method gave good results in our laboratory and in the hands of exceptionally competent analysts. However, in order to make the method work in routine labs, it was necessary to make seven simplification changes.

There also appears to be a tendency among analytical chemists to use statistics as an indicator of precision and accuracy rather than as a valid tool for the evaluation of a method. There is too much tendency to discard outlier results. This can be devasting in a round robin for evaluation of a method. If one uses as a tool the comparison of the within-lab error with the among-lab error, it can be quite useful. In one example where this was done, a factor of 3 to 10 difference in these error parameters was observed. A careful examination of the procedure showed some critical points in the method.

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DETECTION OF SYSTEMATIC ERRORS BY THE ANALYSIS OF PRECISION

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The extension of accuracy in trace element analysis to the ppb level cannot be based directly on the study of Standard Reference Materials because most of these are certified only at the ppm levels. Control of accuracy at such low levels may be achieved by an analysis of precision which serves to detect unknown sources of variation in replicate measurements. This approach was used to locate errors in connection with the determination of arsenic in human serum by neutron activation analysis. Successive applications of the analysis of precision led to the detection of two unknown sources of variation. In addition, the analysis of precision indicated that the current evaluation of small photopeak areas was not entirely unbiased. The apprehension of these errors led to changes in the methodology which in turn yielded both better precision and improved accuracy at the ppb level of concentration.

Keywords: Accuracy; analysis of precision; *a priori* error; *a priori* precision; *a posteriori* precision; chi-square test; precision; precision of analytical method; ultratrace level.

I. Introduction

The value of an analytical result is strongly dependent on its precision, while its validity is determined by its accuracy. Both attributes are indispensable for satisfactory reporting of analytical work.

Accuracy and systematic errors are accepted as characteristic of the analytical method, whereas precision and random errors are traditionally considered to be rather more characteristic of the results. With a sufficiently accurate specification of the analytical technique [1], precision also becomes truly characteristic of the analytical method. This view

becomes more and more appropriate as the automation of analytical work increases, and the interaction between analyst and equipment is reduced.

The precision of an analytical method has been defined [2] as a set of quantitative instructions for the estimation of the precision of a single analytical result.

The resultant estimate for a particular analytical result is referred to as its *a priori* precision [3], in contrast to the variability of actual results calculated *a posteriori*. In the analysis of precision [2] the *a priori* precision is compared with the actually observed variation between individual results for identical samples.

II. Precision of Analytical Methods

The problem of establishing the precision of an analytical method usually has to be solved in two steps. First a determination by whatever procedure deemed appropriate of the magnitude of all known or suspected random errors, as well as of their contribution to the overall precision. Then a verification of the estimated *a priori* precision for a series of duplicate, analytical results, by the analysis of precision with a chi-square test [2].

A. Classical Methods

For many classical analytical methods with incomplete theoretical bases, the overall random errors are determined—along with possible systematic errors—by repeated analysis of samples to which known quantities of determinand are added [4], or of Standard Reference Materials with certified concentrations of determinand. In this case the analysis of precision may be used to justify the assumption that the *a priori* precision thus determined, is also applicable to analytical results for real samples.

The range of such methods is limited to the range of concentrations and matrices for which experimental tests of precision and accuracy have been made [5]. The methods may therefore be suitable for trace analysis, but not for elements at the ultratrace level, *i.e.*, < 10 ppb, a concentration below which no Standard Reference Material has as yet been certified for any element.

B. Contemporary Methods

For analytical methods with a precisely known theoretical basis, the individual sources of variability are well defined, and their corresponding contribution to the overall precision may be determined by specific measurement, instead of implicitly by actual analysis of samples.

Here the analysis of precision serves to verify the algorithm for the estimation of overall precision from knowledge of individual variance components. This is again most properly done by repeated analysis of Standard Reference Materials at different concentrations and in different matrices.

If good agreement is achieved in all cases between actual and estimated variability of results, then the precision of the method is well established. Hence it can be expected to give accurate estimates of *a priori* precision also outside the range of materials and concentrations for which experimental tests were made.

These methods are therefore to be preferred for analysis when no suitable Standard Reference Material is available, or indeed for elemental determinations at the ultratrace level, where not even an acceptable blank can be found.

C. Activation Analysis

For nuclear activation analysis with carrier addition and radiochemical separation, the *a priori* precision is completely independent of the concentration of the determinand. The precision of a single, analytical result must, however, be supplemented with an estimate of the precision of the counting process based on Poisson statistics.

With the inclusion of this so-called counting statistics in the overall precision, the analysis of precision based on duplicate analysis of actual samples may again be performed with a chi-square test [2].

Particularly neutron activation analysis, with its enormous range, benefits from the constancy of *a priori* precision. This may be verified by the repeated analysis of Standard Reference Materials at the trace level or even higher concentrations, where the contribution from counting statistics is negligible. The adequacy of estimates of counting precision may be tested separately if necessary.

The extension of precision to the ultratrace level should therefore be a matter-of-course.

III. Control of Accuracy at Ultratrace Levels

Although perhaps a dozen different analytical techniques can claim sensitivities high enough to measure elemental concentrations at the ultratrace level (< 10 ppb), the accuracy of results obtained is often difficult to ascertain.

No Standard Reference Materials have so far been certified at this level, and calibration by the addition of determinand to a real sample may not be a very reliable technique at these low levels. In addition, the absence of truly representative blanks makes a distinction between matrix effects and small contents of determinand quite difficult.

The greatest problem is, however, the risk of contamination, to which all methods are susceptible, except activation analysis. Nuclear activation analysis with carrier addition and radiochemical separation is therefore the method of choice for ultratrace element analysis [6]. In addition, calibration may be based on comparator standards with several orders of magnitude higher concentration of determinand than the real samples, and the precision of the method may be checked by the analysis of Standard Reference Materials at similar, high concentrations.

This does not mean, however, that activation analysis automatically yields unbiased estimates of true concentrations of determinand in actual samples. A brief search of recent literature demonstrates that a method for the detection and elimination of erroneous results at the ultratrace level is still greatly needed.

A. Rejection of Results

Elimination of unusual results is practiced under various guises by virtually all analysts, perhaps more or less consciously:

a. Chauvenet's criterion permits an observation to be discarded if the probability of its occurrence is less than or equal to 1/2N, where N is the number of observations.

b. Dixon's test for outliers assumes a normal distribution, and permits the rejection of observations with a probability below some appropriate level chosen in advance.

c. Duplicate analysis which permits elimination of the highest of each pair.

While the rejection of results on purely statistical grounds is not permissible, the application of a. or b. may well draw attention to sources of error, which may then be eliminated. However, only the most perspicuous errors are detected, and the mere absence of outliers does not ensure adequate control of accuracy.

Duplicate analysis with rejection of the highest result does in fact reduce the influence of contamination, but doubles the analytical effort – perhaps needlessly.

B. Analysis of Precision

Results of duplicate analysis of M different materials are much more efficiently utilized in the analysis of precision, where the statistic T was shown to be approximated by a chi-square distribution,

$$T = \sum_{1}^{M} \frac{(Y_{1m} - Y_{2m})^2}{\hat{\sigma}_{1m}^2 + \hat{\sigma}_{2m}^2} \tag{1}$$

with M degrees of freedom [2].

Here Y_{1m} and Y_{2m} are the duplicate results for material m, and $\hat{\sigma}_{1m}$ and $\hat{\sigma}_{2m}$ their estimated standard deviation, calculated as the combined effect of *a priori* precision and counting statistics.

The detection of unknown sources of variation by this method usually results in the detection of unsuspected systematic errors. On the other hand, if the presence of unknown sources of variation is not indicated, the number of duplicates to be analysed may be reduced considerably.

According to Heraclitus it is not possible to step twice into the same river, which means that duplicate samples are not always easy to obtain. On the other hand, samples that cannot be duplicated are probably not worth analysing; the analytical effort is then not only halved, but wasted.

IV. Analysis of Human Serum for Arsenic

The measurement of arsenic concentrations by neutron activation analysis has been carried out in this laboratory by essentially the same method for a number of years [7], although additional steps have been introduced from time to time to increase the number of elements determined in the same sample.

Samples of serum are irradiated for 30 minutes in the pneumatic tube system of the Danish reactor DR 2 at a thermal neutron flux density of 7×10^{12} n · cm^{-2} · s^{-1}.

Multiple carrier addition with 1 mg of arsenic is followed by sample decomposition with sulphuric and nitric acid. After a cupferron scavenging, the arsenic is precipitated with thioacetamide, redissolved in ammonium sulphide and transferred to a counting vial.

After counting in a NaI(Tl) scintillation detector, the samples are re-irradiated for 10 minutes, and the chemical yield is measured in an ionization chamber [8].

A. PHASE 1970

The accuracy and precision of results were studied in the concentration range 2 to 200 ppb by the analysis of Bowen's Kale and by repeated analysis of 18 different actual samples covering this range [9].

Assuming that the standard deviation of a single result could be expressed as

$$\hat{\sigma}^2 = S_a^2 + s_r^2 + s_c^2 \qquad (2)$$

with S_c^2 representing the contribution from counting statistics, an attempt was made to resolve the remaining variance into contributions from absolute and relative, random errors. By successive approximations it was found that the observed variation between duplicate results could be expressed as Eq. (2) with an absolute, random error of 2 ppb and a relative error of 5 percent.

The precision of the method was not established at that time, but later studies showed that random differences in the neutron flux density to which sample and comparator were exposed in both irradiation and re-irradiation, gave rise to a relative, random error of 5 percent [10]. No source of variation in the analytical method was found to account for an absolute random error of 2 ppb, and its origin was ascribed to contamination under the primitive field conditions, under which these samples were taken.

B. PHASE 1971

The precision of the method was therefore assumed to contain only contributions from counting statistics together with the applicable coefficient of variation in irradiation conditions. This was tested by repeated analysis of two Standard Reference Materials, NBS-SRM 1571 Orchard Leaves and NBS-SRM 1577 Bovine Liver, for which results are given in table 1 along with their calculated standard deviation and the initials of the particular analyst.

With the results arranged in numerical order the names of the analysts are met in random order, indicating that the personal touch does not significantly influence the results. All results were therefore pooled, and the

TABLE 1. *Experimental test of accuracy and precision of the analysis for arsenic in standard reference materials*

Standard reference material	NBS–SRM 1571, Orchard leaves		NBS–SRM 1577, Bovine liver	
	Arsenic, ppm	Analyst	Arsenic, ppb	Analyst
	9.32 ± 0.33	TC	54.1 ± 2.9	LR
	9.42 ± .54	KH	55.7 ± 2.9	MH
	9.45 ± .54	KH	56.3 ± 3.0	MH
	9.68 ± .34	TC	56.6 ± 3.1	LR
	9.76 ± .35	TC	57.4 ± 3.0	MH
	9.79 ± .50	ED	60.1 ± 3.3	LR
	9.86 ± .35	TC		
	10.09 ± .42	MH		
Number of results	8		6	
Weighted mean	9.68 ± 0.14		56.6 ± 1.2[a]	
Degrees of freedom	7			
T	2.83			
$P(\chi^2 > T)$	0.90			

[a] This result has been published in Risø–M–1633 (1973).

analysis of precision did not indicate the presence of unknown sources of variation. The weighted mean values of arsenic concentrations in the two Standard Reference Materials were in good agreement with results reported from other laboratories.

The precision as well as the accuracy of the analytical method seemed now under full control, and a series of samples of human serum taken under good, standard hospital conditions was analysed in duplicate.

The results are presented in table 2 along with their calculated standard deviations. The analysis of precision clearly indicates the presence of additional sources of variability, not accounted for in the precision of the analytical method.

The analysis of variance in table 3, last column, estimates a residual random error of 0.84 ppb and indicates that the observed differences between samples may be real; this is a slight improvement in comparison with earlier results for Danish samples [9].

Sampling conditions were tested by assuming the same absolute random error as was found from previous results. Table 3 shows that the variability of duplicate samples is significantly reduced, which means that—as was to be expected—sampling conditions have been considerably improved.

TABLE 2. *Uncorrected duplicate results for arsenic in human serum taken under good, standard hospital conditions*

Sample	Results	
	ng/ml	ng/ml
A	1.29 ± 0.12	1.37 ± 0.12
B	3.33 ± .25	2.71 ± .16
C	3.52 ± .25	2.61 ± .16
D	2.33 ± .15	1.44 ± .12
E	0.65 ± .08	.083 ± .12
F	.53 ± .11	1.98 ± .17
G	1.69 ± .16	0.46 ± .09
H	2.71 ± .19	2.36 ± .15
I	1.52 ± .13	1.79 ± .15
J	5.74 + .36	2.58 ± .17
K	2.79 ± .34	2.34 ± .16
Number of samples	11	11
Degrees of freedom	11	
T	201.6	
$P(\chi^2 > T)$	$<10^{-9}$	

TABLE 3. *Comparison of field sampling conditions with good, standard hospital conditions*

Sampling conditions:	field	standard hospital	
Standard error, ppb:	$s_a = 2$	$s_a = 2$	$s_a = 0.84$
Variation between:	duplicates	duplicates	samples
Number of Materials	18	11	1
Number of Results	39	22	11
Degrees of freedom		11	10
Test parameter		$T = 2.00$	$F = 2.48$[a]
Probability		≤ 0.0015	<0.08

[a] Estimated population variance relative to the *a posteriori* precision of a single determination with 11 degrees of freedom.

Other sources of error were therefore sought, and it was found that in some cases interference from Br-82 was not negligible. Hence, the calculation of results was changed to correct for this influence by evaluation of the 777 keV peak in the sample spectrum [11] and the subsequent elimination of the contribution to the 559 keV peak by means of a Br-82 reference spectrum.

Results corrected for bromine interference are presented in table 4, but it is obvious that all sources of variability have not yet been brought under control.

The analysis of variance gives a residual random error of 0.27 ppb, and the precision of a single result is now sufficient to detect with high significance actual differences between samples.

TABLE 4. *Duplicate results for arsenic in human serum of the 1971 series*

Sample No.	Results[a]	
	ng/ml	ng/ml
270755	2.06 ± 0.17	2.11 ± 0.35
081250	0.31 ± .18	1.11 ± .17
180149	1.40 ± .15	0.70 ± .14
150248	0.36 ± .25	.24 ± .14
190745	1.34 ± .14	1.35 ± .16
230645	1.54 ± .20	2.22 ± .21
230641	0.22 ± .18	0.65 ± .10
170241	1.02 ± .15	1.21 ± .14
040940	2.00 ± .21	2.65 ± .30
260226	2.74 ± .40	2.34 ± .20
120923	2.49 ± .17	2.76 ± .48
110212	0.27 ± .13	0.29 ± .22
Number of samples	12	12
Degrees of freedom	12	
T	37.23	
$P(\chi^2 > T)$	0.00020	

[a] Results published in Risø Report No. 271 (1973).

C. PHASE 1972

Simultaneous determinations of manganese in the same samples of human serum showed that the good, standard hospital conditions were not good enough to avoid significant contamination with manganese [2]. A set of samples taken later under the most stringent conditions was therefore analysed for both arsenic and manganese, and a highly significant improvement was demonstrated for manganese [2].

The corresponding results for arsenic are presented in the left part of table 5, and it can be seen that the reduction of variation between duplicate samples has now reached the point where it is significantly smaller than prescribed by the precision of the analytical method.

TABLE 5. *Duplicate results for arsenic in human serum of the 1972 series*

Sample	Results, corrected by previous method		Results,[a] corrected by alternative method	
	ng/ml	ng/ml	ng/ml	ng/ml
LM 26	0.67 ± 0.12	0.62 ± 0.17	0.79 ± 0.11	0.66 ± 0.11
LKB 49	.91 ± .14	.80 ± .11	.91 ± .13	.84 ± .11
TMN 49	1.70 ± .12	1.81 ± .13	1.77 ± .12	1.90 ± .14
AMH 56	0.34 ± .13	0.36 ± .07	0.49 ± .09	0.44 ± .07
VJL 63	1.62 ± .12	1.48 ± .12	1.75 ± .13	1.57 ± .11
BS 20	0.70 ± .11	0.76 ± .09	0.81 ± .10	0.76 ± .09
PC 47	1.25 ± .15	1.16 ± .21	1.54 ± .16	1.48 ± .16
HBR 50	0.67 ± .20	0.36 ± .39	0.83 ± .15	0.98 ± .22
AS 51	.61 ± .19	.58 ± .09	.97 ± .16	.65 ± .08
CAC 66	1.09 ± .15	1.08 ± .11	1.11 ± .14	.75 ± .14
Number of samples	10	10	10	10
Degrees of freedom	10		10	
T	2.35		9.71	
$P(\chi^2 \leq T)$	0.007		0.53	

[a] Results published in Risø Report No. 271 (1973).

Seeing that the analysis of precision for uncorrected arsenic results indicates the presence of excess variation, just as was to be expected, the procedure for correcting for Br-82 interference was carefully scrutinized.

It was found that the peak boundary selection method currently used [12] introduces a nonrandom component into the evaluation of very small photopeak areas, such as the 777 keV Br-82 peaks in the present spectra. In this respect a recent method for peak boundary selection [13] seemed preferable, and the results were recalculated on that basis.

The revised results are presented in the right part of table 5, and it will be noted that excellent agreement has now been achieved between the precision of the analytical method and of the analytical results.

V. Discussion

The analysis of precision of duplicate results of arsenic determinations in human serum has brought about a deeper understanding of the sources of variation that may influence the results. The process has reached the stage where all significant sources of variation have been brought under control, and the precision of the analytical method accurately predicts the precision of an individual result.

Two unexpected sources of variability were identified in the process: sampling conditions and bromine interference. Both of them influence the results, but in different ways; table 6 shows the summary of results at various stages of the process.

Sampling conditions influence the *a posteriori* precision, which leads to their detection, but they also significantly influence the estimated standard deviation of the population, but barely so the mean. Bromine interference changes the mean value, but has practically no effect on the population standard deviation.

It is characteristic that the analysis of precision is a much more powerful method than a simple comparison of means; such detection of the difference between the previous and the alternative method of correction for bromine interference at the same level of significance would require an infinite number of determinations instead of 10 duplicates.

However, it must be remembered that statistical inference based on repeated calculations on the same original data set is not made at a well-defined level of significance. It is therefore pertinent to refer to the subsequent application of the revised correction method to routine analysis under controlled sampling conditions. Thus, in 1973 a new set of serum samples was taken in duplicate under almost perfect conditions with the

purpose of studying diurnal variations in arsenic concentrations. The results were corrected for bromine interference by the alternative procedure, and the analysis of precision confirmed that all significant sources of variation are under control.

TABLE 6. *Arsenic in serum from normal Danish subjects*

Sampling conditions	Correction method	Number of subjects	*a posteriori* error $\bar{\sigma}$ ng/ml	Mean and s.e.m. ng/ml	Population s.d. $\hat{\sigma}_p$ ng/ml
1970	none	16	2.4	2.4 ± 0.6	1.9
1971	none	11	0.85	2.1 ± .3	0.85
	usual	12	.32	1.4 ± .3	.85
1972	usual	10	.12	0.94 ± .14	.44
	alternative	11	.11	1.07 ± .14	.44

VI. Conclusion

The control of accuracy and precision at the ultratrace level of concentrations cannot be based on Standard Reference Materials certified at trace levels.

The analysis of samples in duplicate is a well tested way of lending greater credibility to uncertain results. In combination with an analytical method of established precision and accuracy, such as our present neutron activation analysis method for determination of arsenic, unexpected sources of variation may be detected by the analysis of precision.

The identification of these unknown sources of variation is no simple task, and their elimination may be even more difficult. However, it is usually possible by changing experimental conditions to confirm or invalidate, by the analysis of precision, the assumption that a particular factor is crucial. Only then may efforts be made to circumvent, reduce, or randomize their effect.

Among unexpected sources of variability, sample heterogeneity is particularly troublesome, but without analytical methods of known and established precision, reports on insufficient homogeneity of well-characterized materials should be taken with some reservation.

The ultimate goal of analytical pursuit is to provide meaningful results for actual samples and, like experiments that cannot be repeated, results that cannot be duplicated are not meaningful. Results that are not

meaningful are often misleading, and it is the responsibility of the analytical chemist to identify such results promptly and not to release them from his laboratory.

The analysis of precision is believed to be an important tool in the performance of this task.

VII. References

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DETECTION OF SYSTEMATIC ERROR IN ROUTINE TRACE ANALYSIS

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The Youden technique has been modified in order to monitor systematic error between runs. When a laboratory appears to have systematic error problems, it is important to know whether or not this is typical of the routine operation. The procedure to be described examines the sum and difference of the results obtained on each of two control samples. Simple statistics permit long-term analysis of the results of such control analyses to separate within-run, or random effects, from the systematic effects occurring between runs. This simple sum/difference approach has been applied in a wide variety of water and wastewater analytical procedures. The blank determination in trace analysis has been implicated, in many cases, as the most significant source of systematic error.

Keywords: Analytical performance; between-run; calibration; precision; quality control; systematic error; Youden plot.

I. Introduction

The need for control of systematic error within the analytical laboratory is well evidenced by the number of interlaboratory comparisons which have shown by means of Youden's technique [1] that it is the most significant source of deviation between laboratories. Many analysts, when queried directly, are unable to comment on the extent or even existence of systematic error in their laboratory. Since intercomparisons are carried out infrequently, insufficient data is available to determine whether an observed error in the result reported by a given laboratory applies only to the run in which the result was obtained or whether it is typical of that laboratory's routine work.

Systematic error can be separated from random deviation by the use of two similar control samples or standards. This technique was applied by Youden to interlaboratory comparison in which several laboratories analyzed each of two control samples. We have applied the same technique to intralaboratory work by analyzing the two control samples once per analytical run over a period of 15 or more runs, and have been able to detect and correct significant systematic error as it occurs.

II. Theory

The object in controlling analytical performance is to ensure immediate detection of any deterioration in instrumentation, technique and/or standardization. Most observed deviations in analytical results can be considered insignificant when measured against routine performance. Unusually large deviations should then occur only rarely as long as the system produces results distributed normally about the mean value. The standard deviation, s, of the available data is commonly used as the measure of performance and is used to infer that deviations larger than $2s$ should occur less than 5 percent of the time. This inference is true if the data is "normally" distributed as is usually the case when it has been obtained under one set of conditions. If the data is provided from different analytical runs, errors made in determining either the blank or calibration slope will result in a broader non-normal distribution of data. However, there is usually insufficient data to demonstrate this, unless two samples run simultaneously show the same error.

If the theoretical relationship between concentration C and instrument response R can be written

$$C = C_0 + kR, \tag{1}$$

where C_o = concentration required for nil response and k = linear slope relating C and R. Then the estimated concentration $\hat{C}$ for an observed response R will be given by the equation,

$$\hat{C} = (C_0 + e_0) + kR(1 + e_k) + c_r, \tag{2}$$

where e_o = systematic error in the blank, e_k = systematic error in the slope and c_r = random deviation. Thus the total deviation can be described by the formula

$$\begin{aligned} c &= (\hat{C}\text{-}C) = c_r + e \\ &= c_r + e_o + e_k C \end{aligned} \tag{3}$$

At levels of analysis close to C_o (*e.g.*, trace analysis) the most signifi-

cant source of the systematic error e is in estimating the blank value C_a and e is independent of concentration. At higher levels e becomes increasingly proportional to concentration due to the term e_k, but since the random component c_r is not necessarily proportional to concentration, the observed total deviation (c_r+e) need not increase linearly with concentration. (For these reasons the common practice of reporting relative standard deviations, rather than absolute standard deviations, can be misleading.)

The separation of systematic and random deviations is achieved in the following manner. When several analyses are performed on each of two control samples, A and B, the deviations a and b, observed for a given pair of results about their mean values $\overline{A}$ and $\overline{B}$, can be separated into their random components a_r and b_r and their systematic error component e. Provided that A and B are close in concentration, the error component e will be common to both A and B. Thus

$$a = (A - \overline{A}) = a_r + e \tag{4a}$$

$$b = (B - \overline{B}) = b_r + e \tag{4b}$$

$$(a + b) = (T - \overline{T}) = a_r + b_r + 2e \tag{4c}$$

$$(a - b) = (D - \overline{D}) = a_r - b_r \tag{4d}$$

where $T=(A+B)$ and $D=(A - B)$. Note that since the values a_r and b_r are each normally distributed and randomly associated (*i.e.*, some pairs of a_r, b_r are at least partially self cancelling), not all of the observed deviation common to A and B is due to systematic error.

By means of the usual formula for calculating variance namely:

$$s_A^2 = \frac{\Sigma(A - \overline{A})^2}{(n - 1)} = \frac{\Sigma a^2}{(n - 1)} \tag{5}$$

it can be shown that

$$s_A^2 = \frac{1}{(n - 1)} \cdot \Sigma(a_r^2 + e^2 + 2ea_r) \tag{6a}$$

$$s_B^2 = \frac{1}{(n - 1)} \cdot \Sigma(b_r^2 + e^2 + 2eb_r) \tag{6b}$$

$$s_T^2 = \frac{1}{(n - 1)} \cdot \Sigma[a_r^2 + b_r^2 + 4e^2 + 4e(a_r + b_r) + 2a_rb_r] \tag{6c}$$

$$s_D^2 = \frac{1}{(n - 1)} \cdot \Sigma(a_r^2 + b_r^2 - 2a_rb_r) \tag{6d}$$

it can be shown that

a) within-run variance $s_r^2 = \dfrac{\Sigma(a_r^2 + b_r^2)}{2(n-1)}$ (7)

$= \frac{1}{2}(s_D^2) + \Sigma a_r b_r/(n-1)$

b) systematic variance $s_e^2 = \dfrac{\Sigma e^2}{(n-1)}$

$= \frac{1}{2}(s_T^2 - s_A^2 - s_B^2) - \dfrac{\Sigma a_r b_r}{(n-1)}$ (8)

c) total variance $s^2 = (s_r^2 + s_e^2)$

$= \frac{1}{2}(s_T^2 + s_D^2 - s_A^2 - s_B^2)$ (9)

$= \frac{1}{2}(s_A^2 + s_B^2) - \dfrac{\Sigma e(a_r + b_r)}{(n-1)}$ (10)

$= \frac{1}{4}(s_T^2 + s_D^2) - \dfrac{\Sigma e(a_r + b_r)}{(n-1)}$ (11)

Provided that there is sufficient data so that a_r, b_r and e can exert their random association, the cross-product terms will be negligible. The actual value of the term in equation (10) can be calculated by comparison to equation (9), but the value of the cross-product term in equation (7) and (8) cannot be determined so that for insufficient data the within-run variance will be over-estimated by an indeterminate amount. Note that equation (9) is to be preferred when calculating total variance using data from different analytical runs.

III. Application

The primary purpose of the two-sample control procedure is to flag those analytical runs which may result in significant systematic error. The control technique involves recording the values of A and B obtained for each of the control samples, calculating their sum T and difference D, and plotting these values in time sequence. If no systematic error is present then s_T will equal s_D but in general this is not found to be true. Warning limits of $\pm 2s_D$ and control limits of $\pm 3s_D$ can be used to define outliers. Outliers in the T plot confirm the presence of significant systematic error whereas outliers in the D plot indicate a significant level of random deviation. In practice control limits are based on a previous set of control data. Care must be taken not to set control limits tighter than those the system is routinely capable of maintaining.

It is also useful to maintain a sequence plot of *B vs A* (Youden plot) in order to clarify the long-term performance of the analytical system. In the absence of systematic error, the lines joining sequential *A*, *B* points will be randomly oriented. As systematic error increases these lines will tend to be oriented from upper right to lower left quadrants and the points will change from a circular into an elliptical distribution along this axis, provided only that variance of *A* and *B* are approximately equal.

Any reasonably stable material can be used to prepare the two control samples. Sufficient material or sample is required to provide for at least 20 to 30 analyses over a period of 1 to 2 months. As few as 10 sets of analyses have proved useful however. Both artificial and natural samples have been used with success, depending upon whether control of instrumentation only, or control of the entire analytical process was required.

The choice of concentrations to be used for *A* and *B* control samples or standards depends upon the relative significance of e_o *versus* e_k type systematic error. Two controls at the upper end of the operating range will control slope error and will be relatively unaffected by blank error, whereas two low-level controls will monitor systematic error in the blank. Since most trace analysis is performed at the low end of the operating range, the control of e_o error is critical if small changes in the environmental levels are to be detected. Notice that the actual strength of the check samples need not be known in order to apply this control technique so that stabilized field samples can be used to provide control at the actual level of interest.

Figure 1 demonstrates the use of this technique to document the effect of systematic error in the taring of 50 ml porcelain dishes (weighing about 40 g), for use in the gravimetric determination of total dissolved solids. Each day two tared dishes arbitrarily assigned A and B were filled with 50.0 ml of distilled water and dried overnight at 103 °C along with the routine samples being analyzed. After cooling in a desiccator the next morning the dishes were reweighed and the "blank" values were calculated. Although most results fell in the range ± 10 mg/l some values were as high as 28 mg/l. Examination of the *T* and *D* and *B vs A* plots points up the significance of systematic error which results in a between-run precision of 10 mg/l (2 1/2 times the within-run precision) as shown in tables 1 and 2. Because of this, the use of specific conductance has been adapted, for natural water samples containing less than 400 microsiemens/cm, as a more precise measure of dissolved solids in this type of sample.

Figure 2 documents systematic error in the analysis of sulphur in vegetative material by x-ray fluorescence. In this case the *A* and *B*

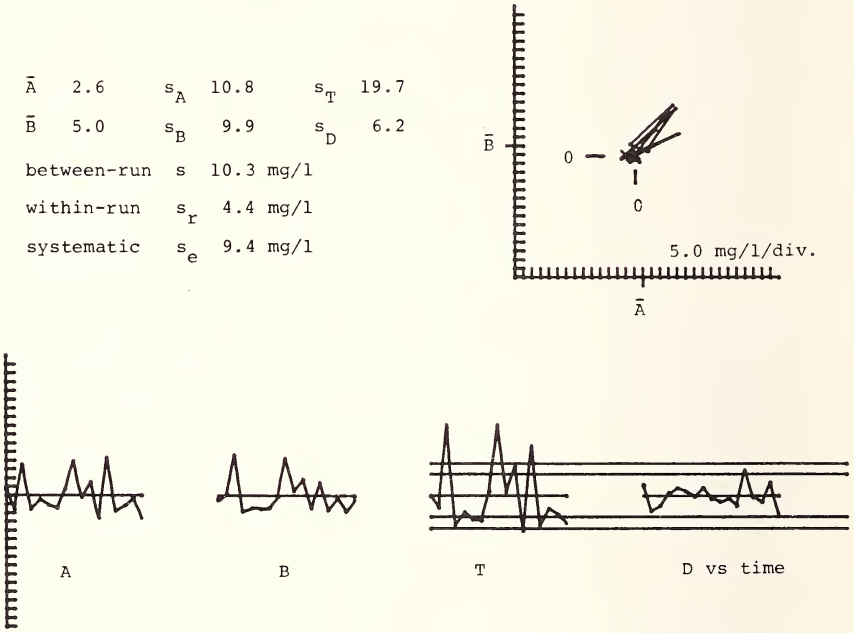

Figure 1. 50 ml distilled water blanks, dried overnight at 103 °C in pretared 50 ml porcelain dishes arbitrarily labelled *A* and *B*, to demonstrate use of *T*, *D* and Youden control charts.

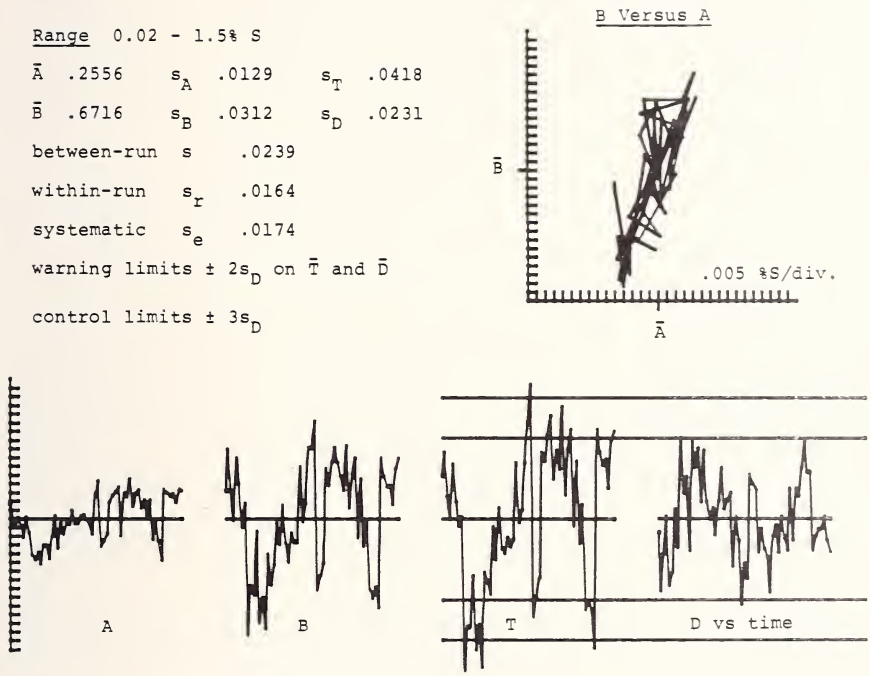

Figure 2. Percent sulphur in vegetation by XRF analysis, over a period of 6 months. Equipment overhauled at time indicated by arrow. Note variation in *A* not equal to variation of *B*.

TABLE 1. *Summary of control data used to plot the figures*

	$\bar{A}$	$\bar{B}$	s_A	s_B	s_T	s_D
mg/l solids (fig. 1)	2.6	5.0	10.8	9.9	19.7	6.2
Percent sulphur						
raw data (fig. 2)	0.256	0.672	0.013	0.031	0.042	0.023
before (fig. 3)	.249	.220	.0092	.0094	.0167	.0083
after (fig. 4)	.263	.227	.0118	.0102	.0218	.0044

TABLE 2. *Calculated level of random versus systematic standard deviation*

	Within-run s_r	Systematic s_e	Between-run s
mg/l solids (fig. 1)	4.4	9.4	10.3
Percent sulphur			
raw data (fig. 2)	analysis not valid	$s_A \neq s_B$	see text
before (fig. 3)	0.0059	0.0073	0.0093
after (fig. 4)	.0031	.0106	.0110

samples were selected to be at about 15 and 45 percent of full scale. Pellets were prepared from each sample and were reanalyzed once per run until they started to disintegrate, at which point fresh pellets were prepared from the same two samples. The data was accumulated over a period of 6 months. It is obvious from figure 2 that systematic error is occurring but since the standard deviation of the data is proportional to concentration, direct analysis of the raw A and B data is invalid. (This is not a common observation for most of the procedures run in our laboratories.)

In order to investigate this system the B data was therefore arbitrarily divided by three, and in addition the data was split into two portions covering periods before and after a major instrument overhaul. The results of this treatment are shown in figures 3 and 4 and tabulated in tables 1 and 2. Examination of the B *vs* A plots demonstrates that the overhaul was successful in reducing sources of random deviation but that systematic error was not eliminated.

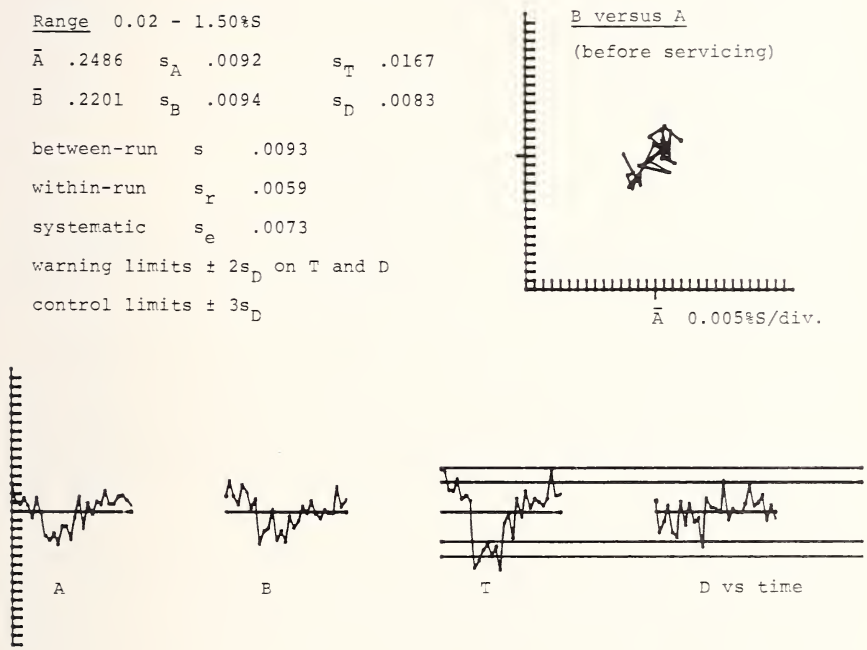

Figure 3. Replot of XRF data prior to overhaul of the instrument (*B* values are 1/3 those shown in fig. 2). Youden plot shows random deviation.

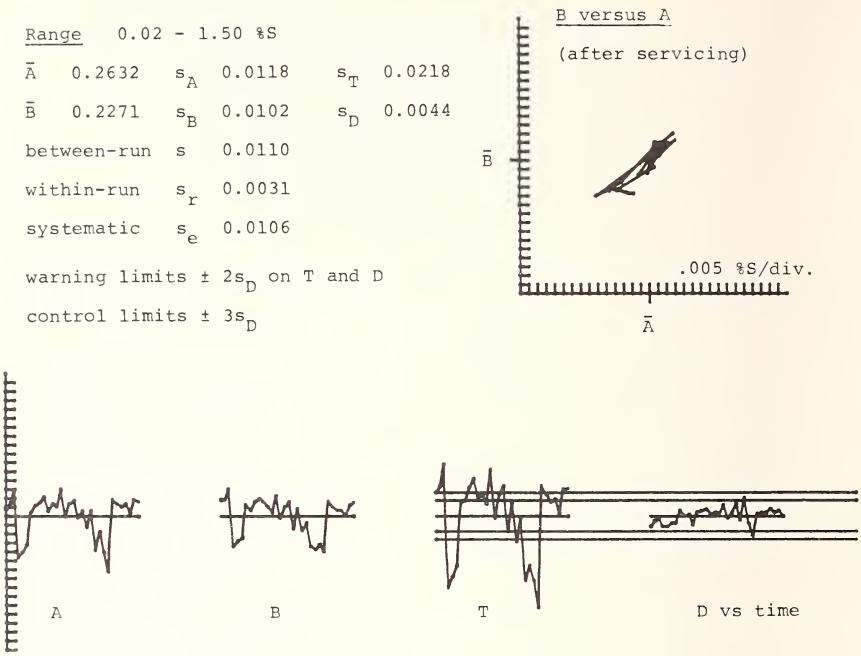

Figure 4. XRF data after equipment overhaul (*B* values are 1/3 those shown in fig. 2). Random deviation has been reduced leaving gross systematic deviation. Note large number of outliers in the *T* plot.

When consideration is taken of the field sampling variability and other effects, the between-run precision of ±4.4 percent in the range 0.25 to 0.75 percent is more than adequate. However, this evaluation suggests that the precision could be improved to ± 1.5 percent if necessary by control of systematic error.

In this XRF study the *A* and *B* data was accumulated only, but it is obvious that, had the need existed, the *A* and *B* samples could have been used to control the instrumental calibration. It is this potential for control *via* daily analysis of the *T* and *D* plots that makes this technique most valuable.

IV. Acknowledgement

The XRF data provided by R. Harris of the Ontario Ministry of the Environment Air Quality Laboratory was greatly appreciated.

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THE APPLICATION OF CLUSTER ANALYSIS TO TRACE ELEMENTAL CONCENTRATIONS IN GEOLOGICAL AND BIOLOGICAL MATRICES

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In the past it has been difficult to accurately interpret the information provided by trace elemental analysis, especially when concerned with biological and geological studies where there may be many variables involved other than simple elemental distributions. Utilizing a combination of hierarchical cluster analysis, factor analysis, and canonical correlation, data is presented showing how these three statistical methods may be combined to study the relationships of trace elemental concentrations in geological and biological matrices. Clusters of elements are found which are not readily apparent from examination of either raw data or simple correlation matrices.

Keywords: Air particulates; blood; chemistry; computer graphics; geochemistry; medicine; pattern recognition; trace elements.

I. Introduction

The field of trace analysis is intimately concerned with finding, measuring, and interpreting trace levels of substances in order to understand or predict the relationships that occur in natural phenomena. A tremendous amount of time and effort is expended by scientists in this endeavor. Pattern recognition, of which cluster analysis, learning machines, factor analysis and canonical correlations are various techniques, will provide new and different insights into the meaning of experimental data. Results of trace analysis treated by the new pictorial methods presented in this paper will greatly increase the scientist's understanding of the interrelationships in his data.

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Pattern recognition [1-6] is a developing branch of artificial intelligence. The technique has been applied to many diverse scientific areas such as computer recognition of handwriting, speech analysis, cell counts in clinical laboratories, photographic processing, and fingerprint identification. In chemistry, pattern recognition has been applied to gamma-ray [7], infrared [8], nuclear magnetic resonance [9,10], and mass spectra interpretation [11], classification of archeological artifacts [12], and structure-activity relationships of drugs [13]. The authors have applied the various techniques of pattern recognition and statistical analysis to trace element concentrations in atmospheric particulate samples from the South Pole [14], surface and sub-surface sea water samples [15], geological sediments from the East Rudolf lake region in Kenya, Africa [16], and in human blood [17]. The correlations and relationships are presented in the new pictorial methods developed by the authors.

II. Method

The computations presented in this paper were performed on an IBM 370/155 computer of the University of Rhode Island's computer laboratory. The graphical representations presented were made on a Broomall 2000 plotter in the same facility. The computer programs used in this work were developed from various sources. The IBM SYSTEM 360 Scientific Subroutine Package, HGROUP (a clustering program authored by D. J. Veldman of the University of Texas) and seven computer programs written by the authors complete the computer programming package.

All of the pattern recognition programs were designed to accept either of two forms of new data input; from cards or from general disk storage data banks. Both columns and rows of the data matrix may be given characteristic names for later ease of identification. Two methods of pattern recognition were used. The first method called FACTOR, is a form of factor analysis in which the linear combinations of a set of correlated data are developed. Various methods of correlating the data, the preliminary step in this procedure, can be used, including product moment correlation coefficient, distance coefficient, cosine coefficient, and Horner coefficient. FACTOR takes these coefficients and determines a set of eigenvalues for the linear combinations, the cumulative percentage of these eigenvalues, the eigenvectors, and finally the loaded factor matrix for each of the eigenvalues. These values in the loaded factor matrix can then be graphically represented with the new routines developed by the authors.

The other method, HGROUP, clusters the variables based on some predetermined definition of similarity. Those most similar will group first, and an error calculation is made to determine the similarity of the clustered variables. A dendrogram can then be made of the series of clusters to once again give a graphical representation of the data.

III. Results and Discussion

Pattern recognition is a mathematical technique that can be applied to any type of experimental data. The clustering programs used in this work can pretreat the raw data with various statistical techniques which develop a new data matrix containing all the information in the original data. A typical method for cluster analysis is to normalize the various rows or columns of the data set using the standard deviation and the mean.

Any statistical method that develops a correlation matrix from the raw data can be used as a preprocessing technique for factor analysis. The resulting output of eigenvectors is used by the new three-dimensional routine for representation of the experimental data. Types of correlations are listed in the Methods section. The correlation method used in the following examples is the product moment correlation coefficient:

$$r_{x,y} = \frac{\Sigma(x_i - \bar{x})(y_i - \bar{y})}{[\Sigma(x_i - \bar{x})^2]^{1/2}[\Sigma(y_i - \bar{y})^2]^{1/2}}$$

between each x and y which are the experimental variables and $\bar{x}$ and $\bar{y}$ which are the means. The authors have applied all the various techniques discussed in this paper to experimental data from trace element values in atmospheric particulates, clinical variables in cancer patients, trace element levels in sediments from the East Rudolf lake area in Kenya, Africa, and the elemental composition of particulates in sea-surface and sub-surface sea water samples.

To completely illustrate all the various techniques that can be used for this type of data analysis is outside the scope of this paper. These pattern recognition techniques do not replace conventional data analysis methods already in use by scientists; rather, they supplement and extend the investigator's understanding of his experimental data. Three examples have been chosen to demonstrate the advantages of these techniques.

The first six figures depict the typical graphical output of the programs. The experimental data presented in these figures is from the trace element

concentrations in atmospheric particulates collected at the South Pole courtesy of Zoller *et al.* [14]. Figures 1, 2, and 3 are normal 2-dimensional plots of the interrelationships for the first three eigenvalues of the loaded factor matrix. Figures 4 and 5 present the new pictorial method of representing three eigenvalues on one graph. The peaks at the rear three corners (figs. 4 and 5) represent 0.0 on a -1.0 to $+1.0$ scale, as do the other two axes. The advantages of figure 4 and 5 over the first three are readily apparent when one realizes that all of the information of figures 1, 2 and 3 is contained in figure 4 or 5.

It is possible to rotate the 3-dimensional representation in any of the three axes with respect to the viewer. The best view is data dependent because cluster representations can mask each other. Interpretation of the interrelationships among the 15 elements in the samples becomes much clearer with this technique. The first three eigenvectors represent about 70 percent of all the variation in this data set, whereas each of the first three vectors alone contained only about 25 percent.

The elements Al, Sc, Co, and Fe exhibit similar patterns in their variation from sample to sample. The tentative hypothesis one could develop from this figure 4 is that Al, Sc, Co, and Fe have the same source and/or undergo similar physical-chemical processes in the atmosphere. Similar hypotheses can be drawn for the pairs: Ce and Zn; Na and Mg; Se and Br. The variation of the elements in the sample (loaded factor matrix) is quite different from each other, and suggests that each grouping has experimental or natural variables that are different and/or independent from each other. Relating these correlations to actual physical-chemical processes in the atmosphere is not difficult.

The correlations suggest that each grouping has a unique source and/or undergoes markedly different physical-chemical processes in their transport by the atmosphere.

Figure 6 is a dendrogram of the experimental data. This figure is the typical result of a pattern recognition program using hierarchical clustering. This technique asks if a set of elemental variables can be associated into groups based upon their similarity within the samples. The program groups variables one at a time at consecutively larger and larger relative error until all variables are divided among two groups. The first groupings (fig. 6) are Na-Mg, Al-Sc-Co-Fe, Zn-Ce-V and Se-Sb-Br. These groupings basically agree with the factor analysis presented in figures 1 through 5. The final three groups contain Na-Mg, Al-Sc-Co-Fe-Mn-Eu-Th and Zn-Ce-V-Se-Sb-Br, which could be respectively associated with sea-salt from the oceans, normal crustal weathering and anthropogenic or high-temperature processes.

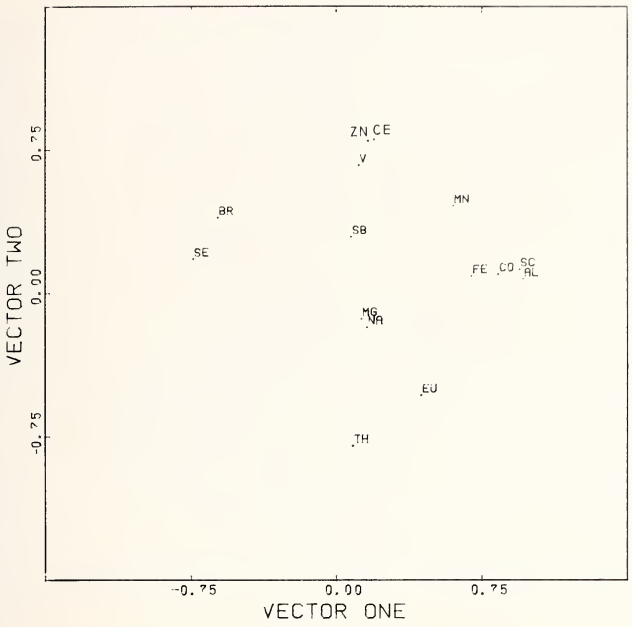

Figure 1. Loaded-factor values of South Pole air samples for principal-axis factors one and two.

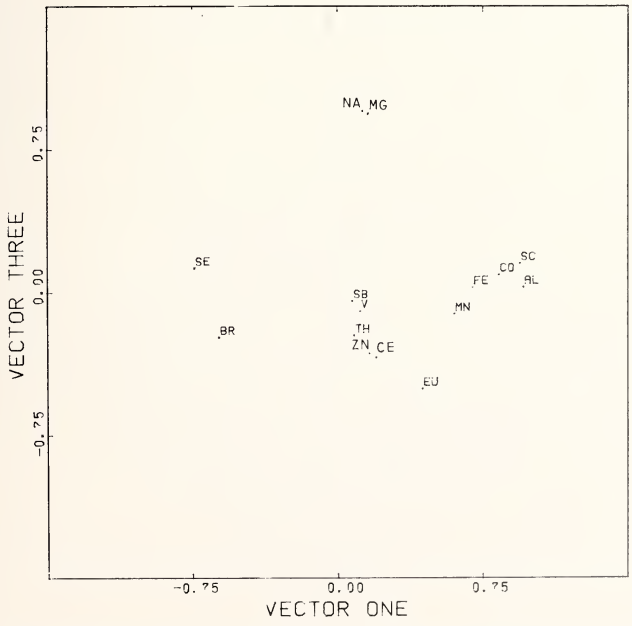

Figure 2. Loaded-factor values of South Pole air samples for principal-axis factors one and three.

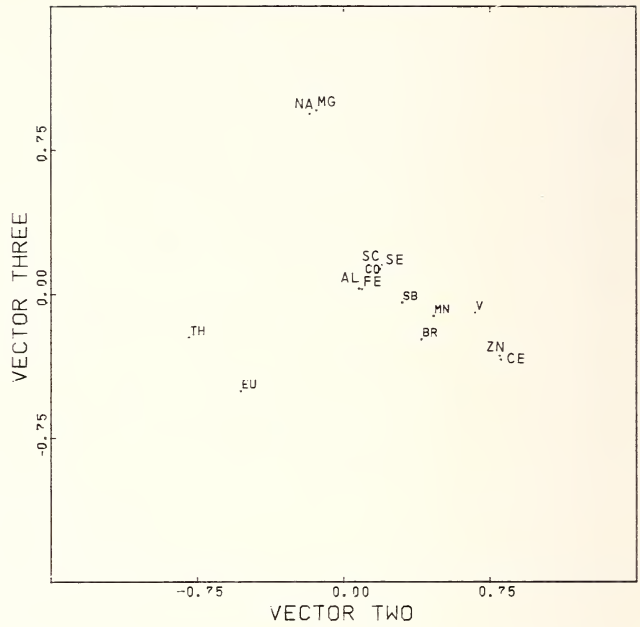

Figure 3. Loaded-factor values of South Pole air samples for principal-axis factors two and three.

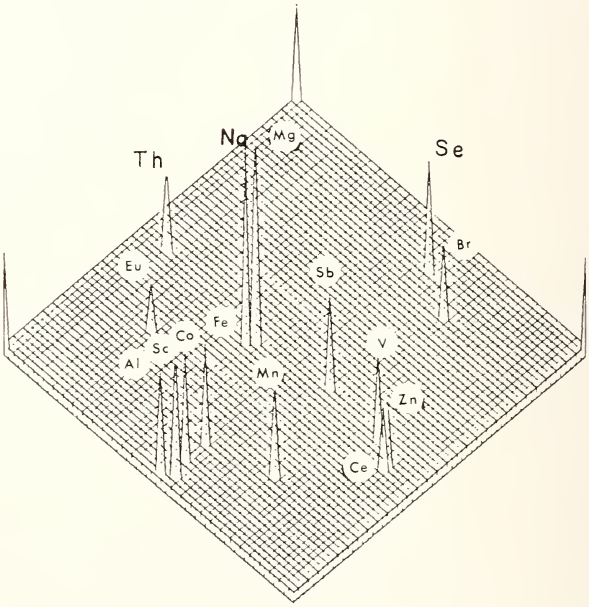

Figure 4. The three-dimensional representation of South Pole air samples for the loaded-factor values of the first three principal-axis factors shown at an angle of 60° by 45°.

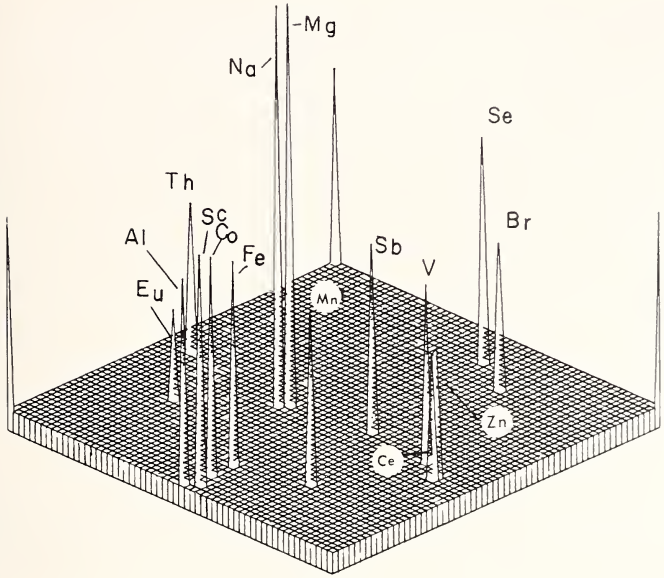

Figure 5. The same representation of figure 4 at an angle of 27° by 45°.

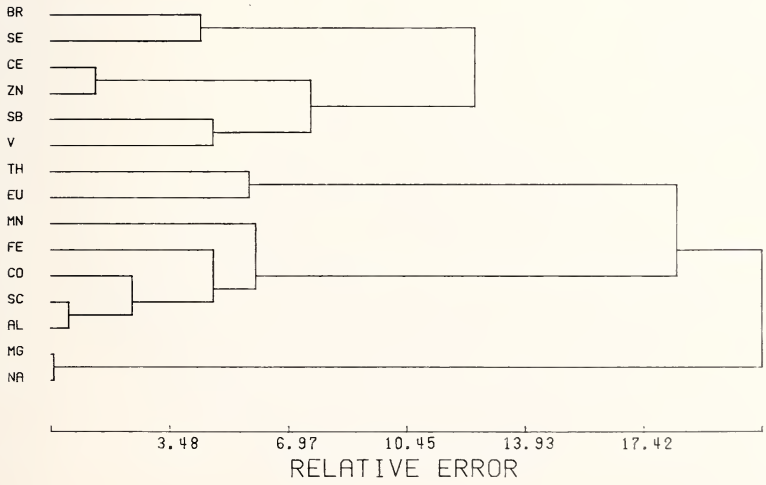

Figure 6. The dendrogram of South Pole air samples with respect to clustering of the 15 elements *versus* the relative error associated with the clusters.

An illustration of the same techniques applied to biological variables in the blood of cancer patients is presented in figures 7, 8, and 9. Figure 7 indicates the relationships between the values of hemoglobin, sex, age, weight, white blood cell counts, platelet counts, sodium values and chloride levels. The associations or similarities are not as well defined in this example as in the first one. Na and Cl levels indicate similar patterns, as do platelet and white blood cell counts. The other four variables indicate little, if any, interdependency. Figure 8 is the dendrogram depicting the relationships given by the clustering program. The groupings are Na-Cl-weight, sex-hemoglobin-age, and white blood cell count with platelet counts. These are exactly the relationships one would expect from these variables. The process can be reversed and the patients (fig. 9) can be studied with respect to their variables (fig. 9). The patients are labeled by sample number on this plot.

The third example is a collection of trace element values in sea-surface and sub-surface (20 cm) sea water samples collected under the sponsorship of NSF-IDOE program. Eight elements (Cr, Cu, Pb, Fe, Cd, V, Mn, and Al) have been determined in 23 pairs of surface and sub-surface sea

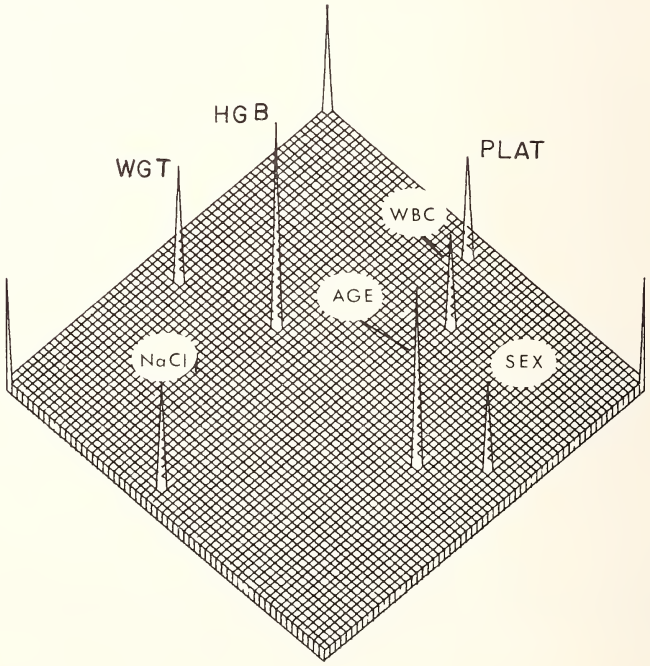

Figure 7. The three-dimensional representation of eight biological parameters using the first three loaded factors.

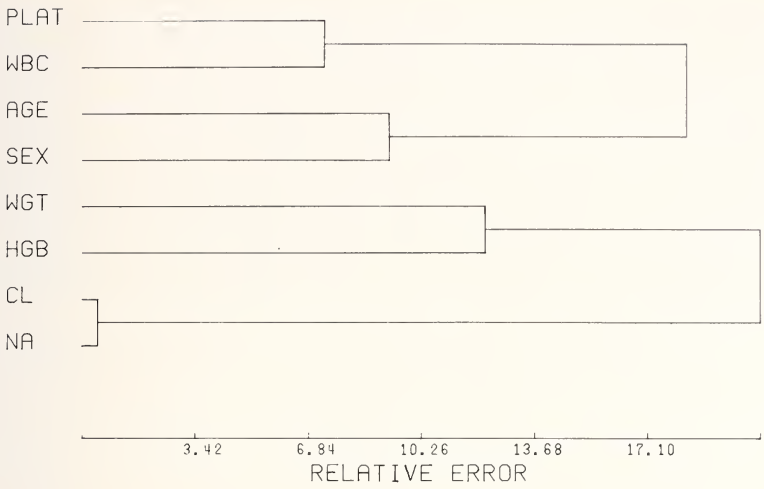

Figure 8. The dendrogram of the biological blood parameters with respect to clustering of the eight variables versus the relative error associated with each grouping.

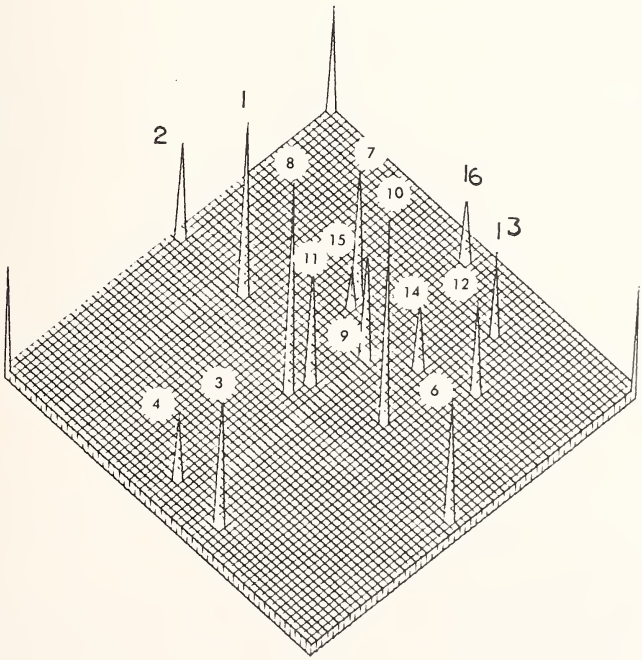

Figure 9. The three-dimensional representation with the cancer patients as parameters using the first three loaded-factors. This is the same experimental data as depicted in figure 7, but with the raw data matrix inverted.

water samples. Figure 10 presents the three-dimensional pictorial of the elements. The similarity of the variation of Pb, Cu, and Fe within the samples is quite obvious. Tentative groups of Al, Mn, Fe, and V also show up in figure 10. Figure 11 shows the dendrogram of the same experimental data. The groupings are composed of Al and Mn; Fe and V; Cu, Pb, Cd and Cr. It is tempting to explain Mn and Al as coming from crustal weathering but that leaves out Fe. The other elements (Cr, Cu, Pb, Cd, V) could be associated with anthropogenic sources. It becomes obvious that there are some samples that have markedly different values than those in the middle.

As with any statistical analysis technique, these are also limited by the accuracy of the experimental measurements. There is no substitute for well-planned experiments or sample collection programs with careful analytical procedures. If the experimental measurements are accurate, these techniques of pattern recognition can greatly improve a scientist's understanding of multi-interrelationships within his experimental data.

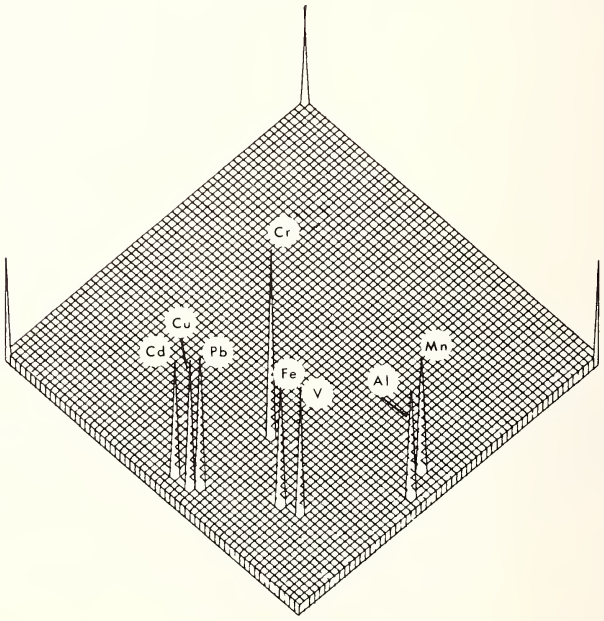

Figure 10. The three-dimensional representation of the eight trace elements in the sea-surface and sub-surface sea water samples using the first three loaded-factor values.

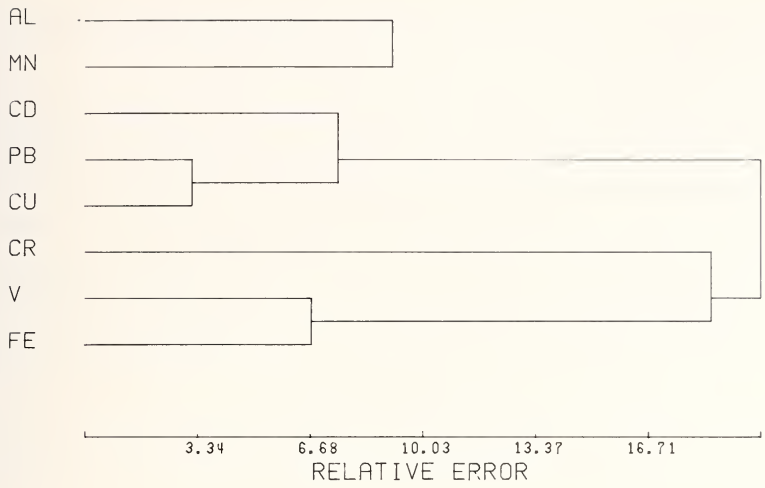

Figure 11. The dendrogram of the eight trace elements in the sea-surface and sub-surface sea water samples *versus* the relative error associated with the groupings.

IV. Acknowledgements

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INDIVIDUAL VARIATION OF TRACE METAL CONTENT IN FISH

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Fifty herring *Opisthonema oglinum* (Le Sueur) were collected from Boquerón Bay, Puerto Rico in one gill net haul. Each whole fish was wet-digested and analyzed as a separate sample in triplicate for Fe, Zn, Pb, Cd, Cu, and Mn using atomic absorption spectrophotometry. Results were correlated to the size of the fish. The concentration of Fe, Zn, Cd, and Cu were significantly higher in the pooled small fish as compared to the pooled large fish. The Pb concentration showed no detectable difference between the size classes. However, the Mn concentration was significantly higher in the pooled large fish as compared to the pooled small fish. The mean concentrations of these metals were statistically compared to the results of a pooled sample of fish collected and analyzed identically and to a pooled sample of thread herring collected in another location at a different time. No significant difference was found between these two pooled samples of medium size fish or between the trace metal values for the individual medium size fish. A determination of the sample size necessary to detect a 15, 20, and 25 percent difference between two significantly different means showed that a minimum sample size to detect that magnitude of difference would be 124, 70, or 50 fish respectively.

Keywords: Atomic absorption spectroscopy; fish analysis; sample size variations; trace analysis; trace metals.

I. Introduction

Considerable emphasis is being placed on the accuracy of trace element analyses for environmental samples [1]. This is, of course, important and necessary. However, often this is done with a considerable effort while no attention is given to adequate sampling and the high natural variation in trace element content among individuals, size classes, and populations of

the same species.

In fish the concentration of trace elements varies from 3 times to an order of magnitude in one population [2-4].

In fallout studies it has been found that the concentration of radioisotopes varies from 2 to 3 times between size and age classes of fish. This was caused by the changes in the diet of the fish [5,6], and in the turnover-time of the radioisotopes and that particular element [7,8] as the fish grow.

The objectives of this study were: 1. to compare the variability of iron, zinc, cadmium, lead, copper and manganese values in samples of a single species of the fish, Atlantic thread herring (*Opisthonema oglinum)* due to analytical procedures and to differences between individual fish; 2. to compare the concentration of trace metals in different sizes of fish and fish from different locations; and 3. to develop criteria for obtaining adequate and representative sample sizes for trace element analysis in fish populations.

II. Methods

Fish were collected with a 3/4 inch gill net in Boquerón Bay on the west coast of Puerto Rico. Fish were caught during three trips. On May 27 and 28, 1974, a total of 108 fish were caught in two net hauls at Station MPS 1 (fig. 1). These fish were assumed to belong to one population. The fish were divided into 50 individual samples of medium size fish (12 to 14 cm long), a pooled sample of 33 medium size fish, a pooled sample of 13 small size fish (10 to 12 cm), and a pooled sample of 12 large size fish (14 to 16 cm). On July 23, 1974, fish were collected at Station MPS 2 (fig. 1) about 1 km away from the first sampling site. These fish were assumed to belong to another school. A pooled sample of 16 medium size fish was selected from this catch. The length and fresh weight of each fish was measured immediately.

The fish were cut in small pieces with a glass knife, dried at 105 °C, weighed and ground. Three subsamples of 2.000 g (dry weight) of each individual fish, 30 replicates of the pooled sample of medium size fish, and 10 replicates of other pooled samples were analyzed with a Perkin-Elmer Model 303 atomic absorption spectrophotometer after a wet digestion procedure. The accuracy of the technique was confirmed by analysis of 12 subsamples of NBS-SRM-1577 (bovine liver).

A 2.000 g of dried samples was boiled at 80 °C with 25 ml of inverse aqua regia (3 parts of concentrated HNO_3 and 1 part of concentrated

Figure 1. Sampling sites in Bahia de Boquerón.

HCl) in a 150 ml beaker until 1 ml was left. The sample was cooled and 30 ml of 30 percent H_2O_2 was added. It was boiled down to 1 ml and 10 ml of inverse aqua regia was added slowly. It was boiled down to 1 ml. After cooling, 10 ml of 30 percent H_2O_2 was added and boiled down to 1 ml. If the solution was not colorless, the inverse aqua regia and H_2O_2 steps were repeated. When colorless, 6 ml of 2N HCl was added and boiled down to 3 ml. This step was repeated. The sample was cooled and centrifuged for 20 minutes at 2000 rpm. The solution was decanted and diluted to 10 ml. The solution was used directly for Cu, Cd, Pb and Mn analyses. For Fe analyses it was diluted 10 times and for Zn analyses 100 times.

All glassware was washed with Alconox®, rinsed with distilled water, refluxed with concentrated HNO_3 and rinsed again with deionized distilled water.

The accuracy was tested by analyzing the NBS-SRM 1577 (bovine liver). The values for Fe were slightly higher, but for Zn and Cu were within the 95 percent confidence limits with values given for the NBS standard (table 1).

TABLE 1. *Determination of accuracy for zinc, copper, and iron using NBS Bovine Liver Standard and method of present study*

Metal		Concentration (μg/g dry wt)		
		Zn	Cu	Fe
Observed value:	$\bar{X}$	141	186	324
	S	24	13	27
	95% confidence limits	±16	±7.9	±16
NBS value:	$\bar{X}$	130	193	270
	95% confidence limits	±10	±10	±20

III. Results

A. Variation Between Size Classes

The concentration of most elements studied was higher in the pooled sample of small fish than in the pooled sample of large fish (table 1). Lead showed no significant differences between any of the size classes. Only manganese was higher in the large fish. This was surprising as small fish usually have a faster metabolism [12] and turnover-time of elements than large fish [7]. As can be seen from table 2, elements seem to behave independently without a similar pattern between closely related element pairs such as Fe-Mn and Cd-Zn.

B. Comparison of Populations

Only iron showed a significant difference ($p < 0.05$, t test) between the pooled samples of the two populations (table 3). The similarity was expected as the locations were similar and reasonably close together (fig. 1).

TABLE 2. *Mean values of different trace metals in the pooled samples of small (12 cm), medium (12 to 14 cm) and large (14 cm) Thread Herring (Opisthonema oglinum) from Boqueron Bay*

	No. of analyses		Concentration (μg/g dry wt)					
			Fe	Zn	Cd	Cu	Pb	Mn
Small	11	$\bar{X}$	261	136	0.69	5.1	8.5	13.6
		S	16.7	8.9	.03	0.5	1.4	0.9
Medium	29	$\bar{X}$	111	91.5	0.54	3.0	8.5	13.3
		S	23.5	14.0	.06	0.5	1.4	1.6
Large	9	$\bar{X}$	91.7	102	0.64	2.6	8.7	16.8
		S	10.3	13.5	.04	0.7	0.7	1.3
Significant difference ($p < 0.05$)			a, b	a, b	b	a, b	d	b, c

Notes: The codes a, b, c, and d show the significant difference ($p < 0.05$, t test) between the size classes.

a = between small to medium
b = between small to large
c = between medium to large
d = no difference between the size classes.

TABLE 3. *Mean concentrations of the elements in pooled samples of Thread Herring in two different populations from Boqueron Bay*

Sample	No. of analyses		Concentration (μg/g dry wt)					
			Fe	Zn	Cd	Cu	Pb	Mn
MPS 1	29	$\bar{X}$	111	91.5	0.54	3.0	8.5	13
		S	23.5	14.0	.06	0.5	1.4	1.6
MPS 2	9	$\bar{X}$	81.6	92.1	0.63	2.9	8.2	17
		S	13.0	21.8	.07	0.2	0.66	2.0

C. INDIVIDUALS VS. POOLED SAMPLE

There was a significant difference ($p < 0.05$, t test) between the mean concentration of Fe in the pooled sample of medium size fish and the 50 individually analyzed medium size fish, but not for the other elements. The standard deviation of individual fish was three times as high as that of the pooled sample for manganese, and nearly 2 times as high for Zn but only slightly different for the other elements (table 4).

TABLE 4. *Mean concentration of trace elements in the pooled sample of the medium size fish and of 50 individuals (3 replicates) of medium size fish*

	No. of analyses		Concentration (μg/g dry wt)					
			Fe	Zn	Cd	Cu	Pb	Mn
Pooled fish	29	$\bar{X}$	111	91.5	0.54	3.0	8.5	13
		S	23.5	14.0	.06	0.5	1.4	1.6
Individual fish	161	$\bar{X}$	90.1	103	0.50	2.8	7.0	15
		S	19.9	24.9	.10	0.5	1.1	5.2

The results of the statistical comparison ($p < 0.05$, t test) of the trace metal concentration between the two different pooled samples of medium size fish (table 3) and the comparison of the pooled and individual medium size fish (table 4) indicated that iron was the only trace metal significantly different. This difference in iron concentration probably indicates contamination rather than actual differences in the iron content of the fish. The results for iron in the NBS bovine liver sample (table 1) and a few high blank values for iron support this hypothesis.

The frequency histograms of different elements (fig. 2) showed a more or less normal distribution (see [10]). The coefficient of skewness varied from zero for Cu to 1.3 for Mn.

To test a possible source of error caused by unequal amount of scales in subsamples, the scales of the medium size pooled fish sample were analyzed separately from the rest of the ground fish sample. Results showed that the concentration of cadmium, copper, lead, and manganese were significantly higher and iron significantly lower in scales than in the rest of the fish (table 5).

TABLE 5. *Mean trace metal concentration in medium size pooled Thread Herring scales and whole fish without scales*

Sample		Concentrations (μg/g dry wt)					
		Fe	Zn	Cd	Cu	Pb	Mn
Whole fish minus Scales	$\bar{X}$	89.0	88.5	0.51	2.8	6.4	18
	S	2.7	12.7	.04	0.32	0.82	1.4
Scales	$\bar{X}$	60.7	95.0	.66	3.3	12	28
	S	10.9	10.2	.06	0.30	1.3	1.8

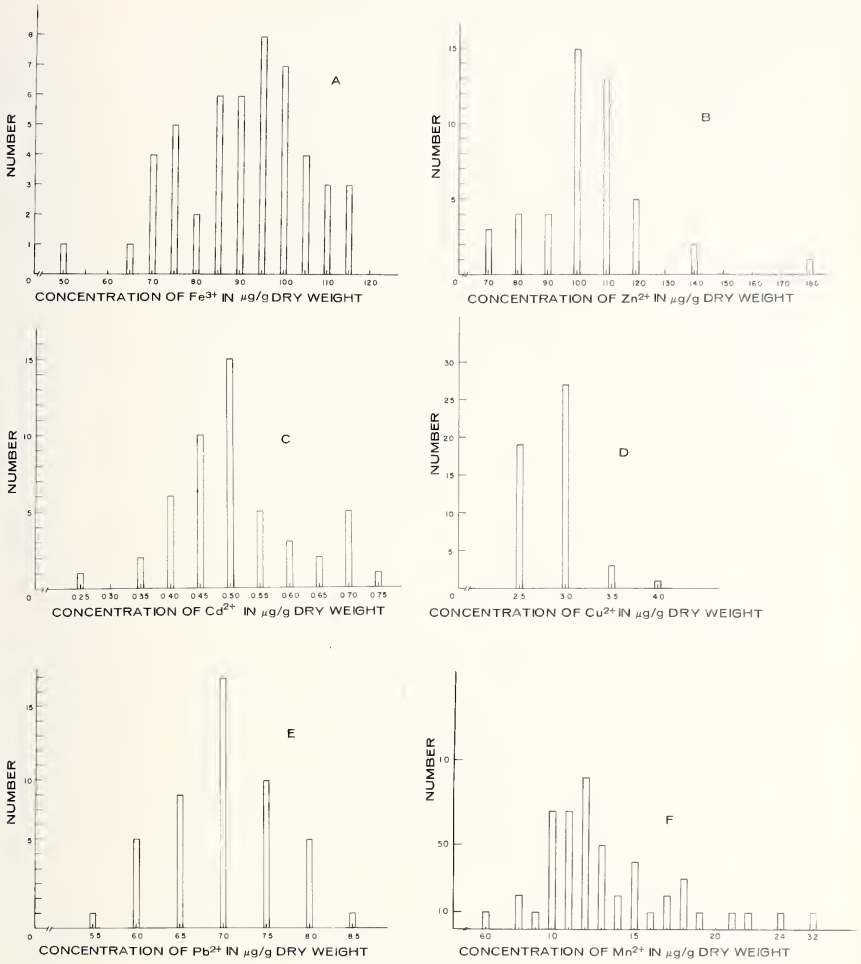

Figure 2. a,b,c,d,e,f: Frequency distribution of Fe, Zn, Cd, Cu, Pb, and Mn, respectively, in individual thread herring. The ordinate scale is number of individuals and the abscissa is the concentration of the metal in $\mu g/g$ dry weight.

Fish scales were difficult to grind completely and great care had to be taken to avoid getting an unequal amount of scales in replicates. In spite of careful mixing some subsamples might have had more scales than others. This could have increased the variability among the samples.

IV. Discussion

The concentration of the six elements studied showed less individual variation than reported by other authors in thread herring [2,3]. This was probably due to the size distribution. In the present study only medium size fish (12 to 14 cm) were used for analyses of individual thread herring, while the above-mentioned studies analyzed a random sample. There were significant differences between different size classes in our fish (table 1). Ting and de Vega [2] found the frequency distribution of trace metals in fish to follow a log-normal distribution. Our data, however, show more of a normal distribution. This discrepancy could be a result of the choice of size class of the different fish analyzed. The concentration of trace metals in thread herring found in previous studies [9] in Puerto Rican waters indicated that the Cd(0.62 μg/g S 0.11, N = 0), Pb(4.7 μg/g) and Mn(28 μg/g, S 17, N = 5) values are very similar but the Fe concentration (1030 μg/g, S 590, N = 10) is very high compared with the present study. However, the relative standard deviation of the former was much higher than the relative standard deviation for this study suggesting Fe^{3+} contamination in the other study.

The concentration of cadmium was about five times as high as the mean concentration of cadmium in New York State fresh water fish [4] when our values were calculated on fresh weight basis (mean wet weight to dry weight ratio for this study was 3.92, S 0.255, N = 50). There was a significant difference in the cadmium content between small and large thread herring (table 1). Lovett *et al.* [4] did not find any correlation between cadmium content and age in lake trout, but their sample consisted of only 11 fish.

The quantity of an element in a fish is determined by the concentration of the element in the water, in the food, and by the turnover rate of the element in the fish [8]. Since small, medium, and large size fish were all collected at the same location, the concentration of the element in the water does not apply. In this case, the individual differences in the element content in thread herring could be caused by differences in the turnover-time of the element and feeding habits of the fish (see [13]). Thread herring is a plankton feeder, but the planktonic species it feeds on vary and the turnover-time of equal size fish for an element may differ two-fold [8]. Both these factors can result in a two- to three-fold difference in the trace element concentration.

The variation between the pooled samples of medium size fish from two different locations was less than the individual variation of medium size fish in one location (see tables 3 and 4).

In general the variation of an element in a fish population is unknown and the *a priori* determination of an adequate sample size is very difficult. However, if the relative standard deviation observed in the present study for the trace metal concentration of individual fish is used in the equation from Sokal and Rohlf [11], it is possible to predict the sample sizes that would have been necessary to detect a 15, 20 and 25 percent true difference with a 90 percent probability of detection (table 6). As can be seen a minimum sample size of 45 individual fish would have been necessary to detect a 25 percent difference between two significantly different ($p < 0.05$) populations whereas a minimum sample size of 70 would be needed to detect a 20 percent difference and finally at the 15 percent level of detection a sample size of 124 would be needed. For the present study it would appear that a sample size of 50 fish was adequate for all metals except Mn, which would need a sample size of 70.

TABLE 6. *Comparison of predicted sample size of Thread Herring based on the observed coefficient of variation in the trace metal content for the individual fish*

Metal	Percentage difference detectable between two population means		
	15%	20%	25%
	Predicted sample size[a]		
Pb	25	15	10
Zn	35	20	14
Cu	35	20	13
Fe	46	27	17
Cd	73	43	28
Mn	124	70	45

[a] The predicted sample size was computed to allow a 90 percent chance of detecting a 15, 20, or 25 percent difference in the means of two populations at the $p < 0.05$ level of significance.

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Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis,
Proceedings of the 7th IMR Symposium,
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ACCURACY OF CHEMICAL ANALYSIS OF AIRBORNE PARTICULATES—RESULTS OF AN INTERCOMPARISON EXERCISE

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Since suitable standard reference materials for chemical analysis of airborne particulates are not available, an intercomparison exercise was carried out among 40 interested laboratories in order to evaluate the accuracy of various trace analysis techniques for this specific application. Six hundred grams of airborne particulates were collected from the inlet filters of the air conditioning installation of a hotel in the center of Milan. The sample was sieved to remove coarser particles, thoroughly mixed, and distributed in 1 to 5 gram aliquots. The homogeneity was checked by relative measurements carried out by three independent techniques. For 40 elements no inhomogeneity was found to exceed the analytical error, which was estimated to be approximately 10 percent. The data of the analytical exercise are being collected and evaluated. Results are available for 56 elements, but to date only 33 have been determined by more than one technique. Activation analysis, emission spectroscopy, atomic absorption, x-ray fluorescence and various wet chemical methods contributed to the intercomparison. No result was received from mass spectroscopic methods and, although analyses were specifically encouraged, very few results were received on the organic components. From a first approximate evaluation a good agreement was found for Al, Fe, Zn, Mn, Ca, Pb, Cl, S, Si, Ti, Mn, while for the other elements no definite conclusion can yet be drawn. An attempt will be made to interpret important cases of systematic errors, a few of which are already evident.

Keywords: Accuracy; activation analysis; airborne particulates; air filter analysis; atomic absorption; emission spectroscopy; environmental pollutants; intercomparisons; multi-element analysis; trace element analysis; x-ray fluorescence.

I. Introduction

During 1972 an experimental activity on the development of analytical techniques for the quantitative analysis of environmental pollutants was started at the Ispra Joint Research Centre (JRC) of the European Communities, within the frame of Environmental Protection Programme. The aim of this action was both to provide efficient analytical techniques for the needs of the Joint Research Centre, and more generally to contribute to the progress of the analytical techniques used for studies associated with the protection of the environment. The evaluation of analytical techniques already used or potentially useful is a part of this programme.

Due to the increasing importance of chemical analysis of airborne particulates both for routine control of air quality and for the identification of pollution sources, an intercomparison exercise was organized. The purpose of the exercise, which was held as a joint effort of the Joint Research Centre and the Health Protection Directorate of the European Communities, was threefold:

a. to characterize an airborne particulate sample as fully as possible, both for its organic and inorganic components,
b. to offer the possibility to the various participants of evaluating the accuracy of their analyses by intercomparison of results,
c. to evaluate the potential of various analytical techniques for the control of chemical compositions of airborne particulates.

II. Sample Preparation and Homogeneity Control

A batch of airborne particulates, weighing approximately 600 grams, was obtained from the Provincial Laboratory for the study of air pollution in Milan. The sample originated from monthly cleaning of special filters installed at the inlet of the air conditioning system of a building located in the central area of Milan. A composite batch was obtained by combining samples already collected during winter months of years from 1962 to 1972.

At the Joint Research Centre the samples were thoroughly mixed; they were then sieved to remove coarser particles through nylon sieve no. 50 of the U.S. Standard Sieve Series (sieve opening 297 ± 12 μm). After sieving, the sample was again thoroughly mixed in a mechanical homogenizer and divided into 5 gram aliquots and stored in Lucite containers at the ambient temperature and humidity.

The homogeneity of the sample was checked at the Ispra JRC by

choosing at random 10 containers and by taking from each of them, after exhaustive mixing, three 150 mg aliquots, which were again partitioned into smaller aliquots and submitted to activation analysis, x-ray fluorescence and emission spectrography.

A. Activation Analysis

Twenty mg aliquots were irradiated with $10^{13}n \cdot cm^{-2} \cdot s^{-1}$ for 40 seconds, 15 minutes, and 8 hours. The activated samples were measured by high resolution gamma-ray spectroscopy. Relative measurements of the radioactivity induced in the 10 samples were carried out on the gamma-ray peaks of the activation products of Ca, Al, Mn, Na, Br, V, Cl, Cu, K, Au, La, Sb, Fe, Sc and Cr. No inhomogeneity was found to exceed the experimental error which was estimated as ± 10 percent.

B. X-Ray Fluorescence

One hundred mg fractions from each sample were analyzed by x-ray fluorescence with a semiautomated x-ray spectrometer, using Mo, W and Cr anode tubes and LiF/Graphite dispersing crystals. Relative measurements of the peak heights of the main K and L spectral lines for the elements Fe, Pb, Ni, Cu, Ba, Sn, Sb, Sr, Zn, Ti, Mn, K, Ca, S and Cl were carried out. Each individual measurement was within ± 5 percent of the mean value for all the reported elements.

C. Emission Spectroscopy

About 25 mg fractions from each sample were arced by a 10 ampere dc discharge under identical experimental conditions. The spectra were recorded photographically. A 7 step rotating sector was used in front of the spectrograph entrance slit in order to obtain spectra with degrading intensities. The following elements were detected in all samples: Fe, B, Si, Mn, Mg, Pb, Sn, Cr, Ga, Al, V, Ca, Mo, Ni, Zn, Ti, Cd, Na. The line intensities of each element from the various samples, when normalized for identical backgrounds, agreed within ± 10 to 15 percent.

As a conclusion, inhomogeneity of the sample should not lead to errors greater than about ± 10 percent in the evaluation of the elements for which the homogeneity was tested, provided the sample weight exceeds 20 mg. This upper limit for the error due to inhomogeneity was considered sufficiently good for the exercise, and no other effort was made to see if

the homogeneity was better than ± 10 percent.

Preliminary semiquantitative elemental analyses were also carried out at the JRC on 25 elements by activation analysis, emission spectroscopy, x-ray fluorescence and wet chemistry, in order to obtain an approximate material balance as an orientation for successive quantitative evaluations. When this preliminary work was completed, samples were distributed to 38 laboratories who accepted to join the intercomparison exercise. The samples were distributed during the fall and winter of 1973, and the analytical campaign was closed on April 30, 1974.

III. Results

Results were reported by 30 laboratories. Three of them reported only on organic components, which are not discussed in the present report, while 27 reported on the elemental composition, with a total of 2012 results obtained on 56 elements. A variety of techniques were employed. In the report they have been grouped as seven groups, although somewhat arbitrarily.

The large number of data obtained on so many elements and by so many techniques make the application of known statistical treatments of data rather difficult and lengthy. The statistical approach used at the JRC for circular analysis, carried out in the frame of the research programme "Standards and Certified Reference Materials" consists of a series of different tests (flowsheet in fig. 1).

First the batch of data for each element, technique and laboratory is checked for normality. If necessary, outliers can be recognized by an appropriate test (Dixon's test). A test on the equality of the variances (Bartlett's test) is followed. Depending on the results of these tests the data is either examined by the one-way analysis of variances or by the Aspin-Welsh test in order to check the equality of the means. Finally for each element, technique and laboratory, confidence intervals for both the mean and the standard deviation are constructed. From this, outlying measurements can be recognized. The final judgement on the significance of outlying measurement is left to the experimenter. The statistical evaluation of the results obtained for individual elements is presently under way, and the results will be included in the final report on the exercise.

The described approach requires, however, that circular analyses be done under experimental conditions which are accepted and followed by all participating laboratories. This was not the case for the described analytical campaign, in which the laboratories were left completely free to adopt their typical working practice. As a consequence and particularly

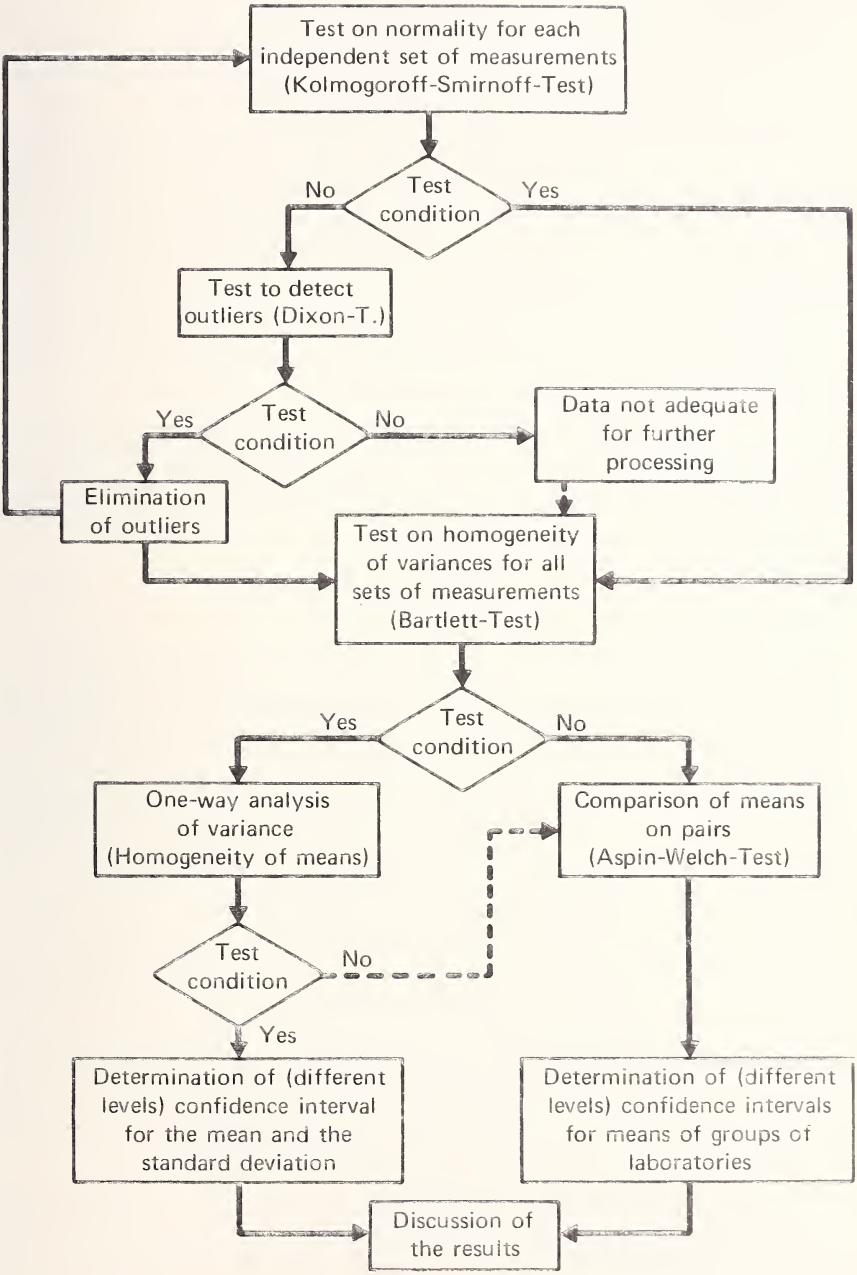

Figure 1. Flowsheet of data treatment used for intercomparison analyses.

for elements on which not many results were reported, the described approach cannot be applied and additional analysis will be required.

In order to allow a first exchange of views among participating laboratories a simpler preliminary data treatment was carried out. The mean value obtained per element and technique was calculated for each laboratory. The overall mean values obtained per element and technique were then calculated by averaging the means of the different laboratories. No data was discarded, unless a large discrepancy was evident. The same statistical weight was given to means of laboratories who performed a different number of replicate analyses.

The results obtained are reported in table 1 as "means of the means," together with the number of contributing laboratories and the relative standard deviation of the means.

A report is given of an attempt to evaluate the results obtained for each group of elements and for each technique. This analysis is obviously preliminary and incomplete. The reader should refer to the final report for a more complete and objective evaluation.

1. Analytical Techniques

Neutron Activation (NA) allowed the determination of the largest number of elements (49). However, among the elements of considerable environmental importance Pb was not analyzed, Cd was analyzed only by one laboratory, and Si by two. The agreement on the results obtained by the various laboratories was usually rather good, except for Mg and In. The few results reported for these two elements showed differences from 30 to over 50 percent. When a comparison with other techniques was possible activation analysis usually agreed rather well with other techniques, with the possible exception of Ca, where the mean result obtained is about 20 percent higher than the average of the other techniques. It is interesting to note, however, that among the five NA laboratories who reported on Ca, the two reporting the lower standard deviation, were also in better agreement with the other techniques. Results obtained by the use of a linear accelerator (LI) (one laboratory only) were kept separated from NA data in table 1. The agreement between NA and LI was good.

Atomic Absorption (AA) allowed the determination of 19 elements. Among the elements of some environmental importance no results were reported for As, Co, Ti, Se, S and the halides, while Al, Si and V were determined by just one laboratory and Hg by two. The agreement between various AA laboratories was not as good as that obtained by NA

laboratories, but still satisfactory, with the exception of Cr, where the data by the various laboratories show variations as high as 300 percent. The mean result obtained for the various elements agreed rather well with the mean values of other techniques.

Emission Spectroscopy (ES) reported the determination of 21 elements, which included those of major environmental importance, with the exception of As, Hg and the halogens. For the other elements the results were reported by only two or three laboratories and the agreement between the various laboratories was worse than for NA and AA. A comparison with the other techniques can hardly be done due to the paucity of data available, but no large discrepancy was evident.

X-ray Fluorescence (XF) reported on 21 elements. Data were not reported for Hg, Mg and Se. The difference between the various laboratories was rather large (frequently 2 to 3 times) and a comparison with the other techniques becomes rather difficult under these conditions.

The results obtained by other techniques included in the broad category of chemical analysis (CA) are too few to allow a comparative evaluation.

2. Elements Analyzed

Group Ia (Li, Na, K, Rb, Cs). Rb and Cs were analyzed only by activation analysis techniques, while Na and K were analyzed by many techniques. Excellent agreement was obtained for both elements between NA and AA. Wet chemical methods and ES gave higher results. The results are, however, too few to yield any definite conclusions. Li was not analyzed by any technique.

Group IIa (Be, Mg, Ca, Sr, Ba). Ca and Ba were analyzed by many techniques, while no result was reported for Sr. NA gave higher results than AA for Ca, while excellent agreement was obtained between AA and a few measurements carried out by ES and wet chemical methods. For Ba the agreement between the various NA laboratories was good, but rather large discrepancies exist among different techniques. Mg was analyzed by many techniques with relatively good agreement in the limits of the few results reported. The agreement between the various AA laboratories is rather good. No laboratory reported results on Be, despite its non-negligible importance for environmental problems.

Group IIIb (Sc, Y, rare earths). Only NA reported results on the rare earth groups. Sc, La, Sm were analyzed by 5 to 7 laboratories with excellent agreement, while larger variations were obtained on Eu which was determined by 3 laboratories. Results for Dy, Lu, Nd and Yb were reported by one laboratory.

TABLE 1. *Chemical analysis of airborne particulates; mean values obtained by different techniques*[a]

Element	NA	AA	ES	XF	CA	LI	CO
Ag	15 (7–23)	9.7 (1–)	6 (1)				
Al	16960 (8– 6)	13500 (1–)	18556 (3–10)		15120 (3–39)		
As	92.8 (7–10)			116 (1–)		116 (1–)	
Au	2.6 (8– 9)						
Ba	1052 (6–16)	558 (1–)	409 (2–80)	809 (2– 2)			
Br	624 (9– 9)			682 (2–30)		752 (1–)	
Ca	43160 (5–13)	38360 (8–16)	37500 (1–)	37060 (5–24)	37940 (2– 2)		
Cd	44.2 (1–)	58 (8–65)	48 (1–)	91 (1–)	73 (1–)		
Ce	150 (8– 6)						
Cl	11480 (9–12)			11500 (1–)	10500 (1–)		
Co	17.5 (10–22)			65.7 (1–)			
Cr	200 (10–15)	253 (6–73)	(333) (3–)	(2520) (3–)	125 (1–)		
Cs	2.5 (6–22)						
Cu	652 (3– 7)	697 (9–14)	648 (3–15)	(845) (4–)	647 (1–)		
Dy	2.6 (1–)						
Eu	0.5 (3– 9)						
F	2428 (1–)				2330 (1–)		
Fe	35040 (11– 6)	32256 (8–12)	32330 (3– 8)	32220 (4–14)	37000 (2– 8)		
Ga	10.7 (1–)						
Hf	1.3 (4–10)						
Hg	23.2 (5–59)	16.5 (2– 1)					

TABLE 1. *Chemical analysis of airborne particulates; mean values obtained by different techniques*[a]—Continued

Element	NA	AA	ES	XF	CA	LI	CO
I	14.3 (1–)					14.2 (1–)	
In	0.5 (2–34)						
K	7540 (8–14)	6596 (5–25)		6800 (1–)	14200 (1–)		
La	83 (7–10)						
Lu	0.17 (2–29)						
Mg	6108 (2–38)	7332 (7– 8)	6183 (3–50)		8000 (2–11)		
Mn	1274 (10– 6)	1290 (8–21)	1528 (4–17)	1695 (5–36)	1080 (1–)		
Mo	31.8 (1–)		(157) (2–)				
Na	9904 (10– 8)	9240 (5–20)	13200 (1–)		19400 (1–)		
Nd	(65) (1–)						
Ni	1410 (5–27)	1517 (7–15)	1824 (4–35)	(2971) (5–)		1540 (–)	
P			1400 (1–)		1445 (2–25)		
Pb		5540 (14–28)	5924 (4–12)	8050 (5–41)	8766 (1–)	6172 (1–)	
Rb	39.3 (1–)					38.5 (1–)	
S	39200 (1–)		44600 (2– 6)	43000 (1–)	45950 (2–20)		47800 (1–)
Sb	283 (11–13)			296 (1–)			
Sc	3.2 (7– 7)						
Se	10.3 (5–15)						
Si	61600 (2– 2)	75300 (1–)	62100 (2– 9)	78000 (1–)	55900 (2– 4)		
Sm	7.8 (5– 4)						
Sn		3100 (1–)	(333) (3–)	175 (1–)			

TABLE 1. *Chemical analysis of airborne particulates; mean values obtained by different techniques*[a]—Continued

Element	NA	AA	ES	XF	CA	LI	CO
Ta	1 (1–)						
Tb	0.73 (1–)						
Th	3.4 (9–21)						
Ti	2746 (5–10)		2163 (3–21)	2466 (3–24)	2550 (2– 3)	3094 (1–)	
U	1.15 (1–)						
V	1990 (9–17)	2952 (1–)	1796 (3–34)	2710 (3–44)			
W	16.7 (4– 9)						
Yb	0.84 (1–)						
Zn	7434 (9–14)	7461 (9–12)	5747 (3–67)	8978 (1–)	9100 (1–)		
Zr						56.6 (1–)	
C							423150 (6– 4)
H					16500 (2– 4)		16560 (1–)
N							21000 (3–13)
O	279000 (1–)						144000 (1–)

[a] Notes: Mean values obtained by different techniques—The first number in parenthesis is the number of contributing laboratories, the second one is σ expressed in percent. When the mean value is within parenthesis σ was exceeding 100 percent.

Analytical methods:

NA = neutron activation analysis
AA = atomic absorption and flame photometry
ES = emission spectrometry
XF = x-ray fluorescence
CA = wet chemical treatment with final gravimetric, volumetric, electrochemical or spectrophotometric measurements
LI = activation analysis with LDIAC
CO = combustion methods.

Group IVb (Ti, Zr, Hf, Th). Only activation analysis techniques reported on Zr, Hf, and Th with relatively large variations (±30 percent among different laboratories). Ti also was analyzed essentially by NA, with good agreement among the various laboratories. A few results obtained by other techniques (XF, CA, ES, LI) were in reasonable agreement with those of NA.

Group Vb (V, Nb, Ta). Results were reported for vanadium essentially by NA, with excellent agreement between nine laboratories. Results reported by AA (one laboratory) were 50 percent higher while larger discrepancies were shown by XF and ES, which reported, however, only a few results.

Group VIb (Cr, Mo, W, U). A few NA laboratories reported on W, with good agreement. A few results were reported on Mo, and only one reported on U. Cr was analyzed by many techniques. While the 10 NA laboratories agreed on a content of 200 ppm ± 15 percent, the AA, Es, and CA data are scattered between 125 and 600 ppm, while XF reported much higher results.

Group VIIb (Mn, Re). Results were reported for Mn by NA, AA, XF, CA, ES, with a good overall agreement. The precision obtained by the various NA laboratories was outstanding.

Group VIIIa (Fe, Co, Ni, Ru, Rh, Pd, Os, Ir, Pt). While no laboratory reported on the noble metals, a large mass of data was obtained for Fe, Co and Ni. Fe was analyzed by NA, AA, XF, ES, CA with excellent agreement among the different techniques. A reasonable overall agreement was obtained between NA, AA and ES for Ni, although the deviations among different laboratories is higher than in the case of Fe. XF reported rather scattered data. Co was analyzed by activation analysis with good agreement between different laboratories.

Group Ib (Cu, Ag, Au). Cu was analyzed by NA, AA, XF, ES and CA with good agreement among the different techniques. The dispersion of XF and ES data was however rather large. Ag was essentially analyzed by neutron activation with relatively large dispersion of data among different laboratories. A few results reported by AA and ES were definitely lower. Au was only analyzed by neutron activation with good agreement between laboratories.

Group IIb (Zn, Cd, Hg). A large mass of data was obtained on zinc, with excellent agreement among the techniques (NA, AA, ES) and very good precision for NA and AA. A rather large dispersion of data was obtained for Cd, for which AA was the most applied technique. Hg was analyzed mostly by NA, and despite of the difficulties usually encountered in the analysis of this element, the agreement obtained was relative-

ly good. A few data reported by AA agreed with NA data.

Group IIIa (B, Al, Ga, In, Tl). Results for this group were essentially reported for Al. The 8 laboratories employing activation analysis reported results in excellent agreement. A few results reported by AA were 20 percent lower, while a few results by ES, and CA were somewhat discrepant. No result was reported for B and Tl, while for Ga and In only a few results were obtained, with relatively large discrepancies for In.

Group IVa (C, Si, Ge, Sn, Pb). A good agreement was obtained on the major constituents C and Si by the various techniques applied. More problems seem to exist for lead analysis, despite of the importance of its determination. The laboratories applying AA and ES gave results in good agreement. A few laboratories, however, reported data which were much higher or lower. Results which were largely different among different laboratories were also obtained by XF. Tin was determined by only a few laboratories and the results were different by an order of magnitude. No laboratory reported on Ge.

Group Va (N, P, As, Sb, Bi). N and P were determined by only a few laboratories but results do not show large variations. As and Sb were essentially measured by activation analysis, with good agreement among laboratories. A result reported on Sb by XF agreed with NA measurements. No real result was reported for Bi.

Group VIa (O, S, Se, Te). S was analyzed by various techniques (NA, XF, ES, CA and CO) with good overall agreement. Se was analyzed by many activation analysis laboratories with relatively good agreement among them. No result was reported for Te. Results obtained by fast neutron activation on oxygen were reported by one laboratory.

Group VIIa (F, Cl, Br, I). Only a few results were reported for F and I, essentially by chemical procedures and activation analysis, with good agreement, particularly for F. Br and Cl were analyzed by many NA laboratories, with excellent agreement among them. A few results reported by XF and CA did also agree.

IV. Meeting of Principal Investigators

On July 7th and 8th a meeting of the principal investigators of the participating laboratories was held in Ispra in order to review the work done and advise on the continuation of the exercise. Critical appraisals of the most important techniques used in the exercise were presented by four experts. The following general observations were made:

Most laboratories analyzed only the inorganic components of the

aerosol (only six laboratories carried out organic analyses and the results obtained were far from being conclusive). Work on the organic fraction was encouraged on an agreed plan of priorities.

Concerning the inorganic fraction the results were considered satisfactory for five elements (Ca, Mn, Fe, Na and Zn).

For 10 elements (As, Al, Br, Cu, C, Cl, Sb, K, Ba, Hg) it was felt that a minor analytical effort could considerably decrease the discrepancies existing among different laboratories and techniques, and a higher priority was given to work on As, Al, Cu, C, Sb and Hg.

For 13 elements (Ni, V, Pb, S, F, P, Rb, I, Mo, Ti, Ag, Mg, Si) the results obtained were not considered satisfactory. Efforts should be concentrated on Ni, V, Pb, S, F, and Ti, and the situation be reviewed again afterwards.

For three elements (Cd, Cr and Sn) the results obtained were rather bad. In view of their environmental importance, major efforts should be done to set up more accurate analytical methods for the three elements.

While it was agreed that the sample distributed for analysis was quite representative of an urban aerosol it was felt that in the future other standard aerosol samples with different compositions, representative of different sources of pollution should be made available. It was further recommended that attempts to obtain a certification of the elements present in the Milan aerosol sample should proceed as quickly as possible so that it could be classified as an "environmental standard" for general use.

V. Contributing Laboratories

The contributing laboratories are given below, with names of principal investigators in parenthesis.

AUSTRIA: Institut fur Analytische Chemie und Mikrochemie
Technischen Hochschule Wien
Wien (H. Malissa)

BELGIUM: Instituut Nucleaire Weteschappen
Rijksuniversiteit Gent
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THE ESTIMATION OF ACCURACY IN TRACE ANALYSIS. RESULTS OBTAINED FROM INTERCOMPARISONS ORGANIZED BY THE I.A.E.A.

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The 1972 and 1973 I.A.E.A. round-robin exercises concerning trace analysis in environmental problems are reported. Paper filters simulating air filters were spiked with known amounts of salts of: As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn, in the order of micrograms of an element per one filter. Only Fe and Pb were present in the order of 200 μg. Twenty-two laboratories returned 922 analytical results obtained by 6 different methods—mostly neutron activation analysis and atomic absorption spectrometry. Tables summarizing the results for each element are presented with special emphasis on the difference between the true value and the reported results. The need and the utility of the Analytical Quality Control programme of the Agency is also discussed.

Keywords: Accuracy; activation analysis; air filter analysis; analytical quality control; atomic absorption spectrometry; environmental analysis; fresh water analysis; intercomparisons; precision; round-robin experiments; trace element analysis.

I. Introduction

The aim of this contribution is to document the status of the quality of trace analysis in environmental problems as it appears in the results from average laboratories in an average country. The data presented in this paper were obtained in two round-robin exercises performed by the

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Analytical Quality Control Services of the International Atomic Energy Agency. The participants in these intercomparisons were recruited on a voluntary basis. The participating laboratories were asked to perform more than two parallel analyses and to return not only a laboratory mean but also the individual results. Most of them did so and it was possible, therefore, to calculate a within-laboratory precision. For the following discussion, however, only the accuracy, *i.e.*, the deviation from the true value, is considered.

Both intercomparisons were performed on artificial samples—the true values were known with an accuracy estimated to be within ±3 percent. All calculations of the overall means for each element, their standard deviation, standard error and accuracy were performed using the laboratory averages rather than the individual results of parallel analyses returned by the participants.

II. Procedure

A. Analysis of Simulated Air Filters

During the second half of 1972 simulated air filters [1] were distributed to 23 laboratories located in the following countries: Belgium, Denmark, Finland, France, Federal Republic of Germany, Hungary, Korea, Mexico, The Netherlands, Norway, Republic of South Africa, United Kingdom, United States of America, and Yugoslavia. Most, but not all of the laboratories are in well developed countries and probably are well equipped. Two thirds of these laboratories are attached to national atomic energy institutes, one third to environmentally oriented institutes. Altogether 922 individual analytical results were returned for 13 different elements: As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn. Neutron activation analysis was used in 53 percent, atomic absorption spectrometry in 27 percent of all analyses and 20 percent were carried out by various other methods. Each participating laboratory received 10 paper filters of 5.5 cm diameter, made of analytical grade paper, which were loaded with 50 μl each of a solution containing known quantities of the above listed elements. The moistened spot which was left for air drying did not have a regular form and did not cover the whole surface of the filter. This fact excluded the use of some direct, nondestructive methods (*e.g.*, x-ray fluorescence) and made necessary the use of the whole filter for any analytical procedure. No blank filters were distributed, but one participating laboratory analyzed the same grade filter from another batch

and found that the blank content of chromium and nickel was in the order of 10 percent of the spike quantity. For all other elements the blank was either lower than 4 percent or completely negligible. Where the influence of blank values on an overall mean could not be entirely neglected (Cr, Ni, Cu, Ba) a second calculation of accuracy was performed including the estimated blank values and the results thus obtained are given in brackets in table 1. No principal elements of aerosol particles were added; this fact made the analysis of filters much easier than for real air filters (especially in the case of nondestructive activation analysis).

The amounts of the elements were of the order of 1 to 5 μg per filter—with the exception of iron, lead and zinc which were added in 100 μg quantities. The errors of preparation of the filters were estimated to be not more than 3 percent. This figure represents the sum of between-filter fluctuation and inaccuracy of the true value of the contents.

A critical view of table 1 shows that the analyzed elements can be classified (somewhat arbitrarily) into three groups:

- —difficult elements: Cr, Ni, Cu, As
- —intermediate group: V, Mn, Se, Cd, Ba, Hg
- —elements, for which the precision and accuracy is satisfactory: Fe, Zn, Pb (Note: the content of these elements was one order of magnitude greater than that of the others).

This classification is based on the magnitude of standard errors and deviations from true value (accuracy) and to some degree on the numbers of outliers. It is a measure of the difficulties in the two analytical methods used, *viz.* activation analysis and atomic absorption spectrometry. The difficulties appearing in the determination of chromium are well known, but the errors in the determination of copper and arsenic are unexpectedly large.

B. Comparison of the Accuracy Obtained in Neutron Activation with that Achieved in Atomic Absorption Spectrometry

For Fe, Zn, Ni, Mn, Cd, Cu and Cr the number of laboratories which worked with atomic absorption spectrometry were comparable with the number of those which used neutron activation [2]. This enabled us to make an intercomparison between the accuracy obtained using these two principal methods for trace elements. Table 2 summarizes the results of the study: for each element and each method the number of laboratories are given which returned results with satisfactory or with nonsatisfactory accuracy. The classification was made without using any statistical

TABLE 1. *Summary of intercomparison of simulated air filters*

Element determined		V	Cr	Mn	Fe	Ni	Cu	Zn	As	Se	Cd	Ba	Hg	Pb
True value (μg/filter)		2.16	1.85 [2.10]	4.2	194	5.3 [6.0]	3.04 [3.12]	74.0	2.04	0.85	3.06	13.8 [14.3]	0.24	153
Number of results reported	indiv. determin.	52	70	91	113	49	74	100	67	33	64	48	35	80
	lab. averages	10	16	19	21	11	15	20	12	7	11	9	6	14
Number of results accepted	indiv. determin.	43	63	91	113	38	60	84	57	33	54	48	35	80
	lab. averages	9	13	19	21	9	12	18	10	7	10	9	6	14
Total range of laboratory means		8.79	6.20	5.35	179	8.37	7.42	68.8	3.70	0.533	3.70	7.52	0.306	101
Range of accepted laboratory means		0.86	1.87	5.35	179	4.30	2.83	35.8	1.64	.533	2.84	7.52	.306	101
Overall mean of accepted laboratory means		2.05	2.13	4.63	191	6.67	3.19	70.7	1.75	.769	3.21	13.0	.259	149
Standard deviation of overall mean	abs.(μg/filter)	0.27	0.54	1.29	41.0	1.41	0.81	9.67	0.49	.160	0.770	2.59	.120	31.8
	rel. (%)	13.1	25.6	27.8	21.4	21.2	25.3	13.6	28.2	20.9	23.9	19.9	46.2	21.2
Standard error of overall mean	abs.(μg/filter)	0.09	0.15	0.29	8.9	0.47	0.23	2.28	0.16	0.061	0.24	0.86	0.049	8.50
	rel. (%)	4.3	7.1	6.3	4.6	7.1	7.3	3.2	8.9	7.9	7.5	6.6	18.8	5.6
Deviation from true value (accuracy)	abs.(μg/filter)	−0.11	+0.28 [+0.03]	+0.43	−3	+1.37 [+0.67]	+0.15 [+0.07]	−3.3	−0.29	−0.081	+0.15	−0.80 [−1.30]	+0.019	−4
	%	−5.1	+15.1 [+1.4]	+10.2	−1.5	+25.8 [+11.2]	+4.9 [+2.2]	−4.5	−14.2	−9.5	+4.9	−5.8 [−9.1]	+7.9	−2.6
Percentage of outliers	%	10	19	.0	.0	18	20	10	16	0.0	9	0.0	0.0	0.0

Note: See explanation in text for values in brackets.

TABLE 2. *Comparison, by elements, of the neutron activation analysis and atomic absorption spectrometry in the air filter analysis*

Element	Content (μg/filter)	Accepted limit of accuracy (%)	Number of laboratories			
			Neutron activation		Atomic absorption	
			Accept. results	Unacceptable results	Accept. results	Unacceptable results
Fe	194	7	4	4	4	4
Zn	74	10	7	3	5	2
Mn	4.2	6	7	3	3	3
Cd	3.06	7	2	3	2	2
Ni	5.3	6	—	3	2	2
Cu	3.04	9	3	4	2	2
Cr	1.85	12	7	2	—	4
Total absorption			30	22	18	19
Percent absorption			58%	42%	48%	52%

test—in most cases there were no doubts about the adherence to the proper group. In most cases also the precision was found to coincide with good accuracy. It appears from table 2, that when the same criterion for accuracy is applied to both methods, atomic absorption spectrometry gives slightly more inaccurate results. Table 3 gives the classification of participating laboratories according to the percentage of returned inaccurate results for the above-mentioned elements and methods. Only a few laboratories returned results which were all acceptable.

TABLE 3. *Comparison, by laboratories, of the neutron activation analysis and atomic absorption spectrometry in the air filter analysis*

Laboratory	Neutron activation analysis			Atomic absorption spectrometry		
	No. of elements determined	Accurate results, %	Inaccurate results, %	No. of elements determined	Accurate results, %	Inaccurate results, %
A	5	100	—			
B	3	100	—	4	75	25
C	6	83	17			
D	7	71	29			
E	6	66	33			
F	3	66	33			
G	3	66	33			
H	6	50	50	7	43	57
I	3	33	66			
J	2	—	100			
K	6	—	100			
L				7	71	29
M				3	66	33
N				2	50	50
O				5	20	80
P				4	—	100

C. ANALYSIS OF FRESH WATER

During the second half of 1973, a round-robin experiment [3] was performed in which 39 laboratories took part. They were located in the following countries: Belgium, Brazil, Canada, Finland, France, Federal Republic of Germany, Hungary, Italy, Korea, The Netherlands, Norway, Pakistan, Poland, Mozambique, Sweden, Republic of South Africa, Turkey, United Kingdom, United States of America, and Yugoslavia. As in the previous intercomparison, most laboratories were located in developed countries; only 54 percent of them were attached to national nuclear authorities or institutes.

Altogether 339 laboratory means were returned, most of them based on 2 or more parallel determinations. The following elements were determined: As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Hg, Mo, Ni, Pb, Zn, Se, U, and V. Evaporation was used for separation or preconcentration in most cases, when the preconcentration method was stated. As the analytical method, atomic absorption spectrometry was used in 53 percent and activation analysis in 21 percent of all determinations. Other methods accounted for 26 percent of the results.

TABLE 4. *Summary of trace element determinations in fresh water*

Element determined		As	Ba	Cd	Cr	Co	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Se	U	V	Zn
True value (μg/l)		10.4	27.6	6.15	11.1	21.5	14.6	444	1.62	8.43	11.9	16.9	305	5.14	2.00	11.9	9.88
Number of results reported	laboratory averages	13	9	34	24	32	34	35	16	30	11	19	24	7	13	13	26
	individual determ.	39	27	106	77	105	110	107	54	94	33	50	78	22	42	39	95
Number of results accepted	laboratory averages	[13]	[7]	23	[23]	24	21	25	13	24	8	9	21	[6]	10	[12]	77
	individual determ.	[39]	[17]	80	[76]	84	70	82	44	82	26	28	71	[20]	36	[37]	65
Total range of laboratory averages		220	385	299	71	59	63	955	5.3	14.6	12	55	528	23.9	22.7	21.8	136
Range of accepted laboratory averages		[22.0]	[50.0]	3.2	[71]	13.8	8.7	149	0.73	5.3	5.0	7.5	193	[3.43]	1.34	[20.2]	9.1
Percentage of outlying laboratories		[0.0]	[22]	32	[4.1]	25	38	29	19	20	27	53	13	[14]	23	[7.6]	35
Overall mean of accepted laboratory averages		[16.4]	[40.8]	6.06	[6.85]	20.2	14.1	454	1.63	7.71	12.4	15.9	288	[2.08]	1.63	[5.6]	9.93
Standard deviation (μg/l)	abs.	—	—	1.08	—	3.3	2.2	38	0.24	1.57	1.8	2.3	55	—	0.52	—	2.38
	rel. %	—	—	18	—	16	16	8.5	15	20	14	14	19	—	32	—	24
Standard error (μg/l)	abs.	—	—	0.22	—	0.7	0.5	8	0.07	0.32	0.6	0.8	12	—	0.16	—	0.58
	rel. %	—	—	3.7	—	3.3	3.4	1.7	4.2	4.2	5.0	4.8	4.2	—	10	—	5.8
Uncertainty limits of the overall mean (μg/l)	abs.	—	—	.47	—	1.4	1.0	16	0.15	0.66	1.5	1.8	25	—	0.37	—	1.22
	rel. %	—	—	7.8	—	6.8	7.1	3.5	9.1	8.6	12	11	8.8	—	23	—	12
Deviation from true value (accuracy) (μg/l)	abs.	[6.4]	[13.2]	−.09	[−4.3]	−1.3	−0.5	10	0.01	−0.72	0.5	−1.0	−16	[−3.06]	−0.36	[−6.3]	0.05
	rel. %	[62]	[48]	−1.5	[−38]	−6.0	−3.7	2.2	.8	−8.5	4.0	−6.0	−5.5	[60]	−18	[−53]	0.5

Note: Values in brackets indicate a normal distribution of results for the element concerned; only the median and its accuracy were computed.

Each participant received three glass ampoules with a concentrated, acidified solution of the appropriate salts. The solution was to be diluted by the participants in 2 liters of double distilled, acidified water. The participants were asked to perform at least two determinations of the blank values of water used for dilution and to return the results of these blanks together with the gross results. Unfortunately in some cases the results for blanks (or rather the lower limit of detection determined on the blanks) were not much smaller than the determined amounts, and a simple substraction of the blanks lead to unrealistic values.

One of the ampoules contained the principal elements of fresh water (SO_4, PO_4, SiO_2, Cl^-, Na^+, Ca^{++}, Mg^{++}, and Al^{+++}) so that the water sample in spite of being artificial was more realistic than the air filters.

The concentration of the above mentioned elements was of the order of 10 μg/l; only that of Fe and Pb was of the order of 300 μg/l; that of U and Hg of the order of 2 μg/l.

Table 4 summarizes the results of the intercomparison. It can be seen that for all elements except As, Ba, Cr, Se, V, and U, the accuracy is satisfactory, but the number of outliers is rather great in all cases. In this intercomparison the outliers were rejected on the basis of a more selective test (t-test) [4] than that used for the air filters [5]. This may explain the rather good accuracy obtained in the determination of Cd, Cu, Fe, Hg, Mo, Pb, and Zn. The large percentage of outliers, generally between 20 and 40 percent of participants, may be due to the fact that about one half of the participants tried to analyze the diluted sample without any preconcentration, *i.e.*, at levels not far from the limit of detection of their equipment.

III. Conclusions

The results of two intercomparisons of trace element analysis in environmental samples have shown that:

- some elements contained in trace amounts in such samples are difficult to determine at the average laboratory (*e.g.*, Cr, As, Ni and Cu; to a smaller extent also Cd, Hg and Se).
- there is an unexpectedly large number of laboratories which returned results outside the expected range, proving that the reliability of the analytical procedures in the average laboratory is too small.

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ACCURACY ASSURANCE IN THE ANALYSIS OF ENVIRONMENTAL SAMPLES

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Three soils, two freeze dried blood pools, two (NBS) plant leaf samples, and one (NBS) freeze dried liver sample were analyzed in an interlaboratory program undertaken by NSF(RANN). Three independent methods produced agreement within ±20 percent for the soils and pooled blood. The values for the plant leaves were within the experimental error as estimated by the standard deviations given for the round robin results. The liver results showed excessive scatter.

Subsequent tests included the use of NBS reference samples, the preparation of secondary reference materials, resubmission of previously analyzed samples, and the use of several methods for the same determination. Methods used for cross checking included atomic absorption with and without chemical separation, nonflame atomic absorption, arc emission spectroscopy, plasma emission, anodic stripping voltammetry, neutron activation analysis and x-ray fluorescence. In most cases the agreement between methods was good, but enough problems were identified and subsequently corrected to establish the value of the program.

The steps taken in the two laboratories represent an adequate, yet practically attainable program to assure accurate results in a centralized analytical support laboratory for a university or other large research organization.

Keywords: Accuracy; anodic stripping voltammetry; arc emission spectroscopy; atomic absorption; environmental analyses; interlaboratory tests; neutron activation analysis; plasma emission; round robin; x-ray fluorescence.

I. Introduction

In any analysis it is always appropriate to question the accuracy of the determination. The analyst must therefore develop and maintain what may best be described as a healthy skepticism concerning his results. If said skepticism develops, quality assurance naturally becomes an important, integral part of the routine and nonroutine operation of the analytical laboratory. In spite of the fact that an analytical chemist deals exclusively with material things, his reward is often only spiritual, *i.e.*, the satisfaction that he should derive from knowing that his results have integrity.

Thirty years ago, Lundell [1] pointed out that there are four important details of concern to an analyst. These are: the sample to be analyzed, the analytical method utilized, the accuracy of the result, and the cast of the analysis. Certainly, the first two have determinative effects on the analytical accuracy. If the analytical sample is not truly representative of the material to be analyzed, the validity of the analytical result for the purpose intended will be degraded even though the method utilized is highly accurate. Neither can the cost of analysis be considered to be totally independent of the other three factors of importance. If large numbers of samples are analyzed as one means of assuring that the sample sets provide accurate representations of the systems under investigation, the cost/time commitments are affected. The necessity of using a method which requires complex instrumentation and/or analytical methodology also affects the economic considerations as does the maintenance of a quality assurance program. Ironically, cost considerations most frequently serve as the excuse for failing to carry out even the most rudimentary quality assurance checks. This general philosophy appears to prevail even though it is indisputable that investigations based on the use of erroneous analytical results may be much more expensive and professionally embarrassing. Laboratories concerned with analysis of environmental samples are perhaps most susceptible to the analytical inaccuracy problem. This derives in part from the fact that a large number of environmental samples delivered for analysis have been collected by individuals from other disciplines who have little or no experience with the difficulties that attend the selection of representative samples or with the steps needed to protect the sample after collection. Many erroneous decisions have been based on samples which were not worthy of the analytical effort required. Additionally, environmental samples are inherently diverse in terms of type and general compositional variability. As Lundell [1] has pointed out, numerous methods work well for the determination of elements which occur alone. Problems with these methods originate because "ele-

ments never occur alone, for nature and man both frown on celibacy." In self-defense then the environmental analyst must be fully aware of the possible effects of compositional variations on his analytical results and he must educate the users of his services regarding appropriate sample selection criteria and handling methods. Both are accomplished by maintaining quality assurance programs, one dealing with the laboratory analyses and the other with the sampling operations. Since the protocol for the collection of environmental samples is highly dependent on the nature and purpose of the investigation program in question, an informative discussion in this area is too complex to be covered here. Quality assurance in an analytical laboratory also represents a complex problem, but the required protocol can be formulated in general terms outside the context of any particular analysis or analysis requirement. The twofold purpose of the present communication is to outline approaches for checking the integrity of analytical methods and demonstrate the utilization of these with actual results.

II. The Question

Quality assurance in an analytical laboratory refers specifically to the question: Are the analytical results valid, accurate, or reliable? Obtaining an answer to this query is complicated by the fact that all measurements are subject to random (indeterminate) errors which always exist and, at the same time, may be subject to systematic (determinate) errors which may or may not exist for the method. Errors are cumulative so those which characterize the accuracy of any analysis result may include the composite effects of: the random errors; the systematic errors inherent in the analysis method used; and the random and systematic errors characteristic of each particular analyst, laboratory, or set of equipment involved in the analysis [2]. Random errors may be sufficiently large to obscure systematic errors. In general, the random errors (the imprecision) must be smaller than the systematic errors (the inaccuracy) if the latter are to be detected and their magnitude(s) estimated. If this cannot be done, any statements regarding the accuracy of the analyses will be limited by the reproducibility of said results. For these reasons, the evaluation of accuracy is not as simple as one might wish. Also, it is well to keep in mind from the start that the cost of the program must be reasonable. Accordingly, an operating laboratory must base its accuracy assurance program on some combination of several possible strategic approaches.

III. The Strategy

A good accuracy assurance program must be based on the analysis of material representative of the general composition and the range of compositional variability of the samples themselves. If this representation is a good one, the quality assurance program can be expected to provide results which will have integrity in the eyes of even the most critical referee. Such materials may be: certified standards such as those provided by NBS, "in-house" (secondary) standards accumulated and documented in one's own laboratory, or actual samples which are merely resubmitted to the laboratory. It is perhaps ironic in this age of environmental concern that the key strategy of operation is one of recycling the materials through the analytical process. By continuing repeat analyses of the material available, it is possible to obtain evaluations of the integrity of the results. As illustrated in figure 1, these evaluations must be based on a combination of approaches which include spike-recovery studies, comparison of independent (in-house) methods, and participation in collaborative test programs. Each of these approaches will provide specific types of information relevant to the overall program. Thus it is appropriate to consider each separately.

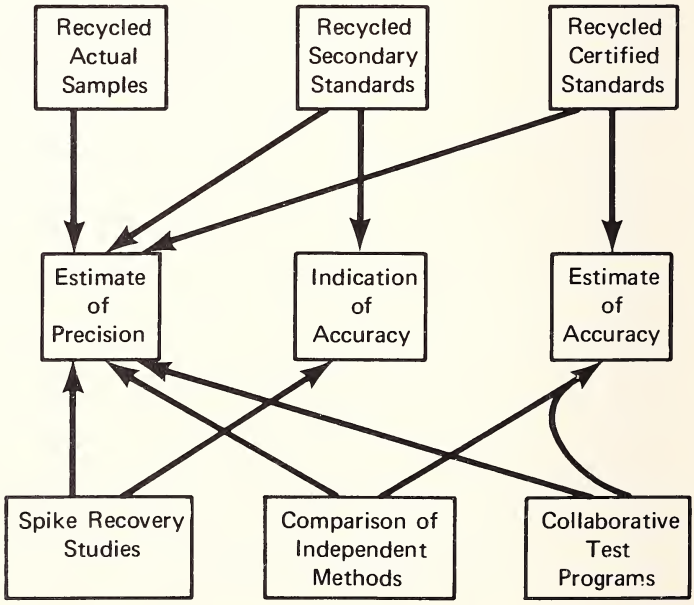

Figure 1. Schematic representation of the steps in an accuracy assurance program.

A. RECYCLING OF SUBMITTED SAMPLES

The principal information that can be derived from the resubmission of user samples to the laboratory is an estimate of the precision of the analytical method used on a day-to-day or week-to-week basis. All analyses to be repeated on any material should be based on resubmission under a disguised sample designation to avoid prejudicing the analyst. Also, the samples resubmitted must come from a homogeneous batch of material or sampling errors may disguise analytical problems. It cannot be assumed that the results are correct unless other independent measurements are carried out for verification purposes because only precision, not accuracy, is checked in this way.

Typical results from a repetitive sample submission program carried out over a period of several weeks at the University of Missouri are given in table 1. Clearly, these results demonstrate that the analytical process is

TABLE 1. *Results of analyses of sample resubmitted to the lab under blind numbers*

Sample submission number	Element concentration, ppm			
	Pb	Cd	Zn	Cu
M10555[a]	0.90	0.096	0.24	0.36
M10589	.90	.092	.29	.39
M10793	.90	.090	.25	.37
M21157	.95	.097	.28	.40
M30360	.85	.090	.25	.36
M30430	1.00	.099	.28	.40
Means	0.91	.095	.26	.37
Relative standard deviation	4.2%	4.0%	7.5%	6.3%
C111A[b]	22	0.43	59	
C112A	24	.41	68	
C113A	21	.42	50	
C211A	22	.40	54	
C212A	17	.38	61	
C213A	20	.32	50	
C121A	25	.43	64	
C122A	25	.46	59	
C123A	21	.44	57	
C221A	24	.41	63	
	22	.21	59	
Relative standard deviation	11.3%	9.4%	10.0%	

[a] All samples designated M were the same water samples run at the University of Missouri Repeat runs were spaced over a period of several weeks.

[b] All samples designated C were the same soil samples run at Colorado State University. Repeat runs were spaced over a period of several weeks.

under control in that the results obtained are reproducible to less than 10 percent.

When two or more analysts are involved in a particular determination, the sample recycling approach may be used to gauge the proficiencies of the respective analysts. If one individual consistently produces results which systematically deviate from the results of others, steps should be taken to correct the systematic errors inherent in his application of the method. An example of this type of check is given in table 2. In this case the fact that analyst C's results were significantly high in several cases was traced to an error in standard preparation.

TABLE 2. *Use of sample recycling as a means of monitoring analyst proficiency*

Sample number	Analyst number	Element concentration, ppm ± std. dev.		
		Cd	Pb	Zn
1	A	0.43 ± 0.01	22 ± 2	58 ± 5
	B	.44 ± 0.01	19 ± 2	56 ± 5
	C	.75 ± 0.08[a]	28 ± 2[a]	70 ± 5[a]
2	A	.42 ± 0.02	238 ± 12	71 ± 6
	B	.35 ± 0.05	225 ± 15	69 ± 7
	C	.75 ± 0.10[a]	250 ± 10	83 ± 10
3	A	.38 ± 0.04	104 ± 8	62 ± 7
	B	.33 ± 0.04	102 ± 8	67 ± 5
	C	.57 ± 0.06	97 ± 10	

[a] Indicates significantly deviant result at the 95 percent confidence level. High results traced to standard preparation error.

B. SPIKE-RECOVERY STUDIES

The practice of analyzing a sample to determine the native concentration of the analyte, spiking it with a known amount of the analyte, and analyzing the spiked material to estimate the accuracy of a method *via* the recovery determination is a widely used method analogous to the use of the method of standard additions. It is generally argued that this practice subjects the analyte spike to the same interference effects experienced by the native analyte and it is usually assumed to be a reliable means of estimating accuracy. This assumption may be valid in numerous instances but can often be rendered invalid by at least three operational hazards. First, the added analyte species may in fact be quite chemically and/or physically different than the native analyte and therefore may not undergo the

same interactions responsible for the interference effect. Second, if the interference in question is dependent on the relative concentrations of the analyte and the interferent, the spike may cause a change in this dependence with a concomitant shift in the extent of the interference. This latter problem is particularly critical when the interference effect is nonlinear in nature. To detect the existence of this type of occurrence, it is advisable to spike the sample at two or more levels. A systematic trend in the recoveries observed, *i.e.*, increasing or decreasing with analyte concentration, generally indicates the presence of an interference of this type. The third type of hazard is an interference which displaces the signal by a constant amount regardless of the analyte concentration. In such cases spike recoveries can be perfect but the analysis may still contain large errors. Uncompensated background effects in atomic emission or absorption methods are a good example of this effect. Spike recoveries will be totally unaffected by the background in spite of the large error it can introduce into the analysis.

A systematic and consistent departure from complete recovery for a particular matrix is a quite rigorous indication of the presence of an interferent in that matrix and appropriate corrective steps can be taken. In essence, spike recovery studies such as those summarized in table 3 are quite easily run and they can be used in a diagnostic sense provided that other quality assurance procedures have indicated the validity of this approach for the types of samples and analytes of interest. With few exceptions, the recoveries in table 3 are quite satisfactory.

C. Independent Method Comparisons

A convenient means of monitoring for possible analytical inaccuracies involves the analyses of samples by two or more independent methods. In this context, the term "independent" strictly denotes that the measurement processes of the methods must be based on totally different phenomena. Flame emission and flame absorption, for example, would not normally be considered independent methods because both measurements rely on the same phenomenon, *i.e.*, the production of a free atom population in a flame. If, however, the sample was delivered to the flame in two different forms for, say, an atomic absorption measurement, the methods might be regarded as independent because the production of the free atom populations would be based on different phenomenological processes. For example, one might compare results obtained by direct nebulization of an aqueous sample with those obtained by nebulization of organic extracts of the same samples (example data are given in table 4).

TABLE 3. *Results of spike recovery studies on complex matrices*

Sample number	Matrix type	Percent recovery			U
		Pb	Cd	Zn	Cu
M0578	dustfall	90	108	98	96
M0588	dustfall	90	92	94	92
M1153	dustfall	97	96	90	95
Mean recoveries		92.3	98.7	94.0	94.3
M1125	humus	103	102	96	95
M1236	humus	104	98	96	91
M1260	humus	100	80	96	90
M0331	humus	116	90	104	92
M0431	humus	110	110	96	—
M0441	humus	104	120	94	100
Mean recoveries		104.5	100	97.0	93.6
M0366	leaves	100	90	104	92
M0386	leaves	100	106	96	100
M0411	grass	105	140	93	102
Mean recoveries		101.7	112	97.7	98.0
C634	soil	98.9	98.1	96.2	97.7
C716	soil	100.6	101.6	98.9	93.0
C802	soil	97.1	101.1	100	102
C871	soil	98.7	100.5	98.8	92
Mean recoveries		98.8	100.3	98.5	96.2

TABLE 4. *Comparison of AAS analyses by direct aqueous nebulization and extraction methods*

Sample number	Cadmium concentration (μg/g)	
	Direct nebulization	Extraction
1170	0.0104	0.011
1172	.0121	.014
1173	.0134	.012
1176	.0147	.015
1178	.0148	.015
1182	.0213	.026

TABLE 5. *Comparison of flame and nonflame AAS results*

Sample number	Lead concentration (μg/ml)	
	Flame AAS	Nonflame AAS
C1001[a]	0.07	0.07
C1007	<.003	.0008
C1014	.007	.005
C1017	.11	.10
C1018	.003	.0018
C1019	<.003	.0004
M1170[b]	.35	.39
M1174	.40	.38
M1177	.70	.39
M11770	.40	.38
M1179	.30	<.40
M1180	.70	.50
M1181	.35	.39

[a] All C samples preconcentrated by a factor of 10 for flame analysis but run directly for nonflame analysis.

[b] All M samples extracted for flame analysis but run direct on nonflame system.

A primary rationale for this type of comparison is based on the separation of the analyte from interferents by the extraction process. Since the results agree well, one may infer that interferences are not prominent for the direct nebulization determination.

Similarly, comparisons in which direct nebulization and nonflame AAS measurements are used for counterchecking results may be considered valid (table 5). Comparisons of this type also demonstrate the relative capabilities of such methods.

In the above cases atomic spectroscopy was used for both measurements. An even greater degree of independence can be achieved by using a completely different method such as neutron activation analysis. Table 6 gives results for thallium in contaminated bird kidney using flame emission and neutron activation analysis (with a chemical separation of the thallium). The good agreement is assurance that both methods are accurate at least in this concentration range.

In-house independent method comparisons serve as a valuable function in an assurance program. Although such cross-checks may be time consuming, it is not essential that a large fraction of the samples be submitted. An additional benefit derives from the possible use of such independent checks as a means of "certifying" concentrations of analytes for in-house standards. The authors routinely maintain method comparison programs to monitor trouble points with analytical methods. The analyst who does not have several methods available is handicapped in this approach but he need not be defeated. Atomic Absorption determination with and without a separation provides a good example of a single method being used in two ways to gain independence.

TABLE 6. *Comparison of thallium in bird kidney by flame emission and neutron activation analysis*

Flame emission	Neutron activation analysis
225 μg/g	232 μg/g
223	219
227	219
	206
	235
225 ± 2	222 ± 12

D. COLLABORATIVE TEST PROGRAMS

Although few analysts will admit that they enjoy participating in collaborative tests, they do serve a useful evaluation function. The Association of Official Analytical Chemists, for example, has the policy of approving an analytical method only after it has undergone a collaborative test. The disadvantage of such a program derives from the fact that the errors characteristic of each of the different participant laboratories,

analysts, instruments, methods, *etc.*, tend to accumulate in the overall results. Too often the values from various laboratories are so variable that one wonders if any valid conclusions from them are possible. However, if the test program is properly designed, it offers a significant advantage, *i.e.*, sufficient analyses are accumulated to provide reliable statistical evaluation of the results. The criteria for, and valuable means of analyzing the results from, a well-designed program have been carefully outlined by Youden [2]. If the sample materials submitted to the collaboratories are available in sufficient quantity and if the results from collaborating laboratories are in good agreement, this means may be used to certify concentrations. The samples can be used in subsequent quality assurance programs with greater confidence than those analyzed by only one laboratory. The results of a limited interlaboratory analysis program involving the determination of lead in simulated blood samples and actual air filter samples are summarized in table 7.

TABLE 7. *Summary results on a limited collaborative test program.*

Sample Number and type	"True" Pb concentration (g/100 ml)	Analytical results[a]		
		Lab 1		Lab 2
		Method		Method
		A	B	A
1—simulated blood	5	7	5	4.8
2—simulated blood	11	14	16	10.7
3—simulated blood	23	29	26	22.1
4—simulated blood	41	43	41	38.2
11A[b]—air filter	—	2.1	—	2.2
11B—air filter	—	1.8	—	2.3
11C—air filter	—	2.3	—	2.0
14A—air filter	—	25.0	—	27.1
14B—air filter	—	28.0	—	24.5
14C—air filter	—	26.8	—	26.-

[a] Dropped figures indicate questionable significance.
[b] A, B, and C designate replicate sections cut from the same filter.

E. Analysis of Certified and Secondary Standards

If standard materials which closely approximate the general matrix composition and the analyte concentration ranges of interest are available, they may be used as a particularly definitive means of characterizing

the accuracy of or delineating problems encountered with analytical methods. An illustrative example may be based on the analysis of the NBS reference material, Bovine Liver, for lead. Initial triplicate analyses indicated a mean concentration of 0.75 μg Pb/gm which was more than a factor of two above the certificate value of 0.34. Subsequent checks initiated because of discordant set of results demonstrated that a reagent in use had mysteriously become contaminated. After the problem was eliminated, repeated recycling of the standard over several weeks produced a mean analytical value of 0.31 ± 0.06.

Comparisons of this type offer the simplest and most reliable means of checking accuracy. The significance of the reference materials supplied by NBS cannot be overemphasized. They have been a boon to quality assurance programs throughout the world. The approach should not be abused by extrapolation of accuracy data obtained on one sample type to another which is entirely different. For the best test of accuracy, any standard submitted to the laboratory should be disguised so it is not given more care and attention than samples.

All of the methods for assuring precision and accuracy add to the work and expense of the laboratory and are likely to be carried out only in centralized analytical laboratories. Many researchers prefer to do their own analytical work but it is difficult to justify the expense of an adequate quality control program unless it can be spread over large numbers of samples. The person supervising the work must be acutely aware of the ways in which the methods can fail and know alternate ways to get the results when a given approach does fail. In the authors' opinion, a centralized analytical facility, equipped for several different methods, staffed by careful analysts, and supervised by a well-trained analytical chemist is one of the better ways to assure quality in the results.

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FOUR-LABORATORY COMPARATIVE INSTRUMENTAL NUCLEAR ANALYSIS OF THE NBS COAL AND FLY ASH STANDARD REFERENCE MATERIALS

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The NBS coal and fly ash Standard Reference Materials (SRM 1632 and 1633) were analyzed for 37 elements in coal and 41 elements in fly ash mainly by the use of instrumental neutron activation analysis (INAA), augmented by instrumental photon activation analysis (IPAA) and direct counting of natural

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γ-ray activity. For most elements measured, there was excellent interlaboratory agreement between the four participating laboratories and with the National Bureau of Standards values for elements measured by them and in this work. In cases of most elements for which comparisons can be made, instrumental nuclear methods used in a round-robin study of the standards provided more accurate average concentrations and smaller interlaboratory dispersions of values than the other major techniques used, atomic absorption spectrometry and optical emission spectroscopy.

Keywords: Analytical methods; coal; environmental samples; environmental standards; fly ash; instrumental neutron activation analysis (INAA); instrumental photon activation analysis (IPAA); natural radioactivity; standard reference materials; trace elements.

I. Introduction

Many laboratories are involved in the measurement of trace-element concentrations in environmental samples. These measurements often present a more severe demand upon analytical methods than most standard industrial or research laboratory analytical problems. In environmental studies, one must often analyze samples that contain a complex mixture of substances, many of which are difficult to dissolve completely. Furthermore, within a given class of environmental samples, there are frequently such large sample-to-sample variations of composition that matrix effects and inter-element interferences also vary greatly. Because of these difficulties, analytical methods that work well with less complex, more easily soluble samples, may not be adequate for some classes of environmental samples. It is thus essential that the methods in use for trace-element analyses be tested *via* the use of standard reference materials that are quite similar to the samples that are to be analyzed routinely. Laboratories engaged in environmental trace-element studies should check their procedures by the use of such standards, since very important decisions may be based upon the results of their analyses, *e.g.*, decisions on trace-element ambient and emission standards having great importance for human health and the spending of enormous sums for pollution controls. In view of these large implications, we must insure that our analytical methods provide accurate answers.

The need for well-characterized environmental standards was demonstrated by a limited round-robin study of coal, fly ash, residual oil and gasoline samples prepared by the Environmental Protection Agency (EPA) and distributed to nine laboratories selected by EPA for their experience in trace element measurements [1]. The results of this round-

robin study, as reported by von Lehmden *et al.* [1], were shocking, as there were often order-of-magnitude ranges of values reported for given elements in particular standards. Although that study demonstrated the problem, it did little to help solve it: since the true elemental concentrations were not known, one could not determine which laboratories and methods provided accurate results and which needed improvement.

In 1972, EPA asked the National Bureau of Standards (NBS) to develop standard reference materials (SRMs) for coal, fly ash, residual oil and gasoline and to help conduct a more extensive round-robin study of trace-element concentrations. The SRMs were distributed to about 75 laboratories and, simultaneously, NBS began measurements for ultimate certification of them for concentrations of about 18 (mostly toxic) elements of prime interest to EPA. Results from the 50-odd laboratories that turned in data and the NBS preliminary values were discussed at a meeting held at EPA in May 1973. This round-robin study was more valuable than the earlier one because, for many elements, we have the NBS values available as a "bench mark" by which to evaluate the accuracy of the various techniques used by participating laboratories.

It should be noted that some critics at the meeting questioned the use of NBS values as the true values for comparison with other reported data. Obviously, one cannot be 100 percent certain that the true values lie within the ranges reported by NBS. However, for all elements certified by them, there must be agreement within experimental errors for the concentrations as measured *via* the use of two distinctly different phenomena. Furthermore, the Bureau's excellent reputation for accurate analyses stands behind their values. Some participants suggested that the average of all round-robin results should be taken as the true value. The fallacy of that suggestion was clearly demonstrated by the round-robin Cd concentrations, which were almost universally greater, often by large factors, than the NBS values (discussed below).

Our four laboratories participated independently in the NBS-EPA round-robin analysis, employing instrumental nuclear methods of analysis. At the meeting at EPA in May 1973, we found that the results from our laboratories for coal and fly ash standards were in generally good agreement with each other and with the NBS values. Since NBS has certified the standards for only 12 and 14 elements of primary interest to EPA, we felt it would be of value to other trace-element laboratories for us to publish our concentrations for about 40 elements in the coal and fly ash standards (SRM 1632 and 1633, respectively). In the course of preparation of the results for publication we have evaluated the accuracy and precision of several methods that were used extensively in the round-robin study. Below we briefly note the experimental techniques used in

this work and evaluate the results obtained both in terms of interlaboratory agreement and in comparison to other methods used in the round-robin study.

II. Experimental Methods

All of our laboratories employed instrumental neutron activation analysis (INAA) as their major technique. In general, samples of coal and fly ash of up to 100 mg each were irradiated in nuclear reactors along with elemental monitors for two or three durations ranging from 30 s up to several hours to emphasize products of various half-life ranges. Following irradiations, γ-ray spectra of the irradiation products in the standards and monitors were taken at several times with Ge(Li) detectors of about 45 cm^3 or greater volume, which produced photopeaks of 2.0 to 2.3 keV full width at half maximum for the 1332 keV line of ^{60}Co. Details of the techniques used have been given previously [2-5]. In addition to INAA, the Maryland group used instrumental photon activation analysis (IPAA) to observe several elements that cannot be measured by INAA and to serve as a cross check for other elements that can be measured by both [6]. Samples were irradiated with bremsstrahlung produced by 35 MeV electrons from the NBS electron LINAC. Also, the Battelle group measured concentrations of K, Th, and U by observing the natural γ-ray activity of these elements in 100-g samples with anti-coincidence-shielded NaI(Tl) multi-dimensional γ-ray spectrometers [7]. Elements measured by the various techniques are listed below in table 1.

III. Interlaboratory Agreement

The total number of laboratory and method values obtained for the various elements measured in this work are listed in table 1. In cases where five values were measured, all four of our laboratories measured the element by INAA and Maryland or Battelle also determined the concentration by IPAA or γ counting of natural radioactivity, respectively. Concentrations of 37 elements in coal and 41 in fly ash were measured. In preparing our recommended values for the concentrations of the various elements in the coal and fly ash standards, we rejected individual laboratory values that were in considerable disagreement with the average of the other laboratory values. It is remarkable that among the total of 230

values reported, only 6 had to be rejected (one value each for Na, Ti and Hf in coal and for Ba, Hf and La in fly ash).

As a measure of the interlaboratory agreement of concentrations measured by our laboratories, we calculated the relative standard deviations (σ/X, also called "coefficient of variation") of laboratory values for all elements for which 2 or more values were determined. The six values rejected from the averages were included in the calculation of relative standard deviations; however, the Sb concentrations in coal were not included, as our sample-to-sample variations of Sb concentration were so large as to indicate an inhomogeneity of Sb in coal for samples of 100 mg or less. (It should be noted that our sample sizes were less than the 250-mg samples in which NBS measured Al and Mn in their homogeneity checks.)

TABLE 1. *Elements measured in NBS Standard Coal (SRM 1632) and Fly Ash (SRM 1633) in this work*

Number of laboratories and method values	Elements	
	Coal	Fly ash
5	K,[a] Ti,[b], As,[b], Th[a]	Ba,[b] Ti,[b] As,[b] Sb,[b] Th[a]
4	Na, Ba, Cl, Br, Sc, V, Cr, Fe, Co, Se, Sb, Hf, La, Eu	Na, K,[a] Sc, V, Cr, Mn, Fe, Co, Hf, La, Eu
3	Rb,[b] Cs, Ca,[b], Al, Mn, Ta, Sm	Rb,[b] Cs, Ca,[b] Al, Se, Ta, Sm
2	Mg, Sr, Ni,[b] W, Ce, Tb	Mg, Sr, Ni,[b] W, Ce, Tb, Yb
1	Zn, Ag, In, Lu, Yb, U[a]	Cl, Br, I,[b] Si,[b] Zn,[b] Zr,[b] In, Pb,[b] Y,[b] Lu, U[a]

[a] Includes one value obtained by observing natural γ-ray activity.
[b] Includes one value by IPAA.

In table 2 are listed the relative standard deviation ranges for the 30 elements in coal and fly ash, except Sb in coal, for which 2 or more values were reported. For 17 elements in coal the standard deviation is less than 10 percent, and for 25 out of 30, less than 15 percent, and all are ≤ 25 percent. For fly ash, the fractional standard deviation was < 10 percent for 16 out of 30 elements, ≤ 15 percent for 26 out of 30, but two values were > 25 percent (W and Yb, both of which are marginal elements by INAA).

TABLE 2. *Relative standard deviations among laboratory values for various elements reported in this work*[a]

Relative Std. Dev. (%)	Coal	Fly ash
0–5	Cs, Cr, Fe, Tb	Rb, Al, Sc, Ti, Cr, Mn, Fe, Co
5–10	Rb, Ca, Ba, Al, Sc, V, Mn, Co, Se, Ce, Th	K, V, Ni, As, Sb, La, Sm, Th
10–15	Na, K, Sr, Cl, Br, Ti, Hf, La, Sm, Eu	Na, Cs, Ca, Ba, Se, Hf, Ta, Ce, Eu, Tb
15–20	Ni, Ta	Sr
20–25	Mg, As, W	Mg
>25		W, Yb

[a] Includes all elements for which there were two or more laboratory or technique values except Sb in coal, which was found to be inhomogeneous.

IV. Comparison with NBS

In table 3 we list the average concentrations measured in this work for elements for which NBS has reported certified values [8]. For both coal and fly ash, there is agreement within the limits of error of the two sets of data for all nine elements measured both by NBS and in this work. For most elements, the ranges of uncertainty are comparable for the two sets of data. Major exceptions are Ni, Zn and As in coal. Nickel is difficult to measure by INAA as it is observed by an (n,p) reaction induced by fast neutrons. In this work we have only two values for Ni, one obtained at Washington State using a Triga reactor (which has a large fast neutron flux) and the other by the less sensitive technique of IPAA at Maryland. Zinc is difficult to determine by INAA in samples having an abundance pattern similar to that of the earth's crust because the 1115 keV γ-ray peak of ^{65}Zn is small compared to the intense 1120 keV line of ^{46}Sc. (By contrast, Zn is so highly enriched relative to crustal material in atmospheric particulate matter in U.S. cities that it can be measured accurately [9].) In the case of As we had five values, three centered near the NBS value and two at 8.0 ppm, but there was no basis for rejecting either group.

For most elements in fly ash, the relative uncertainties are smaller than for coal both for our work and for the NBS values, probably because the

TABLE 3. *Comparison of concentrations observed in this work with NBS values for elements with provisional certification*

Element	Concentration (ppm unless % indicated)			
	Coal (SRM 1632)		Fly Ash (SRM 1633)	
	This work	NBS[a]	This work	NBS[a]
V	36 ± 3	35 ± 3	235 ± 13	214 ± 8
Cr	19.7 ± 0.9	20.2 ± 0.5	127 ± 6	131 ± 2
Mn	43 ± 4	40 ± 3	496 ± 19	493 ± 7
Fe	0.84% ± 0.04%	0.87% ± 0.03%	6.2% ± 0.3%	—
Ni	18 ± 4	15 ± 1	98 ± 9	98 ± 3
Zn	30 ± 10	37 ± 4	216 ± 25	210 ± 20
As	6.5 ± 1.4	5.9 ± 0.6	58 ± 4	61 ± 6
Se	3.4 ± 0.2	2.9 ± 0.3	10.2 ± 1.4	9.4 ± 0.5
Pb	—	30 ± 9	75 ± 5	70 ± 4
U	1.41 ± 0.07	1.4 ± 0.1	12.0 ± 0.5	11.6 ± 0.2

[a] See reference [8].

concentrations of most elements observed are several-fold higher in the fly ash. The ranges of the two values for V in fly ash barely overlap. This is surprising, as the γ-ray peaks of 3.4-min ^{52}V stands out well in spectra taken shortly after irradiations. We suggest the error may be in the timing. The fractional dead time of the analyzer system is often very large in spectra taken within minutes after irradiations and it changes appreciably during counts because of rapid decay of the dominant 2.3-min ^{28}Al and ^{52}V.

V. Comparison with Other Methods Used in the Round-Robin

In table 4 we compare the results of this work with those of the two other techniques for which many data were reported in the round-robin study: optical emission spectroscopy (OES) and atomic absorption spectrometry (AAS). The four elements chosen were the only ones meeting the criteria: provisional NBS values available, large number of values reported from AAS and OES, and element measured in this work. In the upper half of table 4 are shown the fractional errors of the mean values obtained by the various techniques, where fractional error is defined by:

$$\text{Fe } (\%) = \frac{\bar{X} - X_{\text{true}}}{X_{\text{true}}} \times 100,$$

where $\bar{X}$ = mean value and $\bar{X}_{\text{true}}$ is the true concentration, which we take to be the NBS value.

Fractional errors alone do not give a complete evaluation of the reliability of the various techniques. Since our average values agree with the NBS values within limits of errors of the two sets of data and, as our uncertainties are of magnitude comparable to those of the NBS values for most elements, the true value for many elements could lie just as close to our value as the NBS value. Thus, when the two data sets agree so well, the fractional errors become rather meaningless. Nevertheless, we see that in four out of the eight cases (Cr in coal and Cr, Mn and Ni in fly ash) the fractional errors for our values are the smallest listed.

TABLE 4. *Fractional errors of mean values and relative standard deviations of laboratory values for concentrations of several elements in coal and fly ash as measured by three methods*

Element	Fractional error (%)[a]			
	This work	All INAA[b]	AAS[b]	OES[b]
Coal				
V	2.9 (4)	−5.6 (7)	0.4 (6)	22.5 (4)
Cr	−2.5 (4)	−4.2 (7)	16.5 (12)	15 (4)
Mn	7.5 (3)	11.2 (7)	1.0 (14)	2.7 (3)
Ni	20 (2)	—	21.5 (14)	12.0 (4)
Fly Ash				
V	9.8 (4)	1.3 (7)	3.1 (7)	22.9 (3)
Cr	−3.1 (4)	−19.1 (7)	−12.2 (13)	10.7 (3)
Mn	0.6 (4)	5.7 (8)	−15.3 (14)	4.8 (3)
Ni	0 (2)	—	8.2 (15)	12.0 (3)
	Relative Standard Deviation (%)			
	This work	All INAA[b]	AAS[b]	OES[b]
Coal				
V	6.3	15.7	21.5	29
Cr	4.8	24	76	30
Mn	9.5	16	19.5	17
Ni	17	—	34	38
Fly Ash				
V	6.4	11.4	39	29
Cr	3.2	32	60	20
Mn	3.8	15	27	23
Ni	9.3	—	41	22

[a] Values in parentheses indicate number of laboratory values used in computing average and standard deviation.

[b] Reference 10 corrected to latest NBS values [8]. Note that data from our laboratories make up nearly one half of the "All INAA" data.

An equally important test of the methods is the relative standard deviation of all laboratory values for each technique, as given in the lower half of table 4. Here we see that, although in some cases the mean of all laboratory values is close to the NBS value, that may simply represent the average over a very wide range of individual values in which the positive errors happen to balance the negative errors. Take V in fly ash as determined by AAS as an example. The fractional error of the mean value is only about 3 percent, which is quite acceptable; however, the relative standard deviation is 39 percent. This means that there is a 32 percent probability that any particular laboratory value is in error by more than 39 percent! Thus, it is quite important to consider both fractional error and standard deviation in evaluating the methods used.

When considered in view of both factors, it would appear that, with the exception of Ni, the analyses performed by our laboratories, largely by INAA are superior to those done by the other major techniques used in the round-robin. When all round-robin INAA data are included, the fractional errors are somewhat larger and the standard deviations much greater. (It should be noted that nearly half of the "All INAA" data were contributed by our laboratories.) Although the fractional errors for AAS are often reasonably small, the standard deviations in nearly each case are much greater than for the other major methods, especially for Cr. The results for Cd are even worse by AAS. They are not included in table 4 because there are no INAA and OES data available. For coal and fly ash, the fractional errors for Cd by AAS are > 400 and 180 percent, respectively, and the relative standard deviations are 76 and 44 percent! Perhaps surprisingly, the OES technique, which many consider an out-of-date, fairly insensitive method, was intermediate in performance between INAA and AAS for the four elements in table 4.

In view of the large deviations of values discussed above, especially for AAS, we examined the data more carefully to see if perhaps it was just a few participating laboratories that were causing the large deviations. In the upper half of table 5 we show the average fractional errors for each laboratory for up to six elements for which large amounts of AAS values were reported: Cr, V, Hg, Mn, Ni and Pb. Because of the very poor results for Cd, we have not included those values in this analysis. These results indicate that the errors were very large for nearly all laboratories that used AAS, being rarely less than 20 percent. However, two laboratories stand out as doing accurate work with AAS, Laboratories 19 and 22. Although we do not know the identities of those laboratories, the data suggest that they used great care in performing the analyses. It may be noteworthy that neither laboratory reported Cd concentrations as they

TABLE 5. *Average of absolute values of fractional errors (FE) for laboratories using AAS and OES for NBS standards*[a]

Laboratory number	Coal		Fly Ash	
	FE (%)	No. elements	FE (%)	No. elements
AAS[b]				
2	24	2	48	2
4	25	3	16	2
5	59	2	71	2
7	30	5	32	5
8	44	6	27	6
10	37	5	34	6
11	23	3	70	2
12	24	6	26	6
19	9.3	3	16	4
22	12	6	9.5	6
23	26	5	18	5
37	67	4	66	5
47	40	5	18	4
50	80	4	18	4
52	26	4	49	4
OES[c]				
2	27	3	—	—
3	15	3	13	3
3	36	4	19	4
12	20	4	32	4

[a] Based on Akland's statistical analysis [10], updated to present NBS values [8].
[b] Elements considered: Cr, V, Hg, Mn, Ni, Pb; there were also many data for Cd, but agreement was so poor that all were omitted from this analysis.
[c] Elements considered: Cr, V, Mn, Pb.

apparently recognized difficulties with Cd analyses that were not obvious to the other AAS groups that reported values. The good results obtained by these laboratories suggest that it is possible to obtain reliable analyses for the six elements by AAS, but that most laboratories using the method did not use proper care in attempting to do so.

Among the four OES laboratories, Laboratory 3 stands out as being quite accurate and the others had smaller errors than most AAS laboratories.

Average fractional errors for INAA laboratories are given in table 6. For our own laboratories, we have used the values reported during the round-robin rather than our final values that incorporate slight refinements resulting from additional analyses done since the round-robin was completed. For the most part our laboratory errors are much smaller than for laboratories that used AAS and OES. Only the analyses of coal at

Livermore gave average errors > 20 percent. Among the other participating laboratories, Laboratories 6 and 9 had errors comparable with those of our laboratories and only Laboratory 14 had extraordinarily large errors.

TABLE 6. *Average of absolute values of fractional errors (FE) for laboratories using INAA for NBS standards*

Laboratory Number	Coal		Fly Ash	
	FE (%)	No. elements	FE (%)	No. elements
This work[a]				
Maryland	7.1	3	9	3
Washington State	15	3	3.7	3
Battelle	8	4	5	4
Livermore	21	4	11	4
Others[a] [b]				
6	14	2	15	2
9	11	4	14	4
14	75	2	55	2
25	24	3	27	3
44	27	3	33	4

[a] Elements considered: Cr, V, Mn, Se
[b] Based on Akland's statistical analysis [10] updated to present NBS values [8].

VI. Discussion and Conclusions

In the round-robin analysis of the NBS coal and fly ash standards, we have shown that INAA and related nuclear methods can provide reliable analyses of the highly complex, "real world" samples for nearly 40 elements. There was excellent agreement among our 4 laboratories for the 30 elements determined by more than 1 laboratory. There was good agreement between our average values and the NBS certified values for the nine elements in each matrix for which comparisons could be made. Except for Laboratory 14, the results from all of the participating laboratories that used INAA were acceptable.

By contrast, there were very large errors in the data reported by most laboratories that used AAS, especially for Cd and Cr. However, the accurate results obtained by Laboratories 19 and 22 showed that, with considerable care, reliable results can be obtained by AAS. Results from the few laboratories that used OES were intermediate in accuracy between INAA and AAS.

Why do we observe the big difference in reliability between INAA and AAS? The biggest difference is probably that for AAS, one must dissolve the samples—a long, difficult procedure by which volatiles can be lost, insoluble residues may be left behind, contaminants may be introduced in the solvents, or there may be exchange of species with container walls, if proper care is not used. Also there may be interferences between elements during the AAS measurements. There are also some interferences in INAA, *i.e.*, between γ rays of different species or of reactions on different elements that lead to the same product, but these interferences are rather well known, so one can correct for them or not report concentrations for the element affected.

Despite the excellent performance of nuclear methods in this application we would not, of course, suggest that they should replace all other techniques. The nuclear methods have their disadvantages, too: they require access to reactors, expensive equipment, and personnel highly skilled in fundamental nuclear techniques. Furthermore, the turn-around time of analyses for elements with long-lived products is usually at least 2 weeks and there are several important elements for which no adequate nuclear technique is available, notably S, Be and Cd. (Unfortunately, the round-robin results show that there is no other adequate method for Cd, either.) However, despite these disadvantages, the nuclear methods have so many attributes that they can be used very effectively in many difficult trace-element applications.

VII. Acknowledgements

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TRACE ELEMENT STUDIES OF A SELECTED PORTION OF THE MAHONING RIVER SYSTEM

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The Mahoning River flows through a highly industrialized section of eastern Ohio. It is used as a dumping ground for both solid and liquid wastes by both industry and municipalities along its banks.

Both water and sediment samples were collected at predetermined sites on a section of the Mahoning River running from its headwaters into and beyond one of the industrialized areas. The water samples were analysed for trace elements by neutron activation analysis (NAA) employing Youngstown State University's 10 mg Californium-252 neutron source, wet chemical techniques, and atomic absorption (AA). Certain trace elements, Ca and Mg, in the water samples were detected by all three methods. Comparison of results showed good agreement between NAA and wet chemical analysis for most samples. AA data, for the most part differed considerably from both NAA and wet chemical analysis. Subsequent tests have shown that organics, which are plentiful in the Mahoning River, are responsible for erroneous results in AA analysis.

Neutron activation analysis was shown to be the simplest of the three methods for trace element analysis and the location of possible sources of pollution.

Keywords: Atomic absorption analysis; Californium-252; Mahoning River; neutron activation analysis; pollution; sediment; trace elements; water; wet chemical analysis.

I. Introduction

In December 1971, Youngstown State University began trace element studies of the Mahoning River System. This paper summarizes the collection and analysis procedures and results of studies done on a section of that river running from its headwaters to the outskirts of the city of Youngstown.

The Mahoning River has been used for many years as an open sewer by heavy and light industry in the area. Located along its banks are a number of steel mills and metal processing and fabricating plants; until the late 1960's raw and partially treated sewage was also being dumped into the river rystem.

The section of the river chosen for sampling runs from a region of rather clean water to a heavily industrialized section.

Since trace elements can vary both horizontally and vertically in a given river cross section, samples were collected in consistent fashion. The collection of samples consisted of lowering a 10-quart galvanized pail weighted symmetrically with several lead discs into an approximate midstream position from a bridge. At a few collecting sites this procedure was impossible. The alternative procedure involved tossing the bucket into midstream and drawing it to shore.

Containers used for storage of the water samples were 1 liter polyethylene bottles having screw-type caps. These containers were rinsed several times with river water from the collection site before addition of the sample. After a sample addition, the pH of the sample was reduced to two by the addition of reagent grade nitric acid. This was done to avoid precipitation or plating out on the container walls of trace elements [1]. In addition, temperature and pH measurements were made at each location with the use of pH paper and a mercury thermometer. The sample collection sites were distributed in such a way as to test whether or not village and/or urban pollution existed. Data obtained from the on-site analysis is given in table 1.

Analysis of the water samples was performed by three different methods, these being neutron activation analysis (NAA), wet chemical techniques and atomic absorption (AA). A Buchner funnel with a double layer of Whatman No. 1 qualitative filter paper was used with a vacuum flask to filter the water samples.

A. Neutron Activation Analysis

The NAA work on the filtered water samples necessitated the use of 125 ml of each placed in pre-cleaned polyethylene screw-top bottles of uniform size. The samples were irradiated for 1 hour in the Youngstown State University (YSU) Californium storage and experiment facility using a 10 mg Cf^{252} source. After irradiation the samples were permitted to decay for 30 seconds then transferred to a Plexiglas cup surrounding a Ge(Li) detector (the arrangement yielding a very efficient counting

TABLE 1. *Collection site information on samples numbered 1 to 14*[a]

Sample number	Location	Temp. (°C)	Initial pH of water samples[b]
1	Bridge located on Mahoning-Trumbull County Line Road (east branch of Mahoning River)	9.5	~7
2	Bridge located on Portage County Highway #117 (west branch of Mahoning River)	9.0	~6
3[c]	Effluent from North American-Rockwell Plant (east branch of Mahoning River)	16.0	~9
4	Approximately 50 feet downstream from Site #3 (east branch of Mahoning River)	10.0	~7
5	Bridge located on Ohio State Route #5 (after Ohio Turnpike intersection)	9.3	~7
6	Bridge located on Ohio State Route #5 bypass	9.0	~7
7[c]	Bridge located on Parkman Road (U.S. Route #422) in the city of Warren, Ohio	9.0	~7
8[c]	Effluent from Copperweld Steel Fabrication Plant	10.0	~6
9[c]	Bridge located on North West Bridge Street in the city of Warren, Ohio	9.0	~6
10	Bridge located on Market Street in the city of Warren, Ohio	9.0	~6
11	Bridge located on West Park Avenue Extension (west of the city of Niles, Ohio)	10.0	~7
12	River embankment near the intersection of Pratt and River Streets in the city of Niles, Ohio	9.0	~6
13	Bridge located on Belmont Street in the city of Niles, Ohio	9.0	~6
14	Bridge located on Olive Street in the city of Niles, Ohio	12.0	~6

[a] All samples were collected on November 12, 1973.
[b] pH adjusted to ~2 with concentrated HNO_3 to prevent precipitation of aqueous components and/or their "plating-out" onto container walls.
[c] Only water sample collected.

geometry) and counted for 20 minutes using a 400 channel analyser. Quantitative analysis was performed by comparison of peak areas of samples to peak areas of standards. Volume size of the sample, irradiation and decay times, and the geometry of the counting system was the same both for samples and standards.

B. Atomic Absorption

The AA analysis was performed using a Perkin-Elmer Model 107 Atomic Absorption Spectrometer. The water samples were prepared in exactly the same manner as those for the NAA determinations. Both water samples and standards were analysed according to the procedures outlined in the General Information Manual supplied with the spectrometer. Due to the limited number of lamps available only five elements were detected: these were Ca, Cu, Mg, Pb and Zn.

Because the Mahoning River contains many organic compounds it was decided to check the addition of certain organics to standards used in the AA analyser. Table 2 summarizes these results for two elements tested. These studies show that the results of YSU's AA apparatus for Ca and Mg are significantly affected by the presence of certain organics.

C. Wet Chemical Analysis

Wet analysis followed very closely the procedures detailed by Brown *et al.* in U.S. Geological Survey, Book 5 [2].

II. Discussion

Comparison results were obtained for Mg, Cu, Cl, and Ca. In general, excellent agreement was obtained between wet analysis and NAA. The AA data, in most cases, differed considerably from both NAA and wet analysis. The authors believe this to be due to interfering substances such as the organics discussed earlier.

The authors believe the Mg results obtained from wet chemical data by subtraction of calcium values from the total hardness values, show that in the Mahoning River, other elements, particularly iron as iron (II) ion contribute significantly to total hardness.

Distribution of the results shows a significant and abrupt increase in nearly every element detected at site 12, and this is believed to be due to

TABLE 2. *Effects of organic reagents on atomic absorption concentration values of calcium and magnesium standards*

Type of standard utilized	Actual concentration values of standards, ppm	Instrumental display of standard concentrations, ppm			
		Without organic reagents	With phenol[a]	With sodium acetate[a]	With acetic acid[a]
Calcium	0.5	1.01 ± 0.01	1.27 ± 0.01	Not performed	Not performed
Magnesium	.5	0.573 ± .010	0.642 ± .003	0.788 ± 0.038	0.763 ± 0.029

[a] The addition of the organic reagents varied between 0.5 g (in the case of phenol) and 1.0 g (in the cases of sodium acetate and acetic acid) per 50 ml of each standard.

elements introduced into the Mahoning River by Mosquito Creek.

It was generally concluded that of the three methods used, AA results were least reliable and internally consistent as they appeared to fluctuate because of interfering elements and compounds present in the river water.

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ANALYSIS OF 11 ELEMENTS IN BIOLOGICAL MATERIAL. COMPARISON OF NEUTRON ACTIVATION ANALYSIS AND ATOMIC ABSORPTION ANALYSIS

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Seven trace elements (Co, Cu, Fe, Mn, Mo, Se, Zn) and four bulk elements (Ca, K, Mg, Na) were analysed by means of neutron activation analysis and flame or flameless atomic aborption analysis.

The organic material was destroyed by wet or low temperature ashing. Four different possibilities of analysis result from this arrangement. Time of analysis, accuracy, reproducibility and real, not theoretical, detection limit will be reported for each arrangement. The analyses were carried out with the 1577 NBS liver standard and our own liver standard. The techniques will be discussed in reference to clinical and research applications. The reported results will be based on analysis of liver biopsies and animal experiments.

Keywords: Atomic absorption spectroscopy (AAS); comparison between NAA and AAS; neutron activation analysis (NAA); time of analysis in NAA and AAS; trace element content in liver biopsies; trace element content in tumor bearing animals; Wilson's disease.

I. Introduction

Neutron activation analysis and atomic absorption spectroscopy, which are used in our laboratory, are among the most sensitive analytical techniques known for trace element determination. Our objective was to test the precision, or reproducibility, of both methods. A secondary objective was to investigate the accuracy of the techniques, using our standard solutions. These have been used for 1 year. All analyses were per-

formed using the NBS liver standard 1577. All tests were done with the assistance of trained technicians under routine laboratory conditions. We did not intend to retest the composition of the liver standard.

II. Experimental

The NBS liver standard was used for the determination of seven trace elements (Co, Cu, Fe, Mn, Mo, Se, Zn), and four bulk elements (Ca, K, Mg, Na). About 10 g of the standard were divided into 30 portions, each weighing between 150 and 200 mg. Three separate sets, each containing 10 samples, were formed.

Ten samples were placed into quartz containers, which were thoroughly sealed. They were irradiated together with standard solutions in a reactor. The irradiation was carried out in the rotating position of the TRIGA Mark I reactor, at the German Cancer Research Center. The irradiation lasted 50 hours in a thermal flux of 2×10^{12}n cm^{-2} s^{-1}. After a "cooling period" of about 14 days the samples were measured by gamma-ray spectroscopy without further handling. The measuring equipment consisted of a sample changer with a Ge(Li) detector, and a 4000-channel analyser (Didac 4000, Intertechnique) connected on line to a 12K-computer (Multi-8, Intertechnique). The detector was a 60 cm^3 Ge(Li) detector, with 12 percent efficiency and 2.3 keV resolution. The concentration of the elements Co, Fe, Se and Zn was determined using 2 hours counting time. The statistical error was less than 3 percent. All calculations, necessary for the quantitative determination, were performed by the computer; it also controlled the sample changer.

The next 10 samples were placed into polyethylene containers. The irradiation was carried out in the fast pneumatic facility of the above mentioned reactor. The irradiation time was 2 minutes, in a thermal flux of 4.8 $\times 10^{12}$n cm^2 s^{-1}. The elements Ca, Cl, Mg, Mn could be measured after 1 minute waiting time. The counting time lasted 2 minutes. After this the samples were irradiated again in the rotating position for 4 hours. After the end of the irradiation the samples were wet ashed by means of H_2SO_4 and H_2O_2. The volume was adjusted to 5 ml by adding bi-distilled water. The solution was divided into equal parts. The first part was used for direct measurement of the elements K and Na. The concentration of the elements Cu, Mn, Mo and Zn was determined after radiochemical separation [1,2]. The other half of the solution was used to measure the elements Ca, K, Mg, Na, Cu, Fe, Mn, Mo and Zn by means of the atomic absorption technique (see below).

The last 10 samples were placed into wide quartz containers and ashed

in a low temperature asher (Tracerlab LTA 600). The ashing time was 40 hours. The residual was dissolved in 1 ml of 0.1 m HCl and divided into equal parts. The concentration of the elements Cu, Mn, Mo, Zn, K and Na was determined by means of neutron activation analysis, as described above. From the second half of the solution the elements Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na and Zn were measured using atomic absorption spectroscopy. For this technique we used a Perkin Elmer spectrophotometer 300, a carbon rod atomizer HGA 72 and a deuterium compensator. The concentration of the elements Ca, Fe, K, Mg, Na and Zn was determined using an air-acetylene flame. The other elements studied, Co, Cu, Mn and Mo, were measured by using the carbon rod atomizer. All parameters, for the different element determinations, were chosen as recommended by the manufacturer. In an additional experiment we tried to determine which part of the various procedures used in flameless atomic absorption spectroscopy is responsible for the total variance. For this purpose three normal rats were killed, and their livers removed, freeze-dried and homogenized. From each liver, three samples weighing about 150 mg were ashed. After ashing, three dilutions were prepared from all samples. Three single detections for copper were done for each solution. Eighty-one determinations resulted from this arrangement. With the aid of an analysis of variance it was possible to detect where the variability had its source [3]. Error was sought in the animals, the ashing equipment, the dilutions and the single analyses.

III. Results and Discussion

The results of the investigation are presented in tables 1 through 6.

Tables 1 and 2 show the mean and the single standard deviation values of the investigated elements for neutron activation, and atomic absorption analysis respectively. Both methods are in good agreement with each other. High Zn values resulted from atomic absorption spectroscopy using wet ashing. This was not found by low temperature ashing. The results furthermore indicate that no loss or large contamination occurs during the low temperature ashing process. The activation analysis of Ca and Mg can only give a hint as to the element concentration. The counting efficiency for Ca is too low for reliable measurement. Since the photopeak of Mg (840 keV) is superimposed onto that of Mn (845 keV), the sample must be recounted after the decay of Mg. The contribution of Mn in the sample is subtracted from the original counts. This double measurement

TABLE 1. *Analysis of the NBS liver standard 1577 by means of neutron activation analysis (μg/g dry weight)*

Element	Set 1[a] $n = 10$	Set 2[b] $n = 10$	Set 3[c] $n = 10$	NBS[d]
Ca	—	—	80 ± 30	123
Co	—	—	0.21 ± 0.02	0.18
Cu	191 ± 6.2	185 ± 6.8	—	193
Fe	—	—	265 ± 16	275
K	—	—	10400 ± 300	9700
Mg	—	—	659 ± 82	605
Mn	10.2 ± 0.45	10.1 ± 0.5	9.9 ± 0.47	10.3
Mo	3.3 ± 0.3	3.5 ± 0.2	—	3.2
Na	—	—	2330 ± 60	2430
Se	—	—	1.04 ± 0.07	1.1
Zn	124 ± 7.3	127 ± 8.0	123 ± 5	130
Cl	—	—	2550 ± 100	2600

[a] Samples ashed prior to irradiation by low temperature ashing.
[b] Samples wet ashed after irradiation with H_2SO_4 and H_2O_2.
[c] Instrumental neutron activation analysis.
[d] Mean values published by the NBS [4].
[a,b,c] Mean ±1 standard deviation.

TABLE 2. *Analysis of the NBS liver standard 1577 by means of atomic absorption spectroscopy (μg/g dry weight)*

Element	Set 1[a] $n = 9$	Set 2[b] $n = 10$	NBS[c]
Ca	106 ± 3.2	—	123
Co[d]	0.20 ± 0.016	—	0.18
Cu[d]	186 ± 5.5	188 ± 9.8	193
Fe	272 ± 9.5	266 ± 10	275
K	9600 ± 600	—	9700
Mg	605 ± 32	—	605
Mn[d]	10.3 ± 0.36	9.6 ± 0.6	10.3
Mo[d]	3.4 ± 0.15	—	3.2
Na	2400 ± 200	—	2430
Zn	128 ± 3.6	147 ± 7.3	130

[a] Samples low temperature ashed.
[b] Samples wet ashed by means of H_2SO_4 and H_2O_2.
[c] Mean values published by the NBS [4].
[d] Analyzed by flameless technique. a, b mean.
[a,b] Mean ±1 standard deviation.

resulted in a counting error of about 12 percent.

The mean values published by the National Bureau of Standards are listed in the last column of each table [4]. A comparison of these values with those which we found show that the accuracy is better than 10 percent, excepting Ca (13%) and Co (16%). The relative standard deviations are listed in table 3. These make it possible to compare the precision within the different sets. This shows that atomic absorption spectroscopy is slightly more precise than neutron activation analysis. This may be the result of the examination procedure used. One measurement of each sample was made using neutron activation analysis, while three measurements were performed with the atomic absorption technique. Atomic absorption resulted in a high variability of K and Na. This may have been caused by contamination. The results show once again that high precision may not yield good accuracy. Our precision was 3 percent while our accuracy was 13 percent for the Ca determination.

TABLE 3. *Comparison of the relative standard deviations of the described analytical methods*

Element	INAA (%)	AAS (%)
Ca	37	3
Co	9.5	8[b]
Cu	3.6[a]	3[b]
Fe	6	3.5
K	3	6
Mg	12	5
Mn	5	3.5[b]
Mo	9[a]	4.5[b]
Na	3	9
Se	7	—
Zn	4	3
Cl	4	—

[a] Neutron activation analysis.
[b] Flameless atomic atsorption spectroscopy.

The results of the analysis of variance are presented in table 4. The mean squares and the expected mean squares are shown for the copper determination using low temperature ashing and the carbon rod atomizer. The biological variability between the animals influenced the total variance most. The repeated measurements of samples also contributed considerably to the variance. The smallest errors were caused by ashing and dilution.

TABLE 4. *Analysis of variance for the determination of copper using the carbon rod atomizer*

Source of variation	Degrees of freedom	Sum of squares	Mean square	Expected mean square
Animals	2	30.6	15.3	0.55
Ashing	6	2.2	0.4	0
Dilution	18	7.5	.5	.04
Replicates	54	20.5	.38	.38

The time of analysis for 20 samples is recorded in table 5. One man-day was calculated at 6 hours. The values do not include the time for the low temperature ashing or irradiation. The atomic absorption technique is faster, if only one element has to be analysed. The time for a multi-element analysis is slightly less with neutron activation. The last two columns of table 5 show the detection limits for the described techniques. These concentrations result in a measurement error of 10 percent. For neutron activation the limits can easily be lowered two to three powers of ten using longer irradiation and measuring times and when applying NaI detectors instead of Ge(Li) detectors.

The results show that both techniques are comparable in the determination of the investigated elements.

TABLE 5. *Time of analysis and minimum determinable amounts of the investigated elements referring to the described analytical methods*

Element	Time (man-day)		Amount (ng)	
	NAA	AAS	NAA	AAS
Fe		0.5	10,000[b]	200[c]
Zn		.5	500[b]	50[c]
Co	0.5	1	3[b]	1[d]
Se		—	100[a]	—
K		0.5	—	—
Na		.5	—	—
Cu		1	5[b]	1[a]
Mn	2.5	1	1[b]	0.5[a]
Mo		1	100[b]	.5[d]
Zn		0.5	500[b]	59[c]

[a] One man-day equals six hours.
[b] This amount should be in a sample weighing about 10 to 200 mg.
[c] Concentration (ng/ml) for the flame-technique.
[d] This amount of the element is necessary for the flameless-technique.

IV. Application

We use neutron activation analysis for the determination of Cu, K, Mn, Mo, Na and Zn, and atomic absorption spectroscopy for the detection of Ca, Co, Fe, Mg and Zn, because both techniques are available to us. When we work with small samples, we use neutron activation omitting the determination of Ca and Mg.

We use both methods for studying the behavior of trace elements under normal and pathological conditions. We have made extensive studies of the behavior of Cu, Mn and Zn in different organs while experimental tumors were growing in rats [5]. In tumor bearing animals the concentration of Cu was significantly higher in spleen and plasma and lower in the kidneys than in the control groups. The Cu-concentration was identical in the liver of the experimental and control group. The concentration of Mn was significantly lower in the liver and the kidneys of the tumor bearing animals, while the spleen showed no difference to the non tumor group. The concentration of Zn in the experimental group was significantly lower in plasma, higher in the liver and the kidneys. The Zn-content of the spleen showed no difference between the two groups.

TABLE 6. *Cobalt, calcium, iron, manganese and zinc concentration in liver biopsies taken from four sisters (μg/g dry weight)*

Subject	Biopsy taken (year)	Co	Cu	Fe	Mn	Zn
1	1969[b]	—	86	—	—	—
	1970	—	74	—	—	—
	1971	—	63	—	4.0	670
	1972	1.2	42	370	6.5	814
2	1969[b]	—	118	—	—	—
	1970	—	34	—	—	—
	1971	0.91	39	260	8.4	1020
	1972	1.2	31	490	7.7	1170
3[a]	1969[b]	—	1890	—	—	—
	1970	—	1550	—	—	—
	1971	0.81	1140	45	6.2	1660
	1972	.59	1320	160	4.0	1230
4[a]	1969[b]	—	1000	—	—	—
	1970	—	1050	—	—	—
	1971	0.69	1210	108	4.0	1480
	1972	.71	1100	90	6.5	1630

[a] The Cu-determination in 1969 was performed by Dr. I. H. Scheinberg, Albert-Einstein College, New York.

[b] Subjects suffering from Wilson's disease and treated with pencillamine.

Parallel to the animal experiments we are investigating the trace elements Co, Cu, Fe, Mn and Zn in liver biopsies. Up to now we have investigated too few biopsies to draw definite conclusions. Table 6 shows the results of the biopsies of four sisters, two of whom suffer from Wilson's disease [6]. The copper content is typically elevated in the two patients. The high copper concentration could be lowered slightly by treatment with penicillamine. The low concentration of Fe was possibly also the result of the treatment with this drug. The increased Zn values were not caused by contamination. The livers of all four sisters showed pathological alteration, fibrosis, which may have been the reason for the high Zn values.

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DETERMINATION OF ZINC IN ENVIRONMENTAL MATRICES: A COMPARISON OF RESULTS OBTAINED BY INDEPENDENT METHODS

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Zinc determination results in environmental matrices as reported by different laboratories in some recent interlaboratory comparisons, seem to have large biases. In order to achieve a better solution of the problem, an evaluation of four different methods for the determination of zinc content in environmental matrices is underway in our laboratory.

The suggested methods are: a) XRF x-ray fluorescence, b) NAA(I) instrumental neutron activation analysis, c) NAA(RC) neutron activation analysis, *via* radiochemical separation, and d) AAS atomic absorption spectrometry. Each of these techniques gives information on some critical step typical of each different method. XRF and NAA(I) require no sample treatment or dissolution or chemical procedures, and insure no loss of any component and no danger of contamination. Unfortunately some interference due to some matrix content may occur, affecting in both methods the accuracy of the results. NAA(RC) techniques give results free from interferences from contamination, but require wet dissolution of samples or high temperature treatment which may affect the analytical results. AAS analysis may be affected by contamination from reagents and by the difficulties related to the sample dissolution. A critical evaluation of results acquired through these different methodologies on a dust sample is discussed.

Keywords: Accuracy; atomic absorption; environment; neutron activation analysis; precision; x-ray fluorescence.

I. Introduction

As we move down from the field of measurements in interference free or easily soluble samples into applied measurements, the task of making accurate measurements becomes increasingly difficult. This statement seems to be true not only for elements such as mercury, selenium and other highly volatile elements, but also for many "common" metals as zinc.

Zinc determination results in environmental matrices as reported by different laboratories in some recent interlaboratory comparisons [1,2], seem to be affected by large biases. In order to achieve a better solution of the problem, an evaluation of four different methods for the determination of zinc content in environmental matrices was carried out in our laboratory. The methods investigated were: a) XRF x-ray fluorescence, b) NAA(I), instrumental neutron activation analysis, c) NAA(RC), neutron activation analysis, *via* radiochemical separation, d) AAS, atomic absorption spectrometry. The results acquired by each of these techniques give information on some critical steps typical of each different method.

XRF and NAA(I) do not require sample treatment or dissolution or chemical procedures, and insure against loss of any component and against danger of contamination. Unfortunately, interferences due to some matrix content may occur in some element determinations affecting in both methods the accuracy of the results.

NAA(RC) techniques give results free from interference from contamination, but require wet dissolution of samples or high temperature treatment which may affect the analytical results.

AAS analysis may be affected by contamination from reagents and by the difficulties connected with the sample dissolution.

The matrices actually examined with each of these methods were the NBS-SRM 1633 (Fly ash) and an environmental sample of urban particulates, collected by Euratom in the central area of Milan, during winter months.

II. Experimental

A. X-Ray Fluorescence (XRF)

The energy dispersive analysis [3] *via* semiconductor detector was

adopted for the zinc content determination. $A^{241}Am$ 10 mC annular source was used as the exciting radiation. The electronic equipment employed was a Si(Li) semiconductor detector connected to a LABEN 400 channel pulse-height analyzer. The total resolution of the system, FWHM, was 200 eV at 6.4 keV (FeK gamma-ray energy). The method of additions analyses on a single sample was adopted for each determination. Samples of approximately 50 mg were counted in a polyethylene vial stoppered with a Mylar thin film. The samples were counted upside down, with only the Mylar thin film interposed between the sample and the detector.

Each sample was first counted as such and afterwards spiked with a standard zinc solution. After each addition, the sample was freeze-dried and well homogenized by thoroughly mixing with a silica rod and then recounted on the x-ray fluorescence spectrometer.

The base concentrations of zinc in the SRM 1633 and in the Urban Dust sample were determined by fitting a least squares regression to the individual results. The least squares fit gave a base concentration of 213.5 and 7472 respectively. The individual results are given in table 1.

TABLE 1. *Determination of zinc content by x-ray fluorescence, using method of additions*

Sample	Zinc found	
	NBS SRM 1633[a] (ppm)	Urban dust[a] (ppm)
1	210.6	7340
3	213.5	7437
3	215.3	7255
4	212.2	7530
5	216.0	7800
Average	213.5 ± 2.2[b]	7472 ± 210[b]

[a] Content evaluated by least squares.
[b] Errors are standard deviation.

B. INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS NAA(I)

The instrumental neutron activation analysis for the zinc determinations was performed with a high resolution Ge(Li) gamma-ray spectrometer coupled to a LABEN 4096 channel pulse-height analyzer. The total resolution of the system, FWHM, was 2.5 keV at 1332 keV (^{60}Co gamma-

ray energy). The long-lived ^{65}Zn isotope was chosen as the most suitable for this purpose. Samples of approximately 50 mg were irradiated for 10 hours in the sample-holder rotating position of the LENA reactor, at a flux of $\sim 0.5 \times 10^{12} n \cdot cm^{-2} \cdot s^{-1}$. Each set of samples to be analyzed, sealed in plastic snap-top vials, was irradiated together with a primary reference standard of zinc, which was made by adsorbing a known amount of a standard solution on a strip of filter paper and freeze-dried before being sealed in the plastic vial. The irradiation and the counting geometry are of primary importance on the precision of the instrumental measurements. It is not possible to resolve the 1115 keV gamma ray from ^{65}Zn and the 1120 keV gamma rays from ^{46}Sc.

A correction of the ^{46}Sc interference in zinc determination *via* ^{65}Zn isotope is essential for reliable results. Both the ^{65}Zn and ^{46}Sc are long-lived isotopes, and a long decay period of the irradiated samples is not an acceptable solution.

The correction for the ^{46}Sc contribution on the ^{65}Zn 1115 keV gamma-ray photopeak is easy to evaluate *via* the interference free 890 keV gamma-ray photopeak from ^{46}Sc. In this instrumental determination the scandium contribution was taken into account, and the results of the zinc content, after a proper correction for the Sc contribution are given in table 2.

TABLE 2. *Determination of zinc content by instrumentation neutron activation analysis*

Sample	Zinc found	
	NBS SRM 1633 (ppm)	Urban dust (ppm)
1	260.2	7800
2	165.3	7670
3	180.1	7550
4	170.5	7500
5	263.2	7600
Average	208.1 ± 49[a]	7624 ± 117[a]

[a] Errors are standard deviation.

C. NEUTRON ACTIVATION ANALYSIS VIA RADIOCHEMICAL SEPARATION NAA(RC)

The high temperature distillation method [4] was utilized in the radiochemical separation of zinc. Since the wet chemistry dissolution

method is a "must" in atomic absorption spectrometry, a completely different method was adopted for the neutron activation analysis determination of zinc *via* radiochemical separation.

This method consists mainly in a combustion of the sample in an oxygen stream and then a distillation of volatile elements as Zn, Cd and As in a CO stream, which reduces all the metal compounds to elemental form. The distilled elements are collected in a liquid nitrogen cold trap, and counted on a high resolution Ge(Li) pulse-height analyzer.

In this way, any interference from ^{46}Sc gamma radiation is prevented. The results of the analysis carried out with this method are reported in table 3.

TABLE 3. *Determination of zinc content by neutron activation analysis with radiochemical separation*

Sample	Zinc found	
	NBS SRM 1633 (ppm)	Urban dust (ppm)
1	190.2	7400
2	200.3	7300
3	210.5	7300
4	198.2	7200
5	203.4	7400
Average	200.5 ± 7.4[a]	7320 ± 84[a]

[a] Errors are standard deviation.

D. ATOMIC ABSORPTION SPECTROMETRY (AAS)

For the atomic absorption determination, a Jarrel-Ash spectrometer was used. An acetylene-air flame was utilized for the flame atomizer, which was a laminar flow burner. Fifty to 100 mg samples were treated during each analysis and the perchloric-nitric acid process was adopted for the dissolution. The process was completed in a Teflon beaker and a few drops of hydrofluoric acid was added after the first perchloric-nitric attack. A final recovery with hydrochloric acid was adopted, and the solution brought to volume.

The results of these analyses are summarized in table 4.

TABLE 4. *Determination of zinc content by atomic absorption spectroscopy*

Sample	NBS SRM 1633 (ppm)	Urban dust (ppm)
1	190.3	5800
2	170.5	5800
3	180.2	5900
4	185.2	5700
5	177.3	5750
Average	180.7 ± 7.6[a]	5790 ± 74[a]

[a] Errors are standard deviation.

III. Results and Discussion

A comparative review of the analytical results obtained with each single method is summarized in table 5. In this table the quoted errors are expressed as standard deviation in percent.

For the NBS-SRM 1633, the certified value is 210 ppm [4] and some considerations can be made if we compare this value with the experimental values obtained with the four different methods. X-ray fluorescence gives good results both for precision and accuracy. The limitation is the sensitivity for trace content at the ppm level. Moreover the additions method, though the most accurate and reliable, is time consuming and not handy for routine determinations.

The instrumental neutron activation analysis seems to give good results when the interference from ^{46}Sc in the photopeak used in the determination of zinc is low.

In fact, the larger error in the determination of zinc content in the NBS-SRM 1633 is mainly due to the high content of scandium in the fly ash. The urban dust [5] has a comparatively lower content of scandium and gives better results. More precise results are obtained with the radiochemical separation of zinc, but the results seem to be lower than the certified value.

As for the atomic adsorption results, they give precise results but the experimental values are somewhat lower than those obtained with the other techniques, probably due to some loss of zinc during the dissolution process.

The reported values of the experimental results may be affected by

the quality and the actual conditions of the technical equipment utilized and by the skill of the experimenters in the different processes.

The results of these intercomparisons illustrate the actual situation of the laboratory where the analyses were done.

TABLE 5. *Comparative review of results for zinc determination*

Sample	XRF (ppm)	NAA (I) (ppm)	NAA (RC) (ppm)	AAS (ppm)
NBS SRM 1633	213.5 ± 1	208.1 ± 24	200.5 ± 4	180.7 ± 4
Urban dust	7472 ± 3	7624 ± 2	7320 ± 1	5790 ± 1

[a] Error expressed as percent standard deviation.

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RELIABILITY OF TRACE METAL DETERMINATION IN FRESHWATER BY FLAMELESS ATOMIC ABSORPTION (GRAPHITE TUBE ATOMIZATION) IN COMPARISON WITH OTHER CHEMICAL AND PHYSICAL METHODS

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An interlaboratory study of trace metal analyses involving seven different laboratories has been carried out, using river water samples and "synthetic" standards. The analytical precision for the flameless atomic absorption with graphite tube atomization was also established for eight different metals at various trace concentrations. The flameless atomic absorption method with graphite tube atomization is compared with 16 other techniques and methods used in the interlaboratory study, and the conclusion is reached that for a number of commonly determined metals the flameless atomic absorption method of measuring trace concentrations in dilute fresh water is as accurate a method as other more established methods. The relative standard deviation for interlaboratory determination of traces of zinc is consistently large, which indicates that this metal is relatively more difficult to measure accurately than most other metals that were investigated. The available analytical data for arsenic is small but sufficiently discrepant to indicate that arsenic is a most difficult metal to measure accurately at trace levels by most of the methods tried.

The accuracy of determination for most of the metals investigated would appear to lie within 20 to 45 percent, at the concentration levels prevalent in unpolluted fresh water.

Keywords: Accuracy; flameless atomic absorption; freshwater; interlaboratory comparison; method evaluation; trace metal analysis.

I. Introduction

An interlaboratory study was conducted in which seven different laboratories participated. The purpose of the study was to compare trace metal determinations on dilute water samples by flameless atomic absorption using graphite tube atomization with determinations by other frequently used methods, and in this way obtain an estimate of the accuracy of this method for the direct determination for trace metals in fresh water. Useful previous evaluations of this method exist. Segar and Gonzales [1] evaluated the heated graphite atomizer for its applicability to determining directly several trace metals in sea water. Such an evaluation is not readily extrapolated to the analysis of fresh water. Bernard and Fishman [2] have made an evaluation of the usefulness of this method for routine determination of trace metals in water. In each case the comparison is made with a single analytical method, namely flame atomic absorption. The present study differs from previous studies in that a number of different methods and techniques, rather than a single method, are used as a basis for comparison. The analytical precision of the flameless method for a number of different elements was established by different workers, for example Fernandez and Manning [3,4], as well as others [5,6].

Unlike precision, the accuracy of an analytical method cannot be ascertained by comparison to a single method because no single method can be relied upon absolutely. The constant errors inherent in each instrument and method only collectively assume random character and in this way are revealed. Only by comparing analytical data from different sources obtained with different instruments and different methods can the reliability of a method ultimately be verified and practical certainty be attained.

Some discrimination in the selection of laboratories was exercised. Laboratories that participated in this study all have a well-established reputation in trace metal analysis. The purpose of the study was revealed in advance to the participants, and this probably induced most of them to analyze more carefully and not treat the samples as routine samples. It is therefore reasonable to assume that the analytical results given here represent a best achievement under present conditions. Since the object of the study was not evaluation of performance but evaluation of a method the departure from the more common practice in interlaboratory studies where laboratories are chosen more indiscriminately and the samples are treated as unknowns, is desirable and possibly a necessary condition for an evaluation of this type.

II. Results and Discussion

It is evident from the analytical results, tables 1, 2, 3, 4, that cadmium, cobalt and chromium in fresh water and synthetic aqueous samples can be determined by flameless atomic absorption with graphite tube atomization (Massman, method 6A) as reliably as by any of the other methods. Zinc and lead are determined in some samples (and not in others) somewhat too low by this method, while iron manganese and copper are determined somewhat too high in some instances. However, in three out of four samples zinc and lead determinations by the Massman method agree with the interlaboratory average within the interlaboratory deviation, and for other metals such agreement is only lacking in the case of sample 3 (spiked natural water). With the possible exception of flame atomic absorption with preconcentration of sample by evaporation (method 1) which gives distinctly more concordant results for iron, the Massman method of measuring trace metals in dilute waters by direct injection of the sample without any pretreatment appears to be as good a method as any for the other metals also. It is also apparent that unless the concentration of iron to be measured is relatively low, the flame atomic absorption method in conjunction with complexing, extraction and aspiration of the organic solvent (method 4A) without prior acid digestion, determines iron consistently too low. A difference in preanalysis treatment of the sample can obviously introduce a substantial systematic error which can then denigrate the reliability of the method itself. This emphasizes the importance of maintaining uniformity in preanalysis sample treatment when evaluating a method especially when different laboratories are involved. It may very well be that part of the confusion in the past over the reliability of the flameless atomic absorption method for measuring trace metals was due to variation in pretreatment of samples.

The arsenic concentration in all samples was sufficiently low to preclude successful determination of this metal by flameless atomic absorption by direct injection of the aqueous sample (Method 6A, this work). An arsenic electrodeless discharge lamp which would have perhaps made possible such determinations was not available commercially at the time these analyses were made. The available arsenic data as a whole (table 5) are too small in number and too discordant to give meaningful standard deviations. Means and deviations were therefore not calculated for arsenic. In some cases the arsenic values obtained by different methods for the same sample differ by a factor larger than 1,000. That these discrepancies were analytical and not due to errors in arithmetic or manipulation was verified subsequently with the respective

TABLE 1. *Sample designated sample 1 is a synthetic sample having approximately the composition of river water in terms of major ions. Trace metal determinations by seven different laboratories using a number of different analytical methods are given*[a]

Laboratory	Method	Fe	Mn	Cd	Zn	Cu	Co	Cr	Pb
1	1	114	69	22	31	—	—	—	—
	2	—	78	—	—	81	22	24	4.8
	3	—	—	—	—	—	—	—	—
2	4	93	—	19	14	80	20	—	23
	4A	88	—	19	14	80	19	—	18
	6	170	—	18	13	82	18	—	8
3	7	170	90	—	—	90	25	—	—
	8	—	90	28	—	90	25	13	50
	9	—	—	—	—	—	—	—	—
	10	170	—	—	—	—	—	—	—
4	1	125	84	23	21	80	23	—	27
	12	—	—	—	—	—	—	20	—
5	1A, 4A	100	70	17	11	70	19	20	18
6	9A	<3000	—	21	55	—	22	27	—
	9B	—	100	—	—	—	—	—	—
	5	—	—	—	—	65	—	—	—
This work	6A	131	86	24	15	85	23	20	13
Synthetic	μg/l	120	80	20	21	80	20	20	20
Mean	μg/l	129	83	21	23	80	21	21	21
Number of analyses		8	7	8	7	9	9	5	7
Standard deviation	μg/l	36	11	3.5	16	8.1	2.6	5.3	15
Relative standard deviation %		28	13	17	70	10	12	25	71

[a] All concentrations are in μg/l.

TABLE 2. *Sample designated sample 2 is unpolluted i.e., low trace metal concentration, river water (Old Crow River, Yukon, Canada), Trace metal determinations by seven different laboratories using a number of different analytical methods are given*[a]

Laboratory	Method	Fe	Mn	Cd	Zn	Cu	Co	Cr	Pb
1	1	218	5	—	21	—	—	—	—
	2	—	5.6	0.4	—	38	<1	<1	1.4
	3	—	—	—	—	—	—	—	—
2	4	220	—	1	12	36	ND	—	3
	4A	190	—	1	10	34	ND	—	3
	6	330	—	0.4	20	33	ND	—	1
3	7	370	5	—	—	30	<0.5	—	—
	8	—	<5	<5	—	30	—	—	<5
	9	—	—	—	—	—	—	—	—
	10	370	—	—	—	—	—	—	—
4	1	290	8	<2	14	31	4	—	12
	12	—	—	—	—	—	—	<10	—
5	1A, 4A	250	4	<1	12	30	1	<1	1
6	9A	2000	—	<7	17	—	<1	1	—
	9B	—	20	—	—	—	—	—	—
	5	—	—	—	—	10	—	—	—
This work	6A	300	12	<1	9	39	4	5	2
Mean	μg/l	280	8	0.7	15	30	4	1	4
Number of analyses		8	6	4	7	9	1	2	6
Standard deviation	μg/l	71	6.1	0.34	4.3	8.1	—	—	4.2
Relative standard deviation	%	25	77	49	29	27	—	—	117

[a] All concentrations are in μg/l.

TABLE 3. *Sample designated, sample 3, is river water (Old Crow River) spiked with trace metals. Trace metal determinations by seven different laboratories using a number of different analytical methods are given*[a]

Laboratory	Method	Fe	Mn	Cd	Zn	Cu	Co	Cr	Pb
1	1	189	71	22	42	—	—	—	—
	2	—	81	—	—	84	20	19	16
	3	—	—	—	—	—	—	—	—
2	4	160	—	18	24	92	20	—	23
	4A	138	—	18	25	87	20	—	24
	6	220	—	11	48	104	25	—	23
3	7	245	90	—	—	120	25	—	—
	8	245	90	27	—	120	25	15	24
	9	—	—	—	—	—	—	—	—
	10	245	—	—	—	—	—	—	—
4	1	220	92	19	28	89	19	—	25
	12	—	—	—	—	—	—	16	—
	13	—	—	—	—	—	—	—	—
5	1A, 4A[b]	140	90	18	30	70	16	<1	19
	11	—	—	—	—	—	—	—	—
6	9A	<3000	—	25	57	—	18	17	—
	9B	—	80	—	—	—	—	—	—
	5	—	—	—	—	33	—	—	—
This work	6A	273	96	18	38	112	25	18	16
Mean	μg/l	200	85	20	36	89	21	17	22
Number of analyses		9	7	8	7	9	9	4	7
Standard deviation	μg/l	45	7.8	4.9	13	27	3.3	1.7	3.3
Relative standard deviation	%	23	9.2	25	36	30	16	10	15

[a] All concentrations are in μg/l
[b] Used for low concentrations of metals.

TABLE 4. *Sample designated sample 4 is a synthetic "standard" (U. S. Environmental Protection Agency, Trace Metals Reference Sample) without major ions and was analysed as an "unknown". Trace metal determinations by seven different laboratories using a number of different laboratories using a number of different analytical methods are given*

Laboratory	Method	Fe	Mn	Cd	Zn	Cu	Co	Cr	Pb
1	1	18	11	—	15	—	—	—	—
	2	17	13	1.5	—	11	<1	8.9	29
	3	—	—	—	—	—	—	—	—
2	4	24	—	2	10	10	ND	—	25
	4A	23	—	2	8	10	ND	—	28
	6	—	—	—	0	0	—	—	—
3	7	—	—	—	—	—	—	—	—
	8	35	10	—	—	10	3	—	—
	9	—	—	—	—	—	—	—	—
4	1	30	13	3	14	11	6	—	33
	12	—	—	—	—	—	—	10	—
5	1A, 4A	40	20	2	10	15	<1	10	30
	11	—	—	—	—	—	—	—	—
6	9A	<2000	—	<15	27	—	3	9	—
	9B	—	10	—	—	—	—	—	—
	5	—	—	—	—	5	—	—	—
This work	6A	32	13	2	8	13	3	12	29
Synthetic (EPA)	µg/l	18	13	1.8	10	9.0	—	9.2	28
Mean		27	13	2	14	10	4	9.5	29
Number of analyses		7	6	5	6	7	3	4	5
Standard deviation	µg/l	8.6	3.8	0.5	6.9	2.9	1.7	0.6	2.9
Relative standard deviation	%	32	29	24	49	29	43	6.3	10

[a] All concentrations are in µg/l.

TABLE 5. *Some arsenic values for four different water samples as determined by different laboratories using different analytical methods*

Laboratory	Method	Sample 1	Sample 2	Sample 3	Sample 4
1	3	<1	1	25	21
3	8	—	—	—	195[a]
	9	<20	20	10[a]	80[a]
4	13	—	—	10	—
5	11	—	—	20	4
6	9B	—	—	—	200[a]
Synthetic (EPA)		—	—	—	22

[a] In mg/l.

laboratories. This indicates that arsenic is one of the more troublesome metals to measure reliably, and that not all the popular means of measuring arsenic are satisfactory.

In table 6, are given some operational parameters for the flameless atomic absorption method (Method 6A) and analytical precision estimates for this method as obtained by the author for three different concentrations for each metal. The estimates were obtained from repetitive injections (10 to 12) of demineralized water-metal solutions of the respective metals. Except for Pb, the precisions are worst at the lowest concentrations, and in general improve with increasing concentration provided the upper concentration limit of the linear response range is not exceeded. As expected, the precision for each metal, even at the lowest concentration level, as given in table 6, is still better (except for Co at the lowest concentration) than the relative standard deviations in tables 1 through 4. The latter deviations were calculated from the individual concentration values obtained by the different methods of analysis and different laboratories. These errors are therefore inter-method errors, and as such they are reasonable estimates of accuracy rather than mere precision.

Considering the total number (30) of relative standard deviations tabulated (tables 1-4) *i.e.*, for each metal and each sample, 80 percent of this number have a magnitude smaller than 45, 73 percent of this number have a magnitude smaller than 33, and 30 percent of this number have a magnitude smaller than 20. The distribution of coefficients of variation is shown in figure 1, where the magnitude of all the coefficients of variation is graphed in increments of 10 percent against the relative number (%) of coefficients of variation within each such increment. The dashed vertical lines delimit the range, 20 to 45 percent, which contains the largest number of coefficients of variation. It would therefore appear that most of

TABLE 6. *Some operational parameters and updated analytical precisions for graphite tube flameless atomic absorption*

Component analyzed	Wave length (nm)	Concentration level (μg/l)	Relative standard deviation[a] (%)	Upper limit of linear working range[b] (μg/l)	Practical lower limit[c] (μg/l)	Absolute detection limit[d] (g)	Sensitivity, g to give 1% absorption[d]
Fe	284.33	10	16	150	5	2×10^{-11}	5×10^{-11}
		30	11				
		50	3.8				
Cu	324.75	5	6.9		2.5	1×10^{-11}	7×10^{-11}
		10	6.3	300			
		25	5.2				
Pb	283.31	5	3.3		2.5	1×10^{-10}	5×10^{-10}
		15	5.2	1000			
		30	9.5				
Zn	213.86	5	18		1	1×10^{-12}	2×10^{-12}
		10	3.6	20			
		20	1.7				
Cd	228.80	5	11	20	1	1×10^{-12}	3×10^{-12}
		10	15				
		20	2.9				
Co	240.72	10	77		5	1×10^{-10}	8×10^{-11}
		30	5.5	100			
		50	4.2				
Cr	357.87	10	5.9		5	2×10^{-10}	3×10^{-11}
		30	2.4	150			
		50	4.0				
Mn	279.48	10	15				
		30	9.2	50	5	2×10^{-11}	5.5×10^{-11}
		50	5.3				

[a] Based on 10 to 12 determinations (for each concentration level) of demineralized water-metal solutions.
[b] Using a 50 μl injection volume.
[c] Still useful measurements with a single 50 μl injection volume.
[d] From Perkin Elmer analytical methods manual for Atomic Absorption Spectroscopy using HGA70 unit.

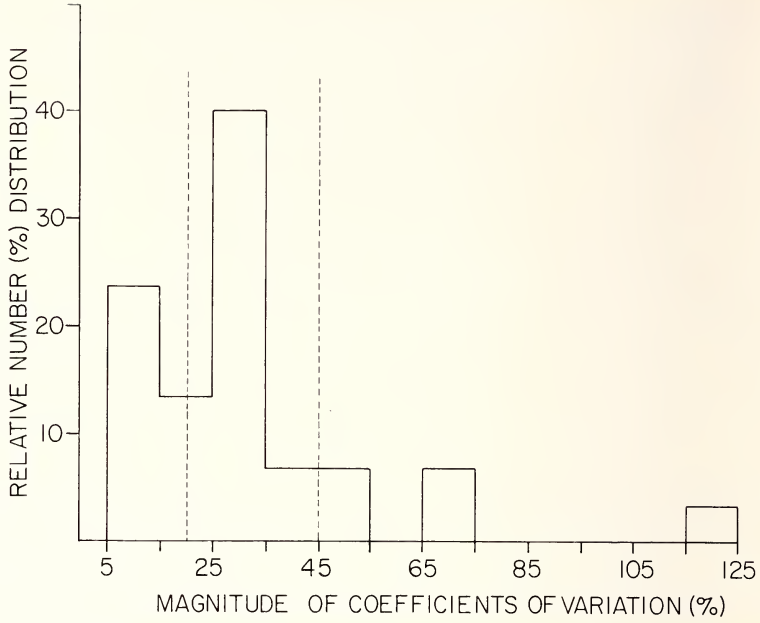

Figure 1. The magnitude of coefficients of variation of all metals and samples in successive increments of 10 units (%) *vs.* the relative number (%) of coefficients of variation within each such size increment.

the metals analyzed for can be determined with an accuracy of 20 to 45 percent at the concentration levels generally prevailing in unpolluted fresh water.

III. Acknowledgements

The author thanks the participating laboratories for their analytical work which most of them performed without charge. Without their cooperation this investigation would not have been possible.

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APPENDIX I

DESCRIPTION OF SAMPLES DISTRIBUTED FOR ANALYSIS

Sample 1

A 20 liter water sample containing trace metals and approximating the composition in major ions (except nitrate) of natural water was made from the appropriate metal nitrates, chlorides and sulfates. The water sample was acidified with nitric acid to pH 3.3. Major ion concentrations (synthetic) are given in the accompanying table. Distilled, demineralized water was used. The acidified bulk water sample was stored for 4 months in the cold room (4 °C) and 1 week at room temperature prior to distribution for analysis. The synthetic trace metal concentrations are listed in the table of analytical results for Sample 1.

Concentrations of major ions synthetic in sample 1.

Aluminium	120 μg/l
Calcium	45 mg/l
Potassium	2.0 mg/l
Magnesium	12 mg/l
Silicon	1.2 mg/l
Sodium	30 mg/l
Chloride	18 mg/l
Sulfate	87 mg/l
Nitrate	2806 mg/l

Sample 2

Forty liters of river water were collected from the OLD CROW river (Yukon) and stored for 4 months in the cold room at 4 °C. The entire sample was then filtered through 0.45 μm membranes, acidified with nitric acid to pH 2.8, and stored for an additional 2 months in the cold room. The sample was equilibrated at room temperature for 2 weeks prior to distribution for analysis. Major ion concentrations (analytical) are given below.

Concentrations of major ions in sample 2

Calcium	5.0 mg/l
Potassium	0.24 mg/l
Magnesium	1.8 mg/l
Silicon	2.9 mg/l
Sodium	1.1 mg/l
Chloride	5 mg/l
Sulfate	101 mg/l

Sample 3

Sample 2 was divided into two equal portions after filtration and acidification. One portion was spiked with trace metals. The spiked bulk river waters sample constitutes sample 3. This sample was otherwise treated in exactly the same way as sample 2.

Sample 4

This is a synthetic water sample supplied by the U.S. Environmental Protection Agency and designated by them as EPA Reference Sample, Trace Metals number 1. The sample is supplied in sealed glass ampoules as a 20 ml concentrate. This sample was made up according to instructions by using 5 ml of the concentrate and 1.5 ml of nitric acid and diluting to 1 liter with demineralized water. The pH of the final solution was measured as 2.96. This sample does not contain major cations found in natural water, but contains trace metals only. The "true" concentrations of trace metals as supplied by EPA, which are the synthetic concentrations are listed in the table of analytical results for sample 4.

APPENDIX II

DESCRIPTION OF METHODS

Method 1: Flame atomic absorption.

Treatment

Sample was acidified with HNO_3, preconcentrated twenty-fold by evaporation to near dryness, made up to volume with demineralized water and aspirated.

Method 2: Flameless atomic absorption using graphite rod atomization. Background absorption corrected with hydrogen continuum radiation from hydrogen hollow cathode lamp.

Treatment

Direct injection of aqueous sample (5 μl) without prior treatment or preconcentration.

Method 3: Flameless atomic absorption as in method 2.

Treatment

Aqueous samples were acidified with HNO_3, photooxidized in quartz vessels for 4 hours, reduced (AsV – AsIII) with sodium metabisulfite and complexed with diethylammonium diethyldithiocarbamate (DDDC) and extracted into CCl_4. The organic phase was injected and analyzed.

Method 1A: Flame atomic absorption.

Treatment

Direct aspiration of aqueous sample without preconcentration or pretreatment.

Method 4: Flame atomic absorption.

Treatment

Sample was digested with HNO_3, complexed with ammonium pyrrolidine dithiocarbamate (APDC) and extracted into methyl-isobutyl ketone (MIBK). The organic phase was aspirated into flame.

Method 4A: Flame atomic absorption.

Treatment

Same as in method 4A, except no prior acid digestion.

Method 5: Neutron activation using a thermal neutron reactor flux of 1×10^{12} n · cm^{-2} · s^{-1}.

Treatment

Pre-irradiation concentration by complexing with (APDC) ammonium pyrrolidine dithiocarbamate (at pH 2.8), extraction of complex into $CHCl_3$ and removal of solvent by evaporation. Residue in polyethylene vials irradiated for 5 hours concurrently with 100 μg of standard deposited on glass-fiber filter. After cooling overnight, sample residue and standard dissolved with aqua regia and HCl respectively, transferred to counting bottle and made up to 15 ml. Activity measured and spectrum analyzed in the same way as in method 9A.

Method 6: Flameless atomic absorption using graphite tube atomization with deuterium arc and nonabsorbing line correction for background absorption.

Treatment

Direct injection of aqueous sample without prior digestion or preconcentration.

Method 6A: Flameless atomic absorption using graphite tube atomization without correction for background absorption.

Treatment

Same as in method 6.

Method 7: Flameless atomic absorption using atomization from a molybdenum filament at 2100 °C.

Treatment

Samples of 5 to 60 μl were directly evaporated on a molybdenum filament at 100 °C. The filament temperature was then raised to 700 °C for 15 seconds to remove organic matter before sample atomization. Hydrogen was the purge gas for all elements except cobalt and nickel. For the latter 5 percent hydrogen in argon was used.

Method 8: Emission spectrography using a graphite electrode impregnated with polystyrene.

Treatment

Samples were concentrated tenfold by evaporation in Teflon beakers. 80 μl aliquots were evaporated in each electrode. Excitation was by DC arc at 10.5 amps for 108 seconds, and spectra were recorded on SA-1 Kodak film.

Method 9: Neutron radioactivation using a reactor neutron flux of 1×10^{13} n · cm^{-2} · s^{-1} was used to determine arsenic.

Treatment

Samples were irradiated in high purity polythene containers for 15 minutes. After separation of ^{24}Na activity by adsorption on hydrated antimony pentoxide the induced ^{76}As activity was determined using a Ge(Li) detector and a γ-spectrometer.

Method 9A: Neutron activation using a thermal neutron reactor flux of 1×10^{14} n · cm^{-2} · s^{-1}.

Treatment

300 μl sample aliquots and corresponding aqueous standard solutions irradiated in sealed thin-walled quartz tubes for 100 hours. After 3 days cooling and removal of ^{24}Na activity by adsorption on hydrated antimony pentoxide, activity was determined with a Ge(Li) detector and spectra analyzed with a computer.

Method 9B: Neutron activation using a thermal neutron reactor flux of 1×10^{12} n · cm^{-2} · s^{-1}.

Treatment

1 ml sample aliquots and corresponding aqueous standard solutions irradiated in polyethylene vials for 10 minutes. Activity determined after 4 minutes to 1 hour cooling, in the same way as in method 9A.

Method 10: Direct colorimetry using 2,4,6-tripyridyl-s-triazine (TPTZ) as the color forming agent.

Treatment

Samples were digested in 0.015 percent hydrochloric – 0.-02 percent thioglycolic acid for 5 hours at 70 °C to ensure solution of any iron present in particulate form. The solution was buffered to pH 5, TPTZ added and the absorbance determined at 590 nm.

Method 11: Flameless atomic absorption using an electrically heated silica tube for atomization. No correction for background absorbance was applied.

Treatment

Arsine was generated from acidified (HCl) aqueous samples by reduction with zinc. Argon gas was used as a carrier medium to sweep arsine into the silica tube for atomization.

Method 12: Direct colorimetry using diphenylcarbazide as the color forming agent.

Treatment

Samples were reduced with H_2SO_4-Na_2SO_3, evaporated to fumes (15 min), diluted and boiled with $KMnO_4$, and excess $KMnO_4$ destroyed with sodium azide. After addition of diphenylcarbazide absorbance read at 540 nm.

Method 13: Colorimetry using silver diethyldithiocarbamate as a complexing agent for arsine.

Treatment

Arsine was generated from acidified aqueous samples by reduction with zinc in the usual way and passed into pyridine solution containing silver diethyldithiocarbamate. The absorbance was read at 510 nm.

APPENDIX III

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THE PREPARATION AND CERTIFICATION OF TRACE MERCURY IN WATER STANDARD REFERENCE MATERIALS

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The study of mercury in natural water supplies requires a Standard Reference Material (SRM) with a certified concentration at the 1 ng/g level. NBS SRM's have been prepared with nominal mercury concentrations of 1.5 μg/g and 1.2 ng/g. Confirmation of these values was obtained by neutron activation, atomic absorption, and isotope dilution-spark source mass spectrometry (IDSSMS). Nitric acid and trace amounts of gold were added to achieve a stable mercury concentration. The precautions observed for cleaning the glass and Teflon containers, preparation of mercury solutions, and the packaging of the SRM's are given. As an example of the care needed in the analysis of mercury at these levels, specific details are presented for the chemistry required to prepare samples for the spark source mass spectrometer (SSMS).

Keywords: Atomic absorption spectrometry; isotope dilution analysis; mercury in water, trace analysis; mercury in water, stabilization of; neutron activation analysis; spark source mass spectrometry.

I. Introduction

Maximum permissable levels for mercury in water have not been rigidly established, however the levels of interest are in the 0.01 to 1.0 ng/g range. Although trace mercury analyses have greatly improved in recent years, most of the levels reported were in the 10 to 1000 ng/g range. Unfortunately, it is at the lower concentration levels where significant analytical problems still exist. Data recently published by the Environ-

mental Protection Agency [1] indicated that below 1 ng/g, mercury analyses, as performed by most water laboratories, had large errors.

Accordingly, the National Bureau of Standards has recently completed work on two mercury-in-water Standard Reference Materials (SRM's) which should help the scientific community in this very difficult area of trace mercury-in-water analysis. SRM 1641 is a concentrate, 1.49 μg Hg/ml, designed for use as a "spike" solution for standard addition methods. SRM 1642, 1.18 ng Hg/ml is intended for use as received as a benchmark trace standard to be used for analytical methods development.

As with all SRM's issued by NBS, two of the most important criteria in developing these standards are the homogeneity and stability of the certified species. While homogeneity is no problem for mercury in water, stability has been a constant problem for anyone storing solutions containing trace mercury. Mineral acids have been employed successfully in the μg/g range and above, but in the ng/g range significant mercury is lost to the container wall even when large amounts of acid are added. A study of various mechanisms to stabilize trace mercury solutions was undertaken and a successful procedure has been found.

Solutions were prepared containing 1 ng/ml of mercury with carrier free mercury-203 added to give a count rate of ~ 30,000 counts/min per ml of solution. One ml aliquots of these solutions were withdrawn at successive time intervals and counted at a fixed geometry to determine the mercury concentrations. Solutions stabilized with acid were found to lose about 20 percent of the mercury content per month (fig. 1) while EDTA stabilized solutions lost about 90 percent of the mercury content per month. After

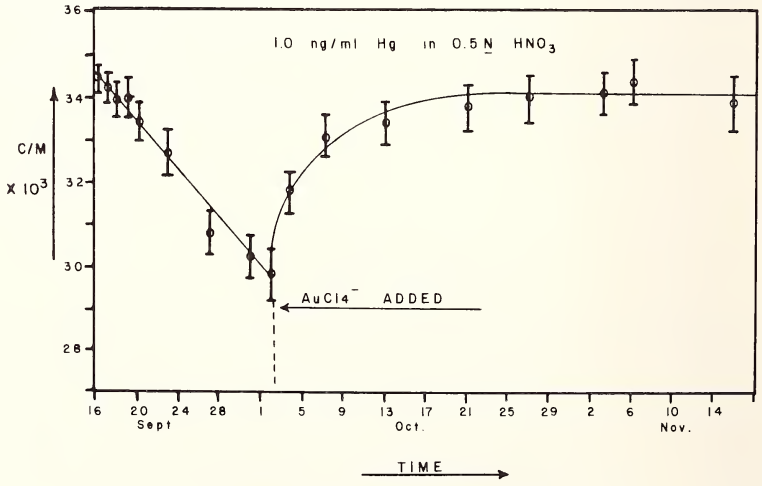

Figure 1. Mercury loss in water.

16 days, the addition of 1 μg/ml of gold tetrachloride solution was found to restore mercury concentrations to their original level in acid stabilized solutions.

Subsequent studies with gold at levels of 0.01 and 1.0 μg/ml in Teflon, borosilicate glass, and polyethylene containers demonstrated the effectiveness of the gold and nitric acid combination (table 1). For more than 1 year, all solutions have maintained constant mercury concentrations within error limits of the mercury-203 count rate. Further, actual analytical results obtained from samples of the prepared SRM's show the same long-term stability. While a period of stability or shelf life of only 1 year is claimed for SRM's 1641 and 1642, their actual shelf life may be much longer.

TABLE 1. *Stabilization of mercury solutions with gold*

Date	Container		
	Teflon[a]	Glass[a]	Polyethylene[a]
9/6/73	1.00	1.00	1.00
10/4/73	0.98	1.00	1.00
10/12/73	1.02	1.00	0.99
10/24/73	1.00	1.01	1.01
1/4/74	1.02	1.02	1.00
3/25/74	0.97	1.00	0.99

[a] Solutions contain—1.0 ng Hg/ml; 10 ng Au^{+3}/ml in 0.5N HNO_3.

II. Preparation of SRM's 1641 and 1642

All acids and distilled water used were high purity materials prepared at NBS by a sub-boiling distillation procedure [2]. Preliminary cleaning of the 25-ml glass ampoules and one liter Teflon bottles was accomplished with dilute (1 + 1 and 1 + 5 respectively, volume acid and volume water) reagent grade nitric acid. Following a rinse, the final cleaning was done by filling the containers with distilled water and storing them for 2 weeks in a clean room. The glass ampoules were dried in a Class 100 clean air [3] oven while the Teflon bottles were air dried in the clean room.

SRM 1641 was prepared by weighing the required amounts of distilled water and nitric acid into a clean 20-liter carboy. The resulting 0.5 mol/l nitric acid solution was then spiked with enough gold tetrachloride solution to give a final concentration of ~ 1 μg/g. After blending the gold with a magnetic stirrer, an aliquot of a standard mercury solution was pipetted

and mixed by stirring for 24 hours. An automatic ampouling machine was used to fill and seal all of the ampoules for SRM 1641. All tubing and pump liquid contact parts were constructed of glass or Teflon and were thoroughly cleaned before use.

For SRM 1642, the mercury solution used for SRM 1641 was carefully diluted by weight into a clean 2-liter Teflon bottle and stabilized with gold tetrachloride. Each 1-liter Teflon bottle was weighed and individually filled by weight with predetermined amounts of distilled water and nitric acid. Sufficient gold was added into the nitric acid to produce a final concentration of ~ 10 ng/ml. A 10.00-ml aliquot of the dilute mercury solution was then added to each of the 1-liter bottles previously filled with 0.5 mol/l nitric acid.

Where used, pipets were calibrated. Conversion from weight/weight units used in the preparation of the SRM's to weight/volume units was accomplished by determining the density of the solution. All of the preceding operations were performed under a Class 100 clean air environment.

III. Certification Testing

Random samples of SRM's 1641 and 1642 were selected by a statistical process. Certification analyses were performed by neutron activation, atomic absorption spectrometry and isotope dilution spark source mass spectrometry (SSMS). Activation analysis used a modification of the combustion separation developed for the determination of mercury in biological materials [4]. Four grams of water containing 10^{-9} g Hg/ml was sealed in a precleaned quartz vial and irradiated for 30 minutes at a thermal neutron flux of $\sim 6 \times 10^{13}$ n · cm^{-2} · sec^{-1}.

Following irradiation, the sample was cleaned of possible exterior contamination, frozen in liquid nitrogen, opened, and placed in a quartz combustion tube. Approximately 5 mg of HgO carrier was added, and the sample was distilled in the combustion tube through a hot silver wool plug and trapped in a liquid nitrogen cold trap.

The distillate was washed into a 50-ml beaker with 5 ml of nitric acid and three successive 10-ml rinses with deionized water. The solution was then neutralized with 5 ml of concentrated NH_4OH and the mercury was precipitated with thioacetamide as the sulfide. The precipitate was then filtered and the 77 keV photon from the decay of ^{197}Hg was counted directly on a 25 cm^3 Ge(Li) gamma detector coupled to a 4096 channel pulse height analyzer. The results were quantified by comparison to standards treated in the same manner. Due to the relatively high level of mer-

cury in SRM 1641, a direct determination of mercury without the combustion process was carried out using a low energy photon system (LEPS) with the detector connected directly to a 1024 channel multichannel analyzer.

The atomic absorption instrumentation and reagent preparations have been previously described by Rains and Menis [5]. The mercury hollow cathode lamp (HCL) was connected to a dc power supply and the current adjusted to 10 mA. The absorption cell was wrapped with a heating tape and with an argon flow through the reduction cell and absorption cell of 0.25 l/min, the surface of the absorption cell was maintained at a temperature of 200 °C with the aid of a variable autotransformer. After the mercury HCL had stabilized, the wavelength of the monochromator was peaked to read 100 percent transmission at the mercury resonance line (253.7 nm).

To prepare a working calibration curve, a mercury solution containing 0.1 μg Hg was added to the reduction cell and the sample volume diluted to 25 ml with a solution containing 1.0 mol/l HNO_3 and 2.4 mol/l H_2SO_4. Then 20 ml of reducing solution (5% $SnCl_2$ and 3% NaCl in 1.75 mol/l H_2SO_4) was added and immediately the system was closed.

The absorbance signal reached the maximum peak within 20 to 30 seconds and then slowly decreased to zero. The time required for the signal to return to background under these conditions was about 2 minutes. To speed up the return of signal to baseline, the aeration tube was removed from the reduction cell after peak absorbance was recorded and inserted into a clean flask containing dilute HNO_3. The analysis was complete within 1 minute if this technique was used.

Aliquots of the samples were transferred to the reduction cell and the procedure was followed as previously outlined. Then the concentration of the unknown solution was determined with a computer using a least square fit.

The spark source mass spectrometer (SSMS) was used in its electrical detection mode rather than its photoplate mode. The size of the sample taken for isotope dilution analysis met several criteria, all of which were aimed at insuring the degree of precision and accuracy necessary for certification of an SRM. The first of these criteria requires that the sample size taken be at least 20 times the measured blank. Another is that the Hg concentration in the sample electrode loaded into the SSMS must be at least 10 to 100 times the "detection limit" concentrations. Finally there must be enough total sample to permit the measurement of a dozen or more replicate determinations of the altered isotopic ratio of mercury. These criteria are met with the procedure used when a sample of ~ 0.3 μg

or more of mercury is available. For SRM 1642 this required the preconcentration of a 300-ml aliquot of the sample.

Thus, samples of SRM 1642 were taken by weight (300 to 400 g) and placed in clean glass beakers. Ten mg of Ag as $AgNO_3$ solution was added to each sample followed by a spike of a known amount of ^{201}Hg. After mixing, H_2S was passed through the solution to precipitate the sulfides. After adding 100 mg of pure Ag powder (< 10 μg/g detectable impurities) to a membrane filtration apparatus, the sample solution was filtered through to collect the sulfide precipitate.

The membrane filter with its contents was removed and allowed to air dry. This, like all previous operations, was carried out under a Class 100 clean air environment. Finally, the mixture of silver powder and the sulfide precipitate was transferred to a vial, mixed in a mechanical shaker, and then pressed into electrodes for the mass spectrometer. To prevent sample loss, all of the chemical operations were performed just prior to the analysis of the sample in the SSMS. The combination of the sulfide precipitate and the pure Ag powder converts the mercury sample into a nonvolatile form which is stable during sparking in the 10^{-6} Pa (10^{-8} torr) vacuum of the SSMS source chamber. Replicate measurements of the mass 201 and 202 mercury isotopic ratio were made and used to calculate the mercury concentrations [2] from the standard formula.

Due to the relatively large ($\sim 4\%$) contribution of the chemical blank (primarily from the Ag powder) and its variability, as many blanks as sample determinations were made for SRM 1642. For SRM 1641 the blank contribution was insignificant and fewer blanks were determined. In general, the procedures used for SRM's 1641 and 1642 were identical, except for the much smaller sample size required for SRM 1641.

Tables 2 and 3 present a summary of the analytical results obtained by these three analytical techniques for SRM's 1641 and 1642. The results show agreement between the analytical techniques to be within their respective error limits as well as in good agreement with the values expected from dilution of the mercury solution from which these SRM's were made. The final certificate values adapted were 1.18 ± 0.05 ng/ml for SRM 1642 and 1.49 ± 0.05 μg/ml for SRM 1641 at 25 °C. Repeated analyses of these solutions have confirmed long-term (1 year or more) stability. The excellent agreement between the analytical methods as well as their good precision have led to two well characterized SRM's for a difficult element. These SRM's should be of considerable value in helping investigators improve the accuracy of trace mercury determinations in water.

TABLE 2. *Comparison of certification analyses - Standard Reference Material 1641*

	Concentration (ng Hg/ml)
Certified value	1.49 ± 0.05[a]
Theoretical value	1.495
Analytical technique	
Neutron activation analysis	1.48 ± 0.02[b]
Isotope dilution spark source mass spectrometry	1.50 ± 0.02[b]
Atomic absorption	1.52 ± 0.02[b]

[a] 95 percent confidence limit.
[b] Standard deviation.

TABLE 3. *Comparison of certification analyses - Standard Reference Material 1642*

	Concentration (ng Hg/ml)
Certified value	1.18 ± 0.05[a]
Theoretical value	1.23
Analytical technique	
Neutron activation analysis	1.21 ± 0.06[b]
Isotope dilution spark source mass spectrometry	1.18 ± 0.02[b]
Atomic absorption	1.17 ± 0.03[b]

[a] 95 percent confidence limit.
[b] Standard deviation.

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THE INFLUENCE OF LIMITS OF LABORATORY ACCURACY AND PRECISION ON THE INTERPRETATION OF BLOOD LEAD ANALYSES FOR INDUSTRIAL LEAD HYGIENE CONTROL

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Good laboratory accuracy in blood lead analyses can be obtained under some conditions but evaluation of the consistency of analyses of blood samples feasibly obtained from workers in industrial lead plants continues to present significant problems. The need for accuracy in blood lead analyses for industrial lead hygiene control is misunderstood because more than 45 years ago, long before there was any knowledge of blood lead levels, control of clinical lead cases had been achieved, and by 1949, 20 years of successful maintenance of such control was reported. The desirability of having an objective criterion, such as a maximum allowable blood lead level, for protection of the health of industrial lead workers is evident. However, adequate consistency of trace element analyses, even in relatively simple inorganic materials, is often not experienced even under some of the best conditions. Therefore, those who are concerned with reducing the lead hygiene control process to a routine, that can be administered in part by paramedical or nonmedical personnel, and those who are concerned with attempts to establish and enforce maximum allowable blood lead levels urgently need a clear statement that specifies the limits of the capability of the present technology to demonstrate the accuracy that would be essential to meet these objectives.

Keywords: Accuracy; blood lead analyses; clinical chemistry; industrial lead hygiene; interlaboratory correlations; lead hygiene control; occupational exposure standard criteria; occupational health control; precision; trace element analyses.

I. Introduction

To determine which interpretations made from blood lead analyses are scientifically valid, analytical accuracy and precision must be considered as fundamental parameters. The accuracy and precision need to be evaluated from empirical data that are derived from appropriately designed experiments, and the data need to be applied with care to avoid invalid interpretations.

Illustrations taken from two particularly pertinent reports are used to demonstrate the importance of these considerations. The demonstrated capability of other trace element analyses is used to illustrate widespread problems that are encountered in making scientifically valid applications of these analyses.

When blood lead analyses in lead hygiene control are used for purposes other than contributing partial diagnostic information to a physician, it is particularly important that the scientific justification for interpretations be carefully evaluated.

Since highly successful control of clinical lead cases among workers in a lead industry can be achieved without any knowledge of blood lead levels, it is easy to misinterpret success in a lead hygiene control program where blood lead data are used by taking the success to imply a higher level of analytical accuracy than is in fact achieved.

There are indications that a maximum critical allowable blood lead level exists and can be identified. If there is a critical level that can be identified, simplified procedures for lead hygiene control are feasible, and the procedure for defining and enforcing protective standards is simplified. The evaluation of the scientific validity of these concepts depends on the analytical quality of individual blood lead analyses; it is therefore important that the limits of capability of the analytical procedures, and that the implications of these limits be clearly understood.

II. Discussion

Good laboratory accuracy in blood lead analyses has been reported [1,2], but evaluation of the consistency (accuracy and precision) of analyses of blood samples feasibly obtained from workers in industrial lead plants continue to present significant problems [3]. A comprehensive report from the U.S. Public Health Service (USPHS) Laboratory [1] reports a mean lead recovery of 97.1 percent and a percent relative standard deviation (%rsd) of replicate analyses of 4.0 to 9.5 percent. A

recent paper by Evenson [2] at University Hospitals, Madison, Wisconsin, which discusses the capability of a carefully controlled application of flameless atomic absorption (AA) spectroscopy, reports lead recoveries in the range of 96 to 103 percent, and within run percent relative standard deviation in the range of 7 to 9 percent.

The value of these analyses for practical use in the control of lead absorption by workers in lead industry is considerably less than the data might seem to imply [3]. The classical method is not only costly to use, but limits replication because it requires blood samples of several milliliters for each determination. Furthermore, the consistency of replicate determinations by the USPHS laboratory over a period of 2 months is considerably less, and the consistency of results between laboratories is even less. The newer, more sophisticated AA method is less costly to use and it requires less than 10 μl of blood. On a between run basis, its reported precision is somewhat less than that reported for the classical method, but the small sample volume and the economy of operation make replicate determinations routinely feasible. Use of this method may, indeed, change the picture when there has been time for sufficient interlaboratory comparisons and when suitable reference materials become available. However, the method does have the practical accuracy and precision limitations which are inherent in micro sampling.

The present active, demonstrated state of the art limits the validity of results from an individual blood sample if they are to be related to an externally specified norm. Not only precision, but the verification of accuracy needs to be improved significantly for the method to be scientifically valid in this type of application.

An industrial lead hygiene control program, reported in 1949 [4], was administered so successfully that there were no cases of clinical lead disability within a group of approximately 100 workers over a period of 11 consecutive years. Blood lead analyses were not used at all; the diagnostic procedure depended on other, generally even less precise, criteria. This success, achieved without blood lead information, makes it clear that success cannot be used as a proof or even as necessarily an indication of accuracy when blood lead analyses are used in such a program. Success in a lead hygiene control program which uses blood lead analyses as part of a diagnostic procedure provides no justification for disregard of other diagnostic factors. Accuracy and precision of blood lead analyses need to be evaluated independently of a lead hygiene control program in order to determine when the quality of the analytical results justifies their use to identify or evaluate a maximum critical blood lead level or to show whether an individual blood sample has more lead than the critical lead concentration.

The desirability of establishing a maximum allowable critical blood lead level and monitoring blood samples from employees for compliance is clearly evident [5]. Enforcement of hygienic regulations would be greatly simplified, and implementation of a control program that could be operated by paramedical or nonmedical personnel would become feasible. An accurately established value for the critical blood lead concentration is indispensable for these advantages to be implemented with scientific validity; it is difficult to verify accuracy without a pertinent certified reference material.

If a maximum critical blood lead level exists, it would be defined most logically as the maximum level which a person may have without being in danger of acquiring symptoms of lead intoxication. The blood lead concentration for such a level can be evaluated only by accurate blood lead analyses of a group of individuals known to be free of unacceptable symptoms plus accurate blood lead analyses of a group of individuals known to have unacceptable symptoms of lead intoxication. The precision of the analytical method used for the analysis must be sufficient to distinguish between the two levels with adequate certainty, and the accuracy must be consistent so that the critical level can be identified with adequate certainty.

Demonstrated, practical analytical capability imposes limitations to progress toward the goals of adequate precision and accuracy to evaluate allowable blood lead levels and to monitor individual samples for compliance. Pertinent examples of analytical capability are provided by the data in the USPHS report and the report by Evenson.

To develop a working description of the demonstrated precision of blood lead analyses, we can go through the calculations for 95 percent confidence intervals, although conclusions must be approached with caution because these parameters will have something less than their full statistical significance because the available data are too few for their rigorous application. A quantity, specifically, t × percent relative standard deviation, is designated the "estimated percent uncertainty at the 95 percent level" (U_{95}) to distinguish it from a more conventionally determined 95 percent confidence interval.

The USPHS report gives individual replicate results for 10 separate determinations on each of their samples. Of the six samples used in their survey, only one is directly pertinent to blood lead analyses for industrial lead hygiene control; it is the sample designated "S" which is determined to contain a total lead concentration of 70 μg/100g (μg percent). The only other one of their samples that contains a lead concentration which does

not differ from the presumed critical level of 80 μg percent [5] by a factor of more than two is totally inorganic and therefore its relevance is indefinite; overall results are not greatly different, however: percent relative standard deviation is 4.3 percent for sample S and 3.7 percent for the inorganic lead nitrate sample.

If the data in table 1 of the USPHS report are assumed to be in chronological order, calculation of a U_{95} for the first five determinations will give an indication of the day to day precision over a 10-day period, and calculation of a U_{95} for the last five will indicate the precision over a period of the next 2 months: these calculations give ± 3 and ± 12 percent, respectively, on the basis of $t = 2.78$ for five observations. In the more recent report, Evenson gives a between run percent relative standard deviation of ± 9 percent for a comparable lead concentration; this is based on 18 observations for which t is close to 2, and a U_{95} calculated from these data would be ± 18 percent. Reporting on a new procedure, Evenson reports no interlaboratory comparisons as were reported in the USPHS report which discussed demonstrated capability of a 25-year-old procedure. Ten laboratories were included in the USPHS comparison. The value of their data has an unfortunate limitation because each determination reported for the 10 cooperating laboratories is the mean of two individual determinations. Therefore, the percent relative standard deviation, 11.1 percent is less than it would be for single individual determinations. For the reported means, of which there are 10, $t = 2.3$, and a calculated U_{95} would be ± 26 percent. The scatter of individual determinations is indeterminate; it would have to be greater than ± 26 percent, but could not be assumed to be as great as $1/\sqrt{2} \times 26$ or ± 35 percent.

Accuracy estimates in the two reports are based on recovery of inorganic lead spikes. There are no available certified reference materials even now, much less for Evenson's investigation, and more so for the USPHS investigation several years earlier. USPHS reports a mean lead recovery of 97.1 percent. Evenson reports a mean lead recovery of 100 percent and with it he reports a range for nine observations of 96 to 103 percent. For nine observations $t = 2.3$, and the calculated U_{95} is ± 6 percent. If the total uncertainty in his individual results is estimated as a root mean square of the analytical uncertainty (precision) plus the calculated estimate of uncertainty in the recovery determinations, the value is 20 percent.

To give illustrations that are close to critical operating conditions in industrial lead hygiene control, the calculated value of U_{95} can be used with the often presumed critical maximum blood lead level of 80 μg percent to see how wide a band of uncertainty would be indicated.

The USPHS replicate analyses over the 2-month period would seem to represent conditions closest to ordinary operating conditions. The value of U_{95} is 12.4 percent; 12.4 percent of 80 is 10; the interval of 80 ± 10 is the 20 unit range from 70 to 90. This would suggest that an analytical result of 80 obtained from an analysis at the USPHS laboratory indicates a blood lead concentration in the range of 70 to 90 μg percent.

If the critical maximum blood lead level of 80 μg percent were to be enforced by such analyses, a worker may be removed from his work with a concentration of a little more than 70 μg percent. Presumably, analysis of a sample with a concentration of 89 μg percent could yield a result as low as 79, and that worker could be left on the job. Analogous data from Evenson would represent a range of 65 to 95 μg percent instead of 70 to 90 μg percent.

Conclusions from the interlaboratory data in the USPHS report have to be more imprecise because the only results given represent the means of two determinations. These data would be expected to represent conditions that approximate our working conditions even more closely because analyses for enforcement would come from several sources rather than one highly experienced laboratory.

We may consider two ranges calculated from these means: the range calculated from the U_{95} of the mean values, which is too small, and the range calculated from these values multiplied by $1/\sqrt{2}$, which is too large. The ranges are 59 to 101, and 52 to 108. To take some intermediate value, we might consider the range of 55 to 105 as being a somewhat likely range.

The conclusion would be that a worker with a real blood lead level almost as low as 55 may have to be moved while one with a blood lead level almost as high as 105 may be left on the job. Statistically, this may not seem highly objectionable, but if our attention is focussed on the individuals who might be involved it is more difficult to remain unconcerned.

It may be also pertinent to note that the USPHS laboratory precision was less over a period of 2 months than over a period of 10 days. We can only wonder what the calculated ranges of uncertainty might be if we had similar 2-month data for the individual participating laboratories.

Furthermore, in these illustrations we have not considered the question of accuracy. Recoveries of lead spikes in the order of 97 to 100 percent and a range of 96 to 103 percent suggest "good" performance; however, it would seem that more information is needed if critical decisions concerning the job assignments and tenure of individuals are to be made from individual analyses by procedures that do not have the benefit of evalua-

tion and analytical quality control by analyses of certified reference materials.

Other published reports give some information on trace element analyses of materials other than blood. Flanagan reports numerous replicate analyses of standard geological rock samples [6]. A group at the National Environmental Research Center reports a comparison of selected analytical techniques for trace element analyses of coal, fly ash, fuel oil, and gasoline [7]. Schnetzler tabulated analyses made by several prominent laboratories for eight trace elements in moon rock samples [8]. Patterson reported problems encountered with the accuracy of analysis of seawater samples for trace quantities of lead [9]. In all these cases, as in the case of blood lead analyses, accuracy and precision are limited, even in materials of a simple inorganic composition. Some of the most outstanding laboratories in the country were involved in the analyses of the moon rock samples.

Finally, attention is directed to the report of an American Industrial Hygiene Association interlaboratory evaluation project [10] in which 61 laboratories participated in the analysis of seven blood samples. For the sample with a target concentration of 85 μg percent, the percent relative standard deviation is ± 36 percent.

III. Conclusions

The evaluation of the accuracy of blood lead analyses is highly uncertain in the absence of pertinent certified reference materials. Estimates of precision realized in practice under critical working conditions are also highly uncertain. However, the inferences that can be drawn from published data suggest strongly that a great deal more has to be learned and accomplished before individual blood lead analyses can be used in a scientifically valid manner for routine industrial lead hygiene control. Also the question seems pertinent: If a maximum allowable blood lead level exists, do the data indicate that it has been or can be evaluated with sufficient accuracy to make it a scientifically justified basis for enforcement of industrial lead hygiene control?

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Part II. SAMPLING

NATIONAL BUREAU OF STANDARDS SPECIAL PUBLICATION 422.
Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis,
Proceedings of the 7th IMR Symposium,
Held October 7-11, 1974, Gaithersburg, Md. (Issued August 1976).

SAMPLING AND SAMPLE PREPARATION AT THE GEOLOGICAL SURVEY OF CANADA—THE WHAT, WHY, AND HOW

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The landmass of Canada, with half of its 4 million square miles underlain by the Precambrian Canadian Shield, encompasses most of the main geological regions of North America, and presents the geoscientist with a wide diversity of geological terrains of varying degrees of complexity, economic interest and inaccessibility.

Among the chief concerns of the Geological Survey of Canada is that of providing a comprehensive inventory and understanding of the geological framework through its extensive field and laboratory studies, with emphasis upon those regions having high potential for the occurrence of additional economic mineral deposits.

Many factors influence the nature of a sampling program, which may vary from the taking of single "grab" specimens to the collection of large numbers of samples on the basis of a sampling pattern laid out according to a statistically devised plan, but the primary consideration is always to obtain a sample that will yield the best answer to the question being asked. Rocks, minerals, stream and lake sediments, soils, glacial deposits, water samples from lakes, streams, springs, muskeg and other sources, all of these have their use as a source of compositional, petrographic, isotopic, mineralogical, textural and other information.

Because of the often unique nature of a sample, the difficulty of obtaining additional samples, the succeeding extensive and frequently costly work to be done on the prepared material, and the conclusions that will be derived from the resulting data, it is of paramount importance that the sample be correctly prepared. Again, various factors must be considered in the choice of method to be used, but the overriding concern must be to ensure that the sample prepared for study is truly representative of the sample submitted.

Keywords: Contamination; geological sample; rocks and minerals; sample preparation; sampling; trace analysis; water sampling.

I. The Area to be Sampled

Within the limits of the continental slope surrounding Canada there are about 5,526,000 square miles, making up 2.9 percent of the surface of the globe [1]. Included in this total are 3,560,000 square miles of mainland and islands, and 292,000 square miles of freshwater lakes, constituting a sampling area of mind-boggling proportions in anyone's language. The main geological regions of Canada, which comprise most of the main geological regions of North America [2] are outlined in simplified form in figure 1. The central part contains the Precambrian Canadian Shield, the outcropping portion of the North American craton which here has an area of 2,146,000 square miles, or more than half of Canada's landmass. While it is usually depicted on geological maps as a single spotted pink unit, it is complex and composed of several structural provinces. Bordering the Shield are three large elongate belts, or orogens, named the Appalachian, Innuitian and the Cordilleran, consisting of both Precambrian and Phanerozoic rocks. Between them and the Shield are four platform areas (St. Lawrence, Hudson, Interior and Arctic) of unexposed Precambrian basement covered by Phanerozoic sedimentary rocks. Simplifying still further, the Canadian landmass is composed of an old and massive core surrounded by a crescent of borderland younger, mainly stratigraphic rocks.

The terrain is as varied physiographically as it is geologically. The Shield is relatively smooth, a glaciated surface with many lakes, ponds and swamps, which is interrupted by rounded hills of generally low relief. However, very rugged topography is found in the Innuitian region, with local relief of as much as 4,000 ft. In the Appalachian region areas of considerable relief are found and, of course, in the Cordilleran region with its three great mountain belts, elevations rise to more than 10,000 ft. Between these extremes there are the plain-like and moderately elevated regions of the platforms or lowlands; the Interior Plains region varies in nature from semi-arid grassland in the south, through tree-covered in the central part, to tundra in the north.

II. The Reasons for Sampling

The purpose of the foregoing, and necessarily brief, discourse on the geology and physiography of Canada is to give you a picture of *what* our geologists must sample if the Survey is to fulfill its role of providing a comprehensive inventory and understanding of the geological framework

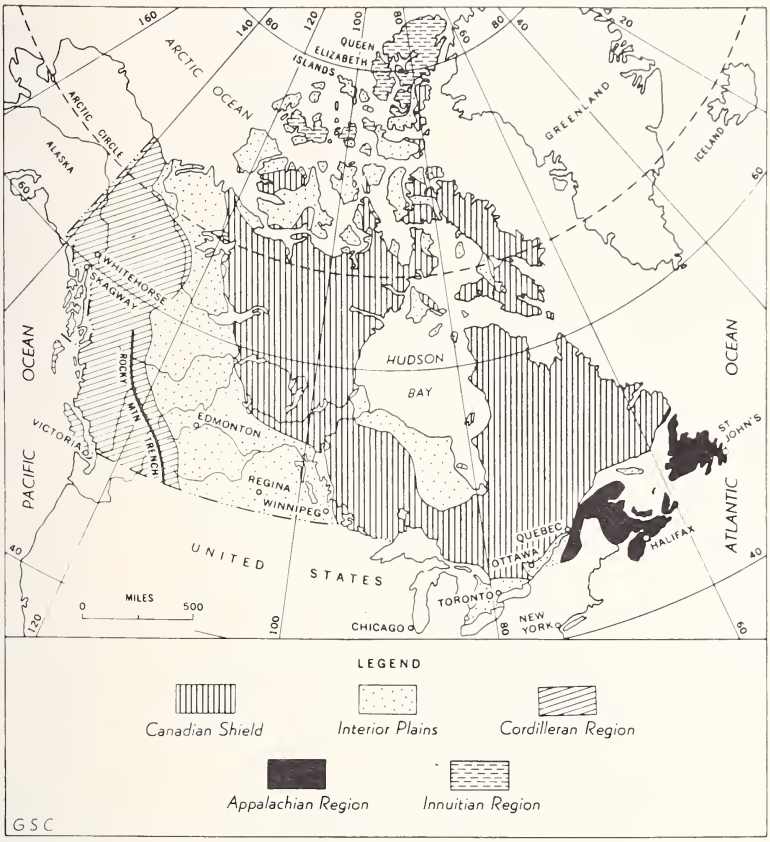


Figure 1. Main geological regions of Canada.

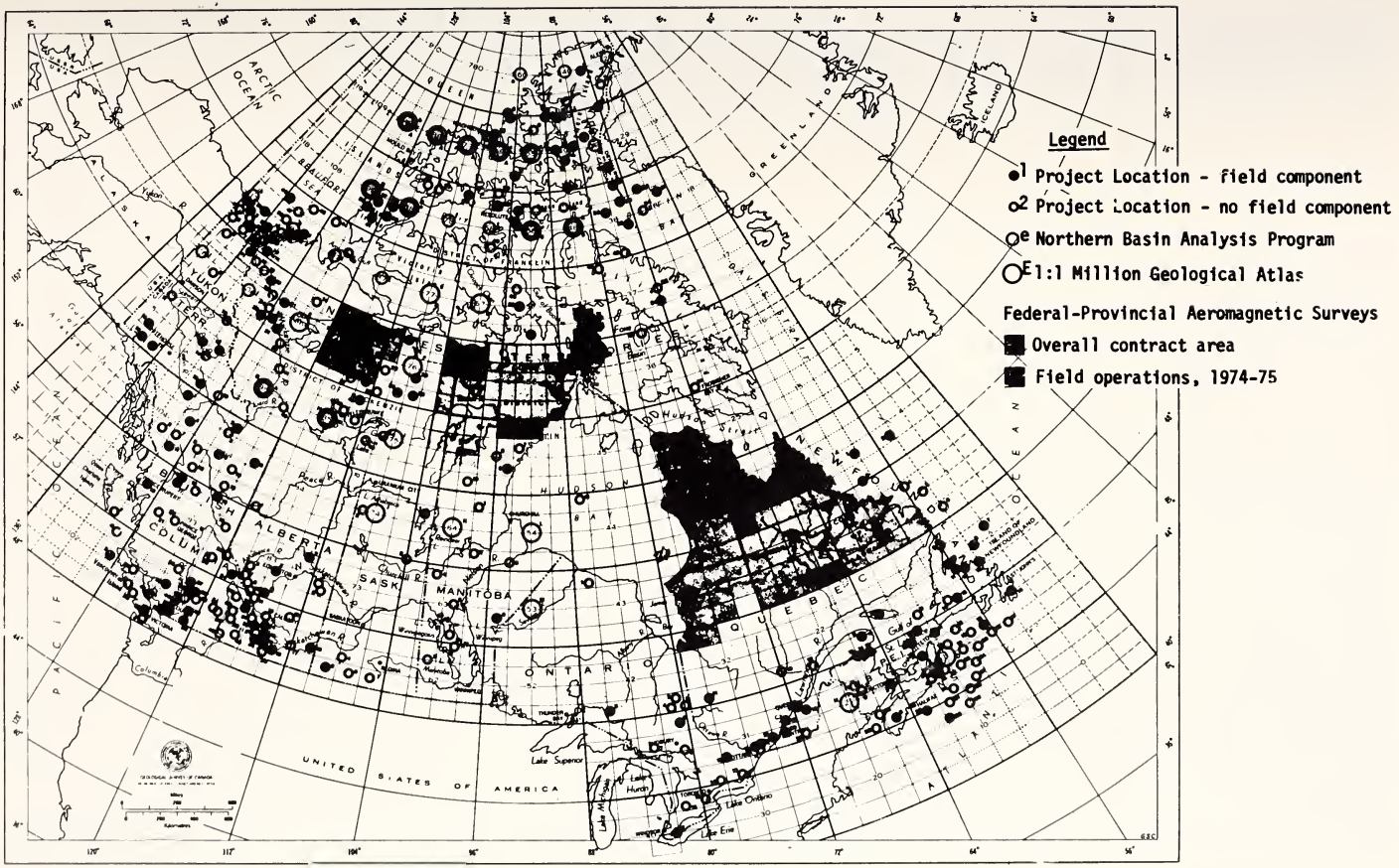
of Canada. When our founder and first director, Sir William E. Logan, was appointed in 1842, he was instructed to make a geological survey of Canada and to organize "...a plan of investigation as may promise to lead to the most speedy and economic development of the mineral resources of the country." The evergrowing demands for energy and natural resources have heightened the need for a more precise knowledge of the geology of Canada and for a greater comprehension of geological processes. These data are required for long range planning, and as a basis for enlightened decisions by government and industry. Thus, some 133 years later, we are still hard at it. The packhorse has, for the most part, been replaced by the helicopter and other modern forms of transportation and the basic reconnaissance of Canada's geology, partly at a scale of 8 miles to the inch and partly at 4 miles to the inch, will be completed by

field work scheduled for 1976. More than 60 percent of this reconnaissance was done in the past 20 years. This past summer the Survey placed 195 parties in the field, as shown in figure 2, to carry out investigations in geology, resource geophysics, geochemistry, geomorphology and physical geology, involving systematic mapping and comprehensive topical studies. In support of these parties the Survey maintains extensive suites of laboratories to provide services and carry out studies in paleontology, geochemistry, metallogeny, mineralogy, petrology, geochronology, geophysics and sedimentology, to mention only a few. The first and fundamental step in the sequence of events which culminate in the production of scientific data by these laboratories is the collection of an appropriate sample or samples in the field, followed by the preparation of the material into a form suitable for subsequent laboratory study.

III. Sampling for a Purpose

Griffiths [3] has defined sampling as an integral part of the general process of problem solving and, in his review of the problems of sampling in geoscience he points out that, because the populations are often very large and the samples are quite small in terms of number of components, the problem of selection becomes critical. While not usually stated in such an elegant manner, this concern for proper sampling is a familiar theme in geological literature, and one given particular emphasis by those to whom the samples are submitted for subsequent study! It is trite to say that an analysis is no better than the sample that it represents, but it is a fact of life that is too often either not fully appreciated, or completely ignored. Much time and labor is expended on samples which are not worthy of the effort; an attempt is then made to justify this unnecessary work by drawing conclusions from the data that, unfortunately, may be misleading or incorrect because of the nature of the sample.

Over the past 20 years, however, there has been a major change in the approach to sampling geological materials, brought about on the one hand by modern field methods which enable the geologist to cover very large areas and to take and transport large numbers of samples, and on the other hand by the development of new methods of geochemical analysis suitable for the processing of large batches of samples. The vastly increased emphasis upon the application of statistical theory to the solution of geological problems has also contributed to a geometric growth in the number of samples submitted for analysis. The day of the "classical" rock analysis, a work of art as much as one of science, appears to have passed,


Legend
1 Project Location - field component
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1:1 Million Geological Atlas
Federal-Provincial Aeromagnetic Surveys
Overall contract area
Field operations, 1974-75
ARCTIC OCEAN
GREENLAND
ICELAND
YUKON
NORTHWEST TERRITORIES
BRITISH COLUMBIA
ALBERTA
SASK.
MANITOBA
ONTARIO
QUEBEC
HUDSON BAY
UNITED STATES OF AMERICA
Lake Superior
Lake Huron
Lake Ontario
Lake Erie

Figure 2

and the "classical" rock analyst with it, unless he happens to be kept around to provide those standard samples so necessary for the calibration and validation of the new methods! As an aside, I might point out that the "classical" methods of rock analysis enjoyed a renaissance during the initial studies of lunar material returned by the Apollo manned lunar missions. One of their objectives was to sample the moon, and England [4] has aptly described this as the granddaddy of all sampling problems undertaken to date.

When one thinks of geological sampling, it is the rocks that come first to mind. The samples taken may vary from the single hand-specimen weighing 1 or 2 lb, and often a "grab" sample because of limitations of time or of outcrop exposure, to bags of small chips taken from all of the representative rock types in an area. The size of sample will be governed by a number of factors, among which are grain size, degree of homogeneity, purpose (*e.g.*, age dating, petrography, mineral reconnaissance, chemical composition), accessibility and ease of transport, to mention but a few. But whatever its size, the extent to which it represents its host material must be understood, in order that the proper limitations can be placed upon the conclusions to be drawn from the resulting data. It is obvious that the geologist can more easily select a sample, or suite of samples, from an outcrop if he is making a detailed petrological study of a batholith, than if he is interested in discovering areas of greater mineral potential in a region of general economic interest. As an example of an approach governed by this latter consideration, let me mention briefly a regional geochemical study being carried out by Dr. R. G. Garrett of our Resource Geophysics and Geochemistry Division [5,6]. This is "rock geochemistry," an approach that has had limited success as a tool for broad mineral reconnaissance in the past because of, in part, an inadequate understanding of the problems of acquiring truly representative samples. Dr. Garrett's work is being done in the northern Canadian Cordillera of east and central Yukon, in an area of about 20,000 square miles, and involves the sampling of about 70 small granitoid plutons having a wide range of compositions, but chiefly granodiorites or quartz-monzonites. The fold belt in which these granitoids were intruded is host to a number of mineral deposits, among them lead-zinc, silver-lead-zinc and tungsten, the latter having a direct relationship to the intrusives. In addition to a reconnaissance of mineral potential, a second objective is to investigate the geochemical relationships of the plutonic rocks to the mineral occurrences associated with them. The samples which were collected each weighed between 2 and 3 lb, made up of *fresh* chips collected over a small area. Ideally, a minimum of 15 sites per pluton were sampled in duplicate, the second of each pair being collected at a distance of some 20 ft from the

first; this number of sites was expected to yield sufficient data to provide meaningful statistics computed for each pluton. In fact, however, the number of sample sites per pluton varied considerably, from many more for large intrusions (usually one site per 1 to 2 square miles) to six or less for small rugged intrusions where sampling is difficult and time is short. The duplicate sampling at each site is to allow the use of analysis of variance to determine if a pluton is internally zoned, and to aid in assessing the overall significance of observed patterns in the data. Of the 12 elements determined in these samples, Si, Al, Mg, Fe, Ca, Na, K, Ti, Mn, Zn, Cu and Pb, 8 of them showed sampling and local geological variability to be more than double the analytical variability, thus underlining the importance of good sampling practice in carrying out effective surveys.

Another approach to the use of rock geochemistry as a reconnaissance tool is the one used to estimate the average abundance of elements forming the surface crystalline rocks of the Canadian Shield [7]. Thousands of samples of these rocks have been collected by Geological Survey of Canada field parties over the past few years and they provide a useful source of identifiable raw material. The investigation involved the study of 27 unit-areas; a sample for analysis was prepared by grouping together the hand specimens of each rock type, as determined for geological mapping, within each unit-area, and approximately 20 g from each hand specimen were combined to form a composite sample for each rock type. About 170 composite rock samples were obtained, including some prepared in duplicate. Special composite samples were prepared from these for three major regions, by mixing portions of the composite powdered rock samples in proportion to the areal extent of these rock units, as measured on the geological maps. Quantitative evaluation of the extent to which the rock types of the various map areas are actually represented in these composite samples is impossible without a special sampling program but, considering the large percentage of any area that is inaccessible to sampling procedures because of water and drift cover, it is believed that the sampling program was adequate for this regional type of chemical study.

Rocks are not, however, the only medium for geological sampling. I mentioned at the beginning that Canada has 292,000 square miles of freshwater lakes, a greater lake area than any other country. These, together with streams, ponds, springs, marshes, muskegs, and other watery accumulations, provide a third dimension to mineral prospecting through the application of limnogeochemical and hydrogeochemical methods. In spite of limitations imposed by high grade metamorphism, low topographic relief, glaciation effects such as disrupted drainage systems, and permafrost, there are areas of the Canadian Shield where

groundwaters circulate deeply, springs are relatively abundant and near-surface mineral deposits are being oxidized, even in zones underlain by permafrost [8]. These waters thus may penetrate great volumes of rock and carry clues suggestive of hidden mineral deposits to the surface, or into underground workings and drill holes as groundwater seeps, and either the water itself, or natural chemical precipitates which tend to scavenge traces of metallic and other elements, can be easily sampled. Particular care is needed to avoid contamination, and to ensure that a truly representative sample is obtained, and many factors must be considered in establishing a sampling program. Water samples should be collected in carefully cleaned plastic bottles which should first be rinsed several times with the water to be sampled; precipitates are collected in plastic bottles or bags. It must be decided upon at the beginning if the water samples are to be acidified or filtered, if the precipitates are to be dried, and if other information such as pH, Eh, and temperature is to be collected at the same time, for it is not possible to sample the same water again. Water in its solid form as *snow* has also been found to provide clues to buried mineralization through the accumulation of such elements as Hg, Cu, Zn, Cd, Mn, Ni and Pb, but its use is limited to *detailed* prospecting in known mineralized areas [9].

The potential of hydrogeochemical methods as a sampling medium for the Canadian Shield has tended to be overshadowed by the relatively greater popularity of sediments, both stream and lake, as a more effective means of geochemical prospecting. Stream sediments have been widely used in regional geochemical surveys in Canada, and in many other areas of the world, and it has been shown that the sampling of material which is related to the drainage system will reflect the overall trace element content of the rocks and associated surface deposits in the drainage basin. Classical stream systems are rarely found in the Canadian Shield, however, and recourse must be had to impeded and disorganized stream systems containing many lakes whose clay-silt sediment provides a composite sample of the drainage system. Its use as a medium for regional geochemical exploration has been reviewed by R. J. Allan [10]. In the southern Canadian Shield, the lake sediment is more usually a grey-green, water-saturated, finely-divided organic-rich ooze, or "gytjja" which also has application as a sampling medium for exploration geochemistry [11]. It is concluded that trace metals are transported, by stream flow and surface run-off, both chemically and physically, in the latter case as elements held on the surface of the particles through the processes of absorption, adsorption, chemisorption and cation exchange. The suspended or physical load is dropped to the lake bottom, and the transported chemical species are sorbed by uncontaminated sediments.

Our Geochemistry Section undertook a major test of methods of geochemical exploration and mapping by the use of lake sediments in 1972, and again in 1973, over 36,000 square miles of the Bear and Slave Structural Provinces in the northern Canadian Shield. Among the objectives of the program were those to 1) outline areas of 20 square miles or greater, containing anomalous contents of one or more trace elements, and 2) to differentiate large areas of similar rock type as to relative economic potential based on the relative abundance of trace and major elements. Because lake sediments are representative of the average composition of the surrounding bedrock it is feasible to collect samples at wide intervals and in this study [12] a sample density of approximately one per 10 square miles was selected. Transportation for two-man sampling teams was by means of three helicopters and the average flying time per site was 10 min; an area of approximately 1,500 square miles could be sampled in 1 day. From seven areas within this region 176 samples of inorganic lake sediment were collected and lake water samples and samples of the various rock types of the areas, the latter in order to be able to relate the chemistry of the lake sediments to the surrounding bedrock, were also taken. The sediment sampling was done by means of a light 6-ft-long post-hole auger which was manipulated from the helicopter float by one sampler when the helicopter had landed in the selected sampling spot on the lake; the layer found at 1 to 2 cm below the water-sediment interface was discarded. The second sampler took two water samples at the same time. Normally, samples were taken near shore, near islands or above reefs, at water depths of 3 to 8 ft. The sediments were air-dried and sieved to -250 mesh, which is the approximate boundary between the sand fraction and the silt fraction, and has a relatively high surface area which provides a fairly uniform absorbing medium. Determinations of some 21 trace elements, including Zn, Cu, Pb, Hg, Ni, Co, and Ag, were made in addition to major elements, and the sediments were shown to be relatively homogeneous within each area, when the -250 mesh fraction was used. While the sample interval was adequate, the question of the optimum interval for lake sediment sampling within the Canadian Shield was not resolved. In a follow-up study in 1973, Cameron and Durham [13] resampled two areas which had been shown to contain prominent heavy metal anomalies, the objective being to determine whether or not these anomalies were indeed related to previously unknown mineralization. Lake sediment, rock and, additionally, soil samples were taken. The 1972 anomalies were confirmed and no new major anomalies were detected. Evidence was found of significant sulphide mineral accumulations.

IV. Sample Preparations

The foregoing has, hopefully, conveyed some sense of the requirements and importance of proper sampling in the field. The cost, or just plain impossibility, of repeating a sampling program because of an unsatisfactory initial attempt, is also another reason for getting it right the first time. Although only casual reference has been made to the application of statistical theory to sampling, this is not to minimize its importance but rather because this subject has been covered in abundant detail by those far more qualified than the writer. *Ne sutor supra crepidam!*[1] Accordingly, sample preparation will be discussed in the same fashion.

Crushing, splitting, grinding, sieving and mixing, preceded if necessary by drying, are the usual steps in the preparation of the sample for use in the chemical and mineralogical laboratories. Thin sections, polished thin sections, oriented cubes and slices, are also used for petrographic studies. Mineral concentrates are prepared for geochronological and mineralogical work. All of this is done with a variety of types of equipment ranging from the very small to the very large, the choice of which is highly subjective. I propose to describe briefly how we prepare samples at the Geological Survey of Canada.

Of the large quantity of samples processed by our sample preparation unit, by far the largest number are aluminosilicates destined for subsequent chemical (including x-ray fluorescence spectroscopy) and/or emission spectrographic analysis. These samples vary in size from 50 g to 20 lb or more, and are first reduced to less than pea-size by means of a ceramic bucking board and muller for the small samples, or in a steel jaw crusher for the large ones. Because of the very nature of rocks and minerals, contamination of the sample during this stage is an everpresent and unavoidable danger, and one must accept that the process is going to change the composition of the sample to some degree, either by introducing extraneous material such as metallic smears or fragments, by the preferential loss of material through dusting, or by oxidation of constituents. It would be ideal if one could do the crushing between two blocks of the same material, but failing this one tries to minimize the unavoidable, or accepts the least harmful contamination. The jaws of the crusher should be of hardened steel, or faced with tungsten carbide if the budget will allow it, and should be replaced when signs of wear are visible.

After reducing the whole sample to less than pea-size, it is thoroughly mixed by rolling it on an appropriately sized paper or rubber mat and then a representative portion of 20 to 40 g (or less for smaller samples) is ob-

[1] An old Roman proverb which can be roughly translated as "Cobbler, stick to thy last!"

tained by the process of coning and quartering. A riffle, or Jones splitter, is also a good way to obtain the final split of the sample, and is available in sizes from those able to handle several pounds of crushed material to microsplitters which are vibrated magnetically. There are other mechanical devices suitable for this purpose as well, such as a rotary splitter, but there is much to be said for having the splitting done manually by an experienced preparator.

The chief source of contamination occurs in the grinding stage. Again there are several very useful devices for achieving a final powder having a particle size of about − 150 mesh, such as mortars, ball mills, cylindrical or cone grinders, hammermills, rotary beaters, and the swing-mill (Schwingmühle) or shatterbox, to name those most familiar to rock analysts. If the ground product is to be used for making mineral separations for mineralogical study, or for age determinations, then steel grinding surfaces may be used, but it is preferable to use ceramic plates or mills wherever possible. Silicon and aluminum are then the only elements introduced into the sample in significant amounts, and for aluminosilicates this is rarely an important consideration. We make extensive use of the "paint-shaker" which consists of six ceramic (mullite) mills clamped in a wooden frame and placed in the jaws of a paint-shaking machine; the grinding is done by mullite balls, and about 45 min is required to reduce a 20-g split to − 150 mesh. The powder is then transferred to a plastic vial without further mixing. Further grinding of subsamples may be done in the laboratory, for analysis by x-ray fluorescence or optical emission spectroscopy; before the contents of the vial are used, however, it is imperative that they be mixed again in order to eliminate any mineral segregation that has occurred in the meantime. Because stored samples are subjected to vibrations, it is possible to obtain some rather remarkable, visible banding in samples containing minerals of strongly contrasting densities. We have not experienced any problems as a result of lengthy storage of powdered geological samples, but we were concerned that the Hg content might change over a period of time, because of the high volatility of this element. It was found, however, that samples of a granitoid pluton collected in 1969, and stored in plastic vials with snap-on caps, were indistinguishable in Hg content from those collected from the same pluton in 1970 [14].

Wet material must, of course, first be dried before it can be processed through some or all of the foregoing stages. Some samples, such as clays, have a high water content which may be considerably altered by the grinding stage (it may be necessary to first dry the material in order to be able to grind it) and the final powder from such samples should be spread out on glazed paper and allowed to come into equilibrium with its sur-

roundings before being bottled. Soils usually require drying, and then must be passed through a coarse screen to remove organic fragments before being crushed.

Mineral samples, unless mono-mineralic by nature, require a concentration stage which usually consists of one or both of two methods, one utilizing the magnetic susceptibility of the mineral, the other its specific gravity.

For magnetic separations the Frantz isodynamic separator is perhaps the best known device. The powdered sample moves down a vibrating chute parallel to the pole pieces of an electromagnet which separates the more magnetic and less magnetic particles into two streams which fall into separate collector vessels at the foot of the chute. Desired separations within these two major groups can be achieved by varying the current strength and/or the inclination of the chute. A preliminary coarse separation, which reduces the amount of material which must be fed to the Frantz, is done with the Carpco direct-roll magnetic separator.

Heavy minerals are concentrated from bulk samples by treatment on a Wilfley table. The powdered sample is carried by a stream of water to an inclined table bearing a number of riffles; the heavier minerals are caught in the riffles, the lighter ones float over them. The superpanner is used for separating minerals whose densities differ only slightly; a thin film of water flows down a long sloping surface and powdered samples are separated into constituent minerals on the basis of different rates of flow for the top and bottom of the water layer.

The differing densities of minerals is the basis also of separations involving heavy liquids. These are organic liquids of known specific gravity, such as bromoform or methylene iodide, and when a mineral concentrate is suspended in one of these some minerals will sink while others remain suspended, thus effecting a separation. Care must be taken to ensure that all trace of the heavy liquid is expelled from the mineral concentrate before subsequent laboratory work is done.

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SAMPLING OF BIOLOGICAL MATERIALS

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As many so-called essential elements are present in biological fluids and tissues in the subnanogram/gram range, contamination-free sampling and storage prior to the actual analysis is primordial. This is particularly the case when an essentially blank-free method like activation analysis is applied.

The used surgical equipment and storage vessels must be checked for their content of elements to be determined and adequate cleaning procedures must be adopted. As the tendency of liquid (or wet) samples to pick up contaminants from the vessel walls is much greater than that of dry samples, a drying (lyophilization) step should be enforced as early as possible after collection. (This will also prevent to a great extent the loss of traces to the vessel.) All samples should also be protected from dust as in an urban, industrial or laboratory environment airborne particles are important pollutants. All sample treatment and storage must be done in a dust-free room.

A typical example (the collection of human blood) will be discussed *in extenso*, together with the design of a dust-free room of simple conception and reasonable cost.

The adequacy and success of these procedures with continuous discipline is illustrated by the very low concentrations in serum obtained for Cr, Mn, and Co, respectively 0.15, 0.5 and 0.1 ng/ml. Furthermore as these elements are essential and the samples practically uncontaminated, the results present a Gaussian frequency distribution with a small standard deviation.

Keywords: Biological materials; chromium; cobalt; contamination; manganese; sampling; serum.

I. Introduction

As biomedical scientists become more and more interested in the role of the trace elements in biological fluids and tissues, methods and techniques of analysis are being developed, improved, and pushed to the extremes of sensitivity. The results obtained by any method, atomic absorption spectroscopy and (neutron) activation analysis the most likely, however, are only valid when they are obtained on "real" samples. The fact of nonobservance of this condition is proved by the continuously decreasing concentrations, by orders of magnitude of *e.g.*, manganese, cobalt and chromium for normal persons in blood serum. It is clear that, in general, not the method nor the analysts but essentially the sampling bears the burden in this situation.

It is the purpose of this paper to follow the history of a sample from the moment prior to it being taken, up to the start of the actual analysis. Dangers encountered, solutions proposed and unsolved problems will be discussed.

As the danger of contamination is naturally related to the concentration to be determined, it can safely be assumed that in every really clean (analytical) laboratory samples can be treated for the determinations in the μg/g range, *e.g.*, Fe, Cu, Zn, Se in blood serum. This opinion can also be formed from the near uniformity, or in any case good agreement, between the results from different origins and over a long period of time in that situation. When concentrations go down to the nanogram or subnanogram/gram range (ng/g), the whole prospect changes and all normal cleanliness or precaution practices in a hospital ward or a chemical laboratory are completely insufficient. This again can be concluded from the spread of values for Mn, Co and Cr in serum found in the literature. It is this last situation that will be the principal consideration from here on.

A. The "Normal" and "Not Normal" Person

It is obvious that the selection and classification of persons to take part in a study on trace element concentrations in biological materials can only be done by physicians, and is in no way the attribution of the analyst. However, some remarks can be proposed.

The selection is never rigorous enough and some outliers amongst well-defined results could probably be explained as a hidden illness or body malfunction instead as a result of statistical probability. When diurnal variations in the concentration patterns are claimed or established, stan-

dardizing the moment at which the sample is taken, *e.g.*, preprandial, postprandial, *etc.*, will improve the ultimate result. When studying hospitalized persons, care should be exercised not to confuse the influence of food or medication. The borderline case of raised Cu-serum concentration in women taking oral contraceptives is a very eloquent example. A strange source of internal contamination can be a surgical intervention whereafter metallosis occurs, as was found in raised Co and Cr levels in blood, urine, and hair from patients with metallic hip replacements [1]. When applying neutron activation analysis, the possibility of the presence of radionuclides, such as ^{75}Se, used for diagnostic radioisotope scanning purposes must always be kept in mind.

B. The Surgical Material

Except when sampling sweat, saliva, or urine, surgical instruments are always needed, such as needles for venepuncture and liver biopsies; blades for sampling skin or (autopsy) tissues as liver, kidney, aorta; scissors for hair or nail clippings; tweezers for placing the samples. Most of these instruments are made from stainless or special steels, and may contain from 8 to 20 percent chromium, 8 to 12 percent nickel, less than 1 percent cobalt, manganese, *etc.* As these elements are present in some biological materials in the ng/g range or below, it is obvious that the enormous concentration gradients which exist will present extremely great risks of contamination when, *e.g.*, blood is flowing through a needle.

Therefore, knowledge of the composition of the surgical material, down to the minor and trace constituents, will help in avoiding these hazards.

To what extent these contaminations do occur is not easily established, but a method used by Versieck [2,3] may give reliable information on contamination introduced when sampling blood or liver. *In vitro* experiments simulating medical practice were performed with neutron activated instruments and the contamination introduced measured by its accompanying radioactivity. Some of these findings are quantitatively expressed as "apparent concentrations" in table 1 and table 2.

Being conscious of the fact that most of the "normal" values listed are at best upper limits, it is obvious from such experiments and results that the studied material is useless for the analysis of chromium, nickel or cobalt in serum or liver. However, in applying this examination procedure to other materials, it was possible to substitute the disposable needles in table 1 by a plastic catheter, thus reducing, for example, the manganese contamination by a factor of 10. It was also feasible to lessen the discomfort for the blood donor by reducing a 3×20 ml flushing of the needle be-

TABLE 1. *Contamination introduced in blood samples (serum) using disposable needles*

		Manganese ng/ml
"Normal": 0.57		
20 ml fraction:	1	0.2
	2	.08
	3	.02
	4	.02
		Chromium ng/ml
"Normal": 0.7–0.2		
20 ml fraction:	1	85
	2	12
	3	10
	4	15
		Nickel ng/ml
"Normal": 40–3		
20 ml fraction:	1	71
	2	10
	3	8
	4	12
		Cobalt ng/ml
"Normal": 0.2		
20 ml fraction:	1	0.9
	2	.2
	3	.1
	4	.2

fore accepting a valid sample to one single 20 ml rinse, with better quality samples.

The use of adequate laser beams on hard or soft tissues could be a solution in the near future [4]. Their possibilities should be further investigated: no addition of material will be made but charring of the tissues could change the weight ratios studied.

For venepuncture another mechanical solution is possible: needles made from Pt-Ir alloy of highest purity would be convenient for most analysis methods, except neutron activation analysis, where the merest trace of Ir would be a great annoyance in γ-spectroscopy, because of the very high sensitivity for this metal together with the long-lived and highly complex γ-decay scheme. This inconvenience could be circumvented by making needles from a Pt-Rh alloy. One advantage of neutron activation analysis on samples taken by regular surgical instruments is the detection of contamination through the presence of ^{182}Ta and ^{46}Sc (from Ti) on the

TABLE 2. *Contamination introduced in liver samples*[a]

	Manganese, ng/g	
	A	B
"Normal": 1500		
1st series of biopsies	600–100	3–1
2nd series of biopsies	40–10	
	Chromium, ng/g	
	A	B
"Normal": 10		
1st series of biopsies	9000	15
2nd series of biopsies	2000–500	
	Nickel, ng/g	
	A	B
"Normal": <60		
1st series of biopsies	12000–6000	60–10
2nd series of biopsies	5000–1000	
	Cobalt, ng/g	
	A	B
"Normal": 20		
1st series of biopsies	230	1
2nd series of biopsies	50	

Notes: A = Menghini biopsy needles.
B = Surgical blades.

sample: these elements not common in biological materials, are usual at low levels in special steels.

C. THE TREATMENT OF SAMPLES BEFORE STORAGE

When the various samples have been obtained in the proper way, dangers now arise in the period of storage prior to the analysis.

It is necessary here to divide the samples in liquid and solid materials. Liquid samples can have altered trace element concentrations through at least two mechanisms:

1) the liquid can leach out traces of material from the walls of the containers. Knowledge about the composition and selection of the right material will drastically reduce this hazard.

2) the second source of error is of the trace element adsorption onto the walls of the containers, lowering the trace element content. Here again selection of materials will help effectively.

Probably the best material (and the most expensive) for storage vessels is quartz of the highest purity available. Again, however, candor on behalf

of the sampler may lead to gross errors. Razeghi [5] found in a very high quality material up to 460 μg/g Mn and 95 and 15 ng/g for Co and Au: levels of impurities not compatible with the "normal" concentrations in tables 1 and 2. Even better material is available, as another commercial brand gives results like 20 ng/g Mn and 3 and 0.01 ng/g for Co and Au, whereas Cr still remained at a 150 ng/g level [6]. Pure polyethylene, with regard to impurities (combined with its low cost), is in most cases a very suitable storage material. It usually contains Mn, Cu, Sb, W, Au, Cr, Cd, *etc.* A contamination of approximately 0.08 ng/g Mn in liquid serum can still be found, however.

Investigations of adsorption onto the container walls usually prove polyethylene to be the better material but for Pb [7] and Cr [8] glass should be preferred.

Since, however, dry, solid samples practically do not present important positive or negative changes in concentration upon storage due to the impossibility of leaching or adsorption, it is a logical decision to transform any liquid sample, as early as possible, in a dry, solid form. The most obvious and practical method is lyophilization, in which, after a fast freezing process, the water content is slowly eliminated. A constant weight usually is reached after 24 hours with most materials. An additional advantage for neutron activation analysis is a concentration gain of a factor of 5 for liver, 10 for serum, 3 for packed cells and 16 to 25 for urine. For atomic absorption methods a drawback is probably the necessity to bring the solid sample in a liquid state again. During lyophilization, escape of highly volatile elements or compounds is possible, mercury being a first choice.

A special problem is presented by blood. Whereas results for whole blood have value only in special situations, the analyses must be performed on the major constituents: serum and cells. The fractionation in centrifuge tubes at 2500 rpm for 1 hour is not a problem. However, an absolute separation is not possible. The danger first encountered is hemolysis whereby part of the elements contained in the red cells will contaminate the serum fraction, thus producing erroneous results later on. Care and routine do alleviate this situation. Too, red cells are never totally free of serum. Radiotracer experiments with ^{131}I-serumalbumen showed 6 percent of the total serum to remain in the packed cells. As the ratio of the concentrations in packed cells to serum varies over a very wide range, 0.6 for copper to approximately 600 for iron, positive and negative errors can result. A 5 percent positive error for Cu and 7 percent negative error for Zn and Mn in packed cells can be calculated.

D. STORAGE OF THE SAMPLES PRIOR TO ANALYSIS

When the ideas and rules presented above are observed, the samples to be analyzed should by now fulfill the desired degree of "normality," *i.e.*, they should represent real "normal" or "not normal" persons without any significant introduction of traces since the sample left the human body.

The containers and centrifuge tubes from the preceding paragraph and the final storage vessels must be exceptionally clean which can be obtained only by vigorous action. Great efficiency is achieved by the following procedures. Quartz tubes are rinsed with deionized water, soaked for 2 days in reagent grade H_2O_2, rinsed again, twice boiled for 3 hours in a HNO_3-H_2SO_4 mixture of Suprapur quality, rinsed again and then steam-cleaned with triple quartz-distilled deionized water and dried thereafter in a specially reserved oven. Their Teflon stoppers are cleaned in an identical way, except for the steam cleaning.

Polyethylene containers are subjected to the same treatment, except the boiling HNO_3-H_2SO_4 mixture. And even this is not always adequate. Analyzing urine for gold at an 0.03 ng/ml level could only be brought to a satisfactory conclusion when an HNO_3-HCl mixture was used for cleaning, with an unacceptable effect, however, on the chlorine content or urine, raising it by 2 mg/ml.

Transport of the samples must be limited as much as possible and should be done in air-tight boxes. Permanent storage is only acceptable in a dust-free room. Further handling of the samples (opening of container, weighing of aliquots, dissolving, *etc.*) must be carried out in boxes, specially designed to keep out all contaminating particles.

The installation of a "dust-free" room is of the utmost importance if reliable "real" results are to be obtained. Indeed, dust particles in an urban or industrial area, even inside a laboratory, contain "huge" amounts of contaminants, *e.g.*, up to 460 ng Mn/m^3 of air have been found. This influence of laboratory and hospital airborne particulates was also detected by Heydorn when analyzing serum for manganese [9].

A not too expensive dust-free room can be installed in the following way: a normal laboratory room is separated from the rest of the building by an air-lock. The room is pressurized (2 to 4 mm water) by absolutely filtered air to keep out exogenous airborne material. Inside the room where "surgery room" clothing and overshoes are worn, an electrostatic air precipitator removes all endogenous material. Lyophilization, storage, and weighing are performed in perspex hoods which have no metal parts on the inside. The same hoods are again pressurized with absolutely filtered air.

As a result of the impossibility of obtaining absolutely clean container material, neutron activation analysis with measurement in the irradiated capsule is mostly senseless for the lowest concentrations encountered. This unfortunate situation makes it necessary to recover the sample, often heavily damaged by the irradiation conditions, by a mineralization process.

E. Contamination Problems in the Analysis

The reasons for obtaining erroneous results with an excellent method in "real" samples are varied, but again are based on the fact that the samples are changed in their constitution before the actual analytical measurement is made.

In atomic absorption spectroscopy the necessity of obtaining, in almost all cases, samples in solution introduces the danger of blank values, but of these all users of the method must be aware, and these nonzero values can be determined with reasonable ease and accuracy.

Efforts made nowadays in applying x-ray fluorescence to biological samples not only suffer from a (maybe temporary) lack of sensitivity but also from the impossibility to find a suitable compound for binding or pelletizing the sample to be analyzed. The most likely material, ultra pure carbon, is completely inadequate regarding purity for the concentrations encountered.

Neutron activation analysis suffers from contamination from the irradiation materials. Recoil effects are often overlooked but positive errors in the accuracy exist by capture of radioactive atoms from the containers by the sample. The analysis of iodine in flour in the ng/g range is hindered by an effective release of an important fraction of the approximately 20 ng of iodine present in the polyethylene container [10]. For chromium-urine analysis, 10 ml samples are lyophilized in small polyethylene bags; upon irradiation recoil ^{51}Cr atoms give important blank values [10]. From cleaned quartz capsules irradiated at high neutron fluxes "blank values" of 0.05 ng Cr and 0.01 ng Co may be measured, necessitating, especially for Cr, important corrections for 100 mg lyophilized serum samples, and thus endangering the establishing of "real" "normal" values.

F. Human Blood Analysis as a Case History

As an illustration of the preceding paragraphs a neutron activation analysis experiment on human serum will be described.

A collaboration was established between the Department of Internal Medicine (Gastro-enterology) and the Institute of Nuclear Sciences of the University; the first partner providing a physician and the persons to be analyzed, the second partner contributing its long-time practice of neutron activation analysis. Thus were assembled the sampler with the awareness developed through experience about contamination and the analyst, used to the determination of subnanogram quantities.

The persons selected as "normal" were usually provided by the hiring service of the University. Their medical history was carefully checked before blood samples were taken. After cleaning with distilled water the place where the venepuncture was to be made, the necessary volume of blood flushed the sampling instrument (this blood was used for more classic or routine investigations). The final samples were collected in three fractions, each in a quartz centrifuge tube and stoppered with Teflon. These were stored in an air-tight polyethylene box of a commercial type and immediately transported to the dust-free room of the nearby analytical laboratory, where all subsequent manipulations prior to transfer to the nuclear reactor were carried out. Fractionation and lyophilization were started immediately, and at the latest 24 to 30 hours after collection. Dry serum or packed cells were transferred to three polyethylene screw cap vials, and while awaiting analysis were kept in one of the dust-free perspex hoods.

In the actual analysis, two of the three samples were analyzed according to two procedures, of which the purely analytical part will not be described here.

1) Manganese was determined, together with zinc and copper, in the institute's own nuclear reactor: for this purpose 50 to 100 mg samples were introduced in polyethylene capsules.

2) Chromium and cobalt were activated in the BR 2 high flux reactor; to do this 100 mg samples, in their quartz irradiation containers, were mineralized at about 500 °C for 24 hours, and then heat sealed; after irradiation the quartz capsules were soaked for 10 minutes in 10M HF to remove, as much as possible, external contamination. Thereafter the analysis started with a mineralization step.

II. Results

The efficiency and quality of the whole procedure is best demonstrated by the results obtained for three elements in serum.

1. Manganese – The serum manganese content for 46 normal persons

has a mean value of 0.57 ± 0.13 ng/ml with a range of 0.38 to 1.04 ng/ml. No statistically significant difference between male and female persons was found and as could be expected of an essential element the frequency distribution is Gaussian.

The concentrations found confirm the results published by three other authors: 0.64 ng/g in 1963 [11], 0.59 ng/g in 1966 [12] and 0.54 ng/g in 1973 [9].

Typical values found in other papers present an extraordinary range of values: 24 ± 33 [13] and 0.5 to 210 ng/ml [14]. Clearly some of these results were obtained by inadequate methods, but others on samples presenting an enormous variation in quality.

2. Cobalt – From the literature it is difficult to establish a "normal" value for cobalt in serum: the mean results varying from 72 to 0.2 ng/ml, the lower level to be preferred. Following the procedure given, for 14 persons a mean value of 0.097 ± 0.042 ng/ml was obtained with 0.048 and 0.185 ng/ml as extreme values. Work in progress will most probably improve these values and confirm the essentiality of Co as a trace element by presenting a Gaussian frequency distribution of the concentrations.

3. Chromium – Again following the strict rules laid down earlier, 18 human sera were analyzed for chromium. Two essential difficulties are at the base of not as satisfactory results as for Mn of Co:

1) the lower sensitivity of the applied method for this element, and

2) the important blank value (see above) representing between 15 and 50 percent of the total amount of chromium detected.

The results range from 0.03 to 0.33 ng/ml with a most probable value of about 0.15 to 0.20 ng/ml, far away from most values hitherto published (table 3).

From the results now obtained two hypotheses may be formulated:

1) the samples analyzed are still partly contaminated, maybe from the beginning, *i.e.*, the persons selected are "not normal" with respect to chromium. There are possibilities for explaining internal contaminations: the extensive use of stainless steel in kitchenware and tableware, food and dairy industries, *etc.*

2) chromium, not presenting the Gaussian distribution, narrow range and small standard deviation as such other essential elements as Mn, Fe, Co, Cu, Zn, and Se lacks the quality of essentiality which has been claimed for it [15].

TABLE 3. *Chromium concentrations in human blood serum*

Neutron activation analysis		Atomic absorption spectroscopy	
Year	Chromium (ng/ml)	Year	Chromium (ng/ml)
1967	2.5	1967	29 (plasma)
1968	1	1969	30
1969	38	1970	20
1969	36	1971	28 (plasma)
1971	3	1972	5 (plasma)
1972	9	1973	5 (plasma)
1972	10	1974	0.6
1972	32	1974	1.6

Other methods

Year	Chromium (ng/ml)	
1961	10	x-ray fluorescense
1962	345	colorimetry
1968	18	spectroscopy
1969	28	spectroscopy
1970	40	gas-liquid chromatography
1971	13	spectroscopy
1971	30	spectroscopy
1972	7	gas-liquid chromatography + mass spectrometry
1972	13	gas-liquid chromatography
1972	2	colorimetry
1974	150	chemiluminescence

III. Conclusions

As can be assumed from the propositions presented above many chances exist for a sample of biological material to be heavily contaminated before it is analyzed. It is therefore remarkable that so few authors give the impression in their papers of being aware of the introduction of unwanted additions in their samples.

It is of the utmost necessity to study carefully the analytical results obtained from the point of view of sample quality. This may be done by lengthy investigation until the lowest concentrations are obtained, assuming that then no more contamination takes place [12]; this may be dangerous as at these subnanogram levels compensation of errors is possible.

A mathematical-statistical approach also can give more security about accuracy and the ways of obtaining it [9]. A third way is to study step by step each degree of progress to the end result [2,3]; this is not always possible, but it is maybe the most "analytical" way. It may be believed that when sample taker and analyst are completely conscious of the problems and pitfalls of contaminating samples, expressions such as "metal free containers," "proved blank material," "chemically clean glass," "metal free nitric acid for preseparation," "all glassware was rendered metal free," *etc.*, no longer will appear in scientific papers. Apart from being analytically meaningless, they do not help along those who know.

As a hopeful consequence of this Symposium, the disappearance from the literature could follow of the three kinds of papers:

1. relating industrious efforts to improve methods and sensitivities, testing them on contaminated samples or on reference materials with 100 times too high concentrations;

2. establishing "normal" values by large scale experiments, invalid because the biological material as such did not qualify in that respect;

3. proving in medical studies the variation of trace element concentrations related to physiological situations, based on erroneous "normal" values or a simple comparison of the efficiency in avoiding contamination by two different sample takers.

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Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis,
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ACCURACY IN AIR SAMPLING

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The physical act of sampling the atmosphere for the subsequent determination of its trace composition is complicated by the fact that the atmosphere is not an equilibrium system. It contains numerous species that are mutually incompatible with one another, but that survive to be measured because of their extreme dilution. These arise from many point sources, many of them sporadic in nature, and hence the atmosphere is poorly mixed, even on a relatively small scale. This leads to serious problems in attempting to isolate a small portion of atmosphere in a form that may be carried back to the laboratory for subsequent analysis – or even analyzed *in situ*.

The planning of atmospheric sampling is further complicated by the statistical properties of the system. Simultaneous or sequential measurements, for example, within a city are not independent numbers, but tend to be highly autocorrelated. As a result, statistical judgments as to the number of necessary measurements to define mean levels within acceptable limits of accuracy have generally been misleading. In point of fact, most measurement networks have been designed around an available budget rather than around statistical ideality, and frequently without a clear notion of the purpose of the resulting data.

Keywords: Accuracy and precision; air; air particulates; air pollution; analysis of air; atmosphere; gaseous pollutants; sampling air.

I. Introduction

The total question of accuracy in the sampling of air for contaminants is extraordinarily complex. In the first place, it subsumes two separate questions: (1) if one assumes that the final analysis is perfectly accurate, what will be the correspondence between the final analytical result and the actual composition of the sampled air? (2) Granted that question (1) is answered completely satisfactorily, what will be the correspondence between the analytical result and the experienced air quality by any receptor of the contaminants in question? These are the questions classically

referred to as sampling accuracy and representativeness. Generally speaking, a great deal of work has been done on the former point, but very little on the latter. These two topics will be considered in turn in the discussion which follows.

II. Sampling Accuracy *Per Se*

An increasing number of instruments are becoming available that are true *in situ* analyzers. Examples are the several types of direct spectrometric analyzers—nondispersive infrared, second derivative ultraviolet, light scattering, *etc.* These analyzers directly transduce concentrations, without alteration of the air, and thus in a real sense do not sample. Where this is actually the case, there appear to be no problems of sampling accuracy in the sense that the term is used here. This is, on the other hand, not true, even for the same sorts of analyzers, where some sort of sample pretreatment is necessary. Where there is, for example, interference in nondispersive infrared analysis from water vapor, then a constant (generally low) humidity level is necessary, and there is the possibility that the concentration of the substance being analyzed may be changed in the process, perhaps by dissolution in the condensed water. Where particulate matter must be removed by filtration prior to analysis, there is always the danger that one or more components in the collected particulate matter will absorb (or desorb) the test gas. The prefilter must also be tested against this possibility; for example, cellulous filters appear to collect a number of acidic gases with rather surprisingly high efficiency. Obviously, the problem is by no means insoluble but needs to be approached with a degree of agnosticism in each new environment.

The bulk of the analytical systems in use, however, respond to the total flow of the analyzed material through the system rather than the concentration directly. It is convenient to differentiate two subcategories. The first of these will be referred to as monitors.

Most monitors are essentially robot chemists that withdraw the substance to be analyzed from the incident airstream, thereby concentrating it, induce a chemical change in it, and measure the result optically or electrochemically. Since analysis is immediate, there is little problem with sample durability. However, the fact that most monitors are intended to operate unattended for greater or lesser periods of time places extreme demands on the accuracy of fluid flow in the sampling systems. Many monitors contain a circulating liquid into which the substance to be analyzed transfers from the gaseous phase. Both this flow and the sam-

pled air flow must be stable over periods comparable with the normal period of unattended operation. The first generation of monitoring devices was virtually without exception deficient in this area. The newer generations of instruments are improved, but this is still a weak point in nearly all monitoring systems, and one that deserves close attention in the design of new instrumentation. It should be noted that, if the instrument can be calibrated with a standard gas mixture, it is not essential that the flows be known accurately – merely that they be extremely stable.

As previously noted, most monitors operate by transferring the contaminant of interest from the gas phase to solution in a liquid. The time available for this transfer is finite, and the transfer is usually not complete, although it may be very high. Unfortunately, there has been very little study in the temperature dependence of these transfer processes in the actual equipment commercially supplied. For many purposes, this is unimportant since the instruments are housed in shelters furnished with heating and air conditioning, and thus their environmental temperatures are stable to within a few degrees. However, if instruments are to be used under less equable conditions, the possibility of a temperature coefficient of collection must be considered and investigated.

For most monitoring systems, however, the greatest source of sampling error is the inlet system. It is astonishing how many monitoring systems of great complexity and sophistication are interfaced to the atmosphere by totally deficient intake manifolds. Yamada [1] reported a survey, actually made during mid-1968, of 34 jurisdictions operating monitoring stations. For practical purposes, not a single one was operating under conditions that would give any confidence that the resulting data were in error by less than 10 or 20 percent. Yamada and Charlson [2] gave parameters of an inlet design that could be defended with respect to its ability to deliver both gases and particles to a sampling port with reasonable assurance that they had not been modified by the manifold itself. Butcher and Ruff [3] subsequently added another constraint with regard to sampling of nitrogen oxides and ozone; under the reasonable assumption that ultraviolet light intensities are less indoors than out during daylight hours, they computed maximum transit times to minimize the effects of the dark reaction between nitric oxide and ozone. It is discouraging to note that (a) this appears to be the sum total of all research done on generalized inlet manifold design, as distinguished from validation of individual, empirically designed manifolds; and (b) there is little evidence that any agency responsible for monitoring has responded to their work by any changes in inlet system.

If the design suggested by Yamada and Charlson is used for the main

manifold, then the material of which that manifold is made becomes relatively unimportant. In fact, as they point out, in the typical urban environment the inner walls of the manifold can be assumed to be made of dust after a very short period of operation.

This is not necessarily true of the individual lines leading from the manifold to the monitors, since they are clearly visible to the operators, and can be cleaned with reasonable frequency. These should be of material that is inert toward the substance being analyzed, and as short as physically possible. For most gases, glass is preferable, and there are hardly any gases that will survive a trip through any finite length of poly(vinyl chloride) intact. If particulate sampling is part of the design of the monitoring installation, it is virtually mandatory that the intake manifold be vertical, and that the instruments be arrayed around it instead of spread laterally across a convenient and esthetic laboratory bench. This arrangement in fact is preferable for gas sampling, since it decreases the rate of dust deposition in the manifold.

When the act of sampling is physically and chronologically separated from analysis—*i.e.*, when samples are taken in the field for subsequent analysis in the laboratory—sampling is usually directly into the collecting media, and the problems of intake manifolds are negated or at least minimized, as are the problems of controlling the flow of continuously pumped reagents. However, sampling rate is still a problem, particularly if sampling is to continue for a long period. Wartburg *et al.* [4] have designed one of the most durable and precise systems for sampling at relatively low flow rates. Clearly, a comparable approach could be taken to higher sampling rates as well, and unquestionably should be.

The sampling of atmospheric particulate matter generally requires a further constraint on sampling; to wit, the achievement of isokinesis in the intake system. For the few individuals not familiar with this problem, the review by Bloomfield [5] is an excellent resource. It should probably be pointed out that Bloomfield's definition of isokinetic sampling is not, strictly speaking, correct. It is true, other things being equal, that sampling in which the velocity inside the sampling probe is equal to the air velocity outside is in fact isokinetic. However, the term means literally that the air within the sampling probe has the same kinetic energy as it had prior to sampling, which is not necessarily quite the same thing.

Several *caveats* are in order. I have seen elaborate precautions for isokinetic sampling in systems intended only to collect gases. This is emphatically unnecessary. There is no point to sampling particles with great care to attain isokinesis, and then lead them through 25 feet of coiled Tygon tubing before sampling them (the picture is overdrawn, but I have seen situations that were almost that bad). Finally, despite the textbooks,

the graphs showing horrible errors resulting from anisokinetic sampling are not quite the whole story. With very extreme mismatches, the errors decrease again. For example, if one is sampling from a jet aircraft, there will probably be less error in using a very small sampling rate than in a fruitless attempt to match the external air velocity. It is far more important in this case to locate the sampling port at some point out of the major shock wave of the vehicle. Unfortunately, there is very little research on errors at extreme mismatches, which makes theoretical corrections of samples taken in this way quite difficult.

Having got the sample into a collecting device, the obvious next problem is to collect it, preferably quantitatively, or failing that, with a known and reproducible efficiency. The previous comments on possible temperature effects are appropriate here. In addition, many gas sampling techniques have a definite threshold of collection. An obvious example is low temperature condensation. For example, let us assume that it is desired to collect a substance that has, at the temperature of boiling liquid oxygen, a partial pressure of 10^{-6} atmosphere. Let us further assume, for simplicity, that it is the only condensable substance in the air sample, so as to be able to ignore interaction effects. This seems a very low vapor pressure. However, a little reflection will make it clear that, if the ambient concentration is 100 ppm, it will be collected with 99 percent efficiency, whereas if the concentration is 1 ppm it will not be collected at all.

Furthermore, the standard and obvious way to determine collection efficiency is to run two identical collectors in series. Then, if the efficiency of the device is E, (expressed as a decimal), then the first collector will capture E of the material present, the second, E (1-E) *etc.* Obviously, the second low temperature collector will collect nothing whatever, and it will be assumed that the first is completely efficient, and that, if the first collector does not collect it, it is totally absent from the sampled air. Unfortunately, many other devices, the reasoning for which is much more obscure, also have collection thresholds.

The collection of particles is also beset with pitfalls. Particles bounce off or shatter in cascade impactors and land on stages corresponding to sizes far smaller than they actually were—or are lost to the walls. Spurny *et al.* [6] have shown that at least some types of filters can have extremely deep penetration maxima for rather narrow ranges of particle sizes.

In this class of sampling for subsequent analysis, one of the serious problems is the durability of the collected sample. In the simplest possible case, in which a plastic bag or syringe is simply filled with the ambient air, it must be recognized that, with the most inert material possible, reactions that were going on in the sampled air will continue to do so. Where the sample is concentrated in some fashion, a still further acceleration of reac-

tion is to be expected, simply because the concentration of possibly reactive materials is increased. In point of fact, frequently the best way to collect samples that will last is to alter them deliberately and in a controlled fashion. This is not always obvious to otherwise knowledgeable people in the field. For example, Hendrickson [7] states without qualification that "precautions must be taken to prevent alteration of the sample after it is collected." However, it should be pointed out that the unique contribution of West and Gaeke [8] to sulfur dioxide analysis was the demonstration that it is possible, in a quantitative fashion, to alter sulfur dioxide into the dichlorosulfitomercurate (II) anion, in which form it is stable for a very long time. Alteration is still more profound in the technique for formaldehyde sorbed on particles reported by Lodge and Frank [9]. Long before analysis is carried out the formaldehyde is irretrievably gone; the reaction product that is finally observed does not contain it. For that very reason, the final evaluation can be done weeks after the sampling, whereas the sorbed formaldehyde is labile and delicate, and certainly could not be preserved as itself for more than minutes after collection—and generally not then.

With all the added hazards of separate collection, transportation and final analysis, the scales may appear to weigh heavily in favor of monitoring on-site as a means of minimizing the problem. Furthermore, "real time" knowledge of concentrations is heady stuff. It would appear far more attractive than knowledge that may not become available for days after the act of sampling. Obviously, if one is in the middle of a city and an air pollution emergency is threatened, this makes good sense. When a further small increase will put the concentration over the threshold of serious health damage, there is no substitute for immediate knowledge of the pollutant concentrations, essentially on a minute-by-minute basis.

However, there are many other situations in which continuous monitoring equipment cannot be used, and many others in which its use needs to be defended in the immediate context, rather than theoretically. In the mountain West, for example, numerous continuous monitoring devices are being emplaced to measure the background concentration of pollutants before the industrial development coincident with the exploitation of western coal and oil shale. With a few exceptions, these monitors are giving magnificent continuous records of instrumental noise level, with occasional fluctuations caused by changes in line voltage. Present concentrations of contaminants are so low that they can only be detected after long periods of integration. Furthermore, it is frequently forgotten that to one degree or another all "continuous" monitors integrate over some time period or other. The old wet chemical analyzers for nitrogen dioxide using the Saltzman reagent integrated for something close to 15 minutes. At

similar concentrations, manual sampling for 15 minutes gives perfectly detectable amounts of nitrogen oxides. The manual sampling required a technician in attendance. The continuous monitors required a technician in attendance, and the hourly rate for electronics technicians is higher than for chemists. It should also be pointed out that extremely fast response times are frequently undesirable; for many contaminants the atmosphere is imperfectly mixed, and the result of truly instantaneous knowledge will be the generation of a good index of atmospheric turbulence and little else. Finally, many of the highly prized "real time" monitors are deliberately hooked to small computers that produce, as their only output, half-hourly averages. I am not opposed to all electronic devices. However, a large capital expenditure for them will frequently produce less data of less validity and at more expense than hiring a few well-trained chemists who know where to take samples.

III. Sample Representativeness

The above list of problems is certainly prepossessing, and their importance cannot be minimized. Nevertheless, it is clear that the entire array of difficulties with sampling accuracy are (at least in principle) susceptible to a technological fix. Proper interfacing of the sampling device with the atmosphere, generation of adequate analytical standards, and rigorous attention to detail will solve most of them. For the rest, certainly the analytical uncertainties can be reduced by a factor of 10 by merely analyzing 100 simultaneous samples (I said that it was theoretically possible, not that it was pleasant).

However, when all of this work is done, all the meticulous analyses completed, and all the calculations done with the latest electronic calculators, what is actually known to high accuracy is the composition of the air that passed through the sampling devices. What is still unknown is the composition of any air that did not do so. For better or for worse, air analysis is a real, not a theoretical problem. The final numbers generated are not abstract information, but are used to answer questions relating to the health and well-being of hundreds, thousands, or even millions of people.

Since I am an Air Pollution Commissioner in my home state, let me personalize the sorts of questions that arise here. I need to know whether the analytical data on which the epidemiological studies supporting the air quality standards and various alert levels are based are at all accurate or reproducible. I need to know with high certainty when dangerously high pollution levels exist in the cities of the state. I need to be able to predict,

and keep the Governor informed, of the probability that this is the year that he is going to have to close all access to downtown Denver for 2 days. I need to know the actual pollution dose encountered by an individual living in Aurora and commuting to downtown Denver. Finally, I need to know whether the substantial sums of money expended by the people of Colorado on pollution control have actually turned the tide and set the cities of the state on a trend of decreasing pollution.

Obviously, what is needed is a technique that will plot with substantial accuracy the pollutant isopleths of the city, and will give me an accurate measure of average pollutant concentrations over the entire city, together with a measure of the dispersion of data around that average.

In the United States, it has been traditional to assume that the distribution of pollutant concentrations over time for space is essentially lognormal, although Georgii *et al.* [10] find that, at least for the data available to them, this assumption is not supported by the chi-square test (nevertheless, in the absence of any other convenient functional form, Georgii *et al.* proceed to use the lognormal assumption as one of their means of conveniently presenting lumped air pollution data).

One of the few opportunities really to resolve this problem lies in the data from the air pollution study of Nashville, Tennessee (Keagy *et al.* [11]; Stalker and Dickerson [12]; Stalker *et al.* [13]). An assortment of air quality data was obtained at stations in networks as closely spaced as one-half mile, to a total of as many as 119 stations for some measurements. With this body of data, it was possible to test the hypothesis that fewer stations would give the same answer, as could less frequent measurements than the continuous or continual measurements actually made. Empirically, it was discovered that a rather small number of stations, sampling rather occasionally would give the same means. However, the authors involved themselves in some unjustified exercises with random statistics to prove that they would have needed, for many purposes, as many as 400 to 700 stations to obtain reasonable mean values.

The answer, of course, is that air pollution data are not random numbers, but higher autocorrelated numbers, both in time and space. That is to say, if the station is not directly in the track of a strong point source, a high value at it will probably connote high values at most stations, and a high value now leads to a high probability that the values were high last hour and will be high next hour as well. After all, this merely bespeaks the meteorological control of pollutant levels, and the well known phenomenon of meteorological persistence. Unfortunately, the possibility of devising some sort of success rating in locating isopleths within the city was passed over as well.

Since that time, there has been no single air pollution study with enough stations to undertake this sort of computer game, and there seems little likelihood that anyone today will pick up the Nashville study and use the data from that.

As a result, neither I nor anyone else can accurately answer the questions raised at the beginning of this section. This is an obvious gap that needs to be filled by our colleagues in the profession of statistics, and filled at an early date. Otherwise, the local official will continue to be without guidance as to how many stations he really needs, and will be entirely at the mercy of the political forces dictating his budget. The original design of the RAPS study in St. Louis called for it to generate a new set of such data. Unfortunately, even it has been designed to a budget rather than being designed for science.

IV. Summary

Numerous technical questions will remain concerning the intrinsic accuracy of the act of sampling the atmosphere. Probably the greatest need is for additional primary standards of substances that can be used to assess the effect of changes in sampling configuration, and eliminate remaining questions concerning sampling bias. One of the most serious problems is in the lack of techniques for generating standard aerosols; another is in the dependable generation of standard mixtures of gases at the levels experienced in the "unpolluted" atmosphere. Techniques now appear to exist for analyzing a number of substances in the part-per-trillion concentration range; however, not one of them has been successfully standardized against a realistic known concentration in the air.

A much more serious and fundamental lack, however, is in the area of sampling representativeness. There are no reliable and theoretically justified techniques for anticipating the number of samplers needed, the optimum frequency of discontinuous sampling, or the proper selection of sampling sites to answer the various hierarchies of problems facing the working air pollution control official.

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Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis,
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THE REDUCTION OF ORDERS OF MAGNITUDE ERRORS IN LEAD ANALYSES OF BIOLOGICAL MATERIALS AND NATURAL WATERS BY EVALUATING AND CONTROLLING THE EXTENT AND SOURCES OF INDUSTRIAL LEAD CONTAMINATION INTRODUCED DURING SAMPLE COLLECTING, HANDLING, AND ANALYSIS

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Most present analytical practices for lead cannot reliably determine lead concentrations at the 1 ng/g level because of a universal lack of familiarity with lead contamination during sample collecting, handling and analysis. Consequently, the great mass of published data on lead in plant and animal tissue and in water is associated with gross positive errors which obscure the meaning of most work dealing with lead at the few μg/g level.

It can be stated unequivocally that for lead concentrations in the μg/g range or less the investigator must know with certainty the magnitude of the contribution of lead from each individual reagent, from air exposure, and from container walls; furthermore he must know the yields for each step in the chemical separation procedure so that he can modify the contamination contribution at any given step caused by yields of less than 100 percent in the preceding step.

Techniques for the collection of uncontaminated samples of fresh water, snow, sea water, and animal tissue are described. A clean laboratory for low level lead analyses is also described and other sources of lead contamination are discussed and means of control evaluated.

Keywords: Accuracy of atomic absorption analytical techniques; accuracy of isotope dilution analytical techniques; errors in lead analyses; lead; lead contamination evaluation and control; lead in animals and plants; pollution, lead; sampling techniques; trace analysis of lead; waters, lead in.

I. Introduction

In most present analytical practices, lead concentrations in plants, animals, and foodstuffs cannot be determined reliably at the 1 μg/g level or in waters at the 1 ng/g level. This includes also the determination of lead in blood. The unreliability of analyses for lead is caused by a universal lack of familiarity with the extent, sources, and control of industrial lead contamination during sample collecting, handling, and analysis. As a consequence, the great mass of published lead data in plant and animal tissues and in waters is associated with gross positive errors and the error noise in lead concentration data below a few μg/g obscures the meaning of most work dealing with lead at these concentration levels.

It cannot be emphasized too strongly that the accuracy of lead, and other heavy metal analyses at these concentration levels, depends primarily upon the ability of the analyst to determine contamination blanks. Low level metal determination instruments such as atomic absorption (AA) spectrophotometers equipped with source furnaces are highly accurate and extremely sensitive when metals, in reasonably pure form, are analyzed in them. Attempts to analyze "garbage" without preliminary chemical separations in which the metals being determined are purified necessitates the use of corrections for interferences which, even under favorable circumstances, generally yield data that barely qualify as usable. Unfortunately, analysts who use such instruments without chemical separations are generally those least experienced and qualified to do so. Analysts who do use chemical separation procedures before determining metals with instruments such as AA, generally fail to use proper clean laboratory techniques and therefore contaminate their samples badly during laboratory processing. Serious errors are generally introduced by contamination during collection, transport, and handling of samples.

An AA instrument equipped with a source furnace can be used to measure quantities of lead as small as tens of picograms. A modest sample of plant or animal tissue collected in the field can easily be contaminated with thousands of nanograms of lead from the hands, clothing, and equipment of the investigator and the sample can be further contaminated by additional thousands of nanograms of lead from the exhaust fumes of vehicles during transport of the sample to the laboratory. An ordinary laboratory which is not pressurized with filtered air is a wind tunnel through which pass millions of nanograms of lead, in the form of submicrometer particles per day. Large fractions of these particles deposit by impact on any liquid or solid surface.

Nuclear geochemists studying the geochronology of U-Pb systems and the isotope geochemistry of lead by means of isotope dilution mass spectrometric techniques have developed, during the past 25 years, a considerable knowledge of methods of obtaining low level lead blanks and of clean laboratory methods. These investigators wished to study ubiquitous mineral systems found in small crystals of common rocks, so they were restricted to microgram sized U-Pb systems. This forced them to solve contamination problems at extremely low levels. A critical constraint was imposed upon their solutions to lead contamination problems. Existing knowledge of chemistry, physics, and geology was interlocked with their work, and errors in evaluating lead blanks were immediately exposed because these errors would yield data which tended to violate known laws of nuclear physics or known benchmarks in the geochronology of the earth.

Principles dealing with the evaluation and control of lead contamination which have evolved from the past work of these nuclear geochemists and which can be used as a guide in setting up clean laboratory facilities are summarized in the last section of this paper. This summary should be used only as a rough guide and should be augmented by consultation with investigators presently using clean laboratory facilities. A partial list of names and addresses of some of these investigators is also included at the end of this paper.

II. The Use of Blanks and Yields in Lead Analysis

It can be stated unequivocally that for the analysis of lead concentrations in the μg/g range or less, the investigator must know with certainty the magnitude of the contribution of lead from each individual reagent, from air exposure, and from container walls; furthermore, he must know the yields for each step in the chemical separation procedure so that he can modify the contamination contribution at any given step in the procedure caused by yields of less than 100 percent in the preceding step.

The following example of tuna muscle analysis illustrates the combined use of yields and individual reagent blanks. In the dissolution step the fresh muscle is exposed to the contamination shown in table 1.

All of this contamination lead is mixed with all the sample lead. This mixture is subjected to a resin column blank of 0.6 ng Pb, so that $\sim$ 0.8 ng of contamination lead is mixed with all the sample lead. Ninety percent of this mixture is then extracted with dithizone. Contamination from this dithizone extraction step is shown in table 2.

TABLE 1. *Lead contamination during acid dissolution*

		Pb, ng
6 ml HNO_3	0.012 ng Pb × 6 =	0.072
2 ml $HClO_4$	.018 ng Pb × 2 =	.036
Dissolution container blank		.05
Air blank	.004 ng Pb × 7 =	.028
Total dissolution blank		0.19

TABLE 2. *Lead contamination during first dithizone extraction*

		Pb, ng
1 ml $(NH_4)_3$(citrate) pH 8	0.15 ng Pb × 1 =	0.15
2 ml KCN	.064 ng Pb × 2 =	.128
1 ml Dz stock soln	.01 ng Pb × 1 =	.01
4 ml $CHCl_3$	.002 ng Pb × 4 =	.008
0.1 ml HNO_3	.012 ng Pb × 1 =	.001
Container blank		.025
Total extraction blank		0.32

All of this contamination lead is mixed with 90 percent of the sample plus contamination lead mixture from the dissolution and resin column step. Ninety percent of this mixture (low yields here are due to film losses in the separatory funnel) is then extracted again with dithizone. Contamination from this second dithizone extraction is the same except for the use of an additional 5 ml of $CHCl_3$, 0.1 ml NH_4OH, and 0.1 ml HNO_3 which makes a total of 0.3 ng contamination Pb.

Ninety percent of this mixture is exposed to additional contamination from evaporation and mass spectrometric filament loading reagents amounting to 0.01 ng Pb. The extraction yields are determined beforehand by analog techniques, while the column yield (the most variable for this particular type of sample) is determined by adding known amounts of Pb^{206} spike (sample Pb^{207} is determined from Pb^{208} spike). An example of the overall expression for the combined yield and contamination effects for 1.00 ng of sample lead is:

$$[\{(\text{Sample Pb} + 0.8 \text{ ng Pb}) \times 0.9 + 0.3 \text{ ng Pb}\} \times 0.9 + 0.3 \text{ ng Pb}] \times 0.9 + .01 \text{ ng Pb}$$

If the sample contains 1 ng Pb, then a total of 1.87 ng Pb will be observed by an AA type instrument. Proper use of yield corrected blanks will give a calculated answer of 1.00 ng sample Pb. However, the commonly used practice of straight blank subtraction gives 0.46 ng sample Pb which introduces more than 100 percent error.

III. Techniques for Collecting Uncontaminated Samples

A. Fresh Water

Industrial lead artifact contamination introduced during the collection of fresh waters from streams and lakes may elevate the lead concentrations observed in such waters by one or two orders of magnitude. For example, some 170 lakes in the High Sierra of California were analyzed for lead by Bradford *et al.* [1]. These investigators found an average of 0.3 ng/g lead in the waters. They collected their samples by bucket from a helicopter. These data were the most reliable available until recently, because previous analyses of similar types of water for lead showed considerably higher values. Fresh stream waters from this same region were collected and analyzed for lead in the Caltech biogeochemical clean laboratory using mass spectrometric isotope dilution analytical techniques. Careful procedures were utilized to prevent contamination during collection of these samples. The average value found by the Caltech investigators was 0.015 ng/g Pb [2]. The use of a leaded fueled helicopter by Bradford *et al.* to collect their water samples is an example of pronounced artifact contamination which probably accounts for part of their high reported lead results. The aircraft used by them burned gasoline containing approximately 0.79 g Pb/l at a rate of about 114 l/h. Their estimated hovering time while collecting a sample was several minutes. During this time approximately 3 g of lead in the form of aerosols 10 to 0.1 μm in diameter were emitted from the aircraft and blown onto the water sample surface. Quantitative considerations of various factors related to determining the proportion of this lead which would end up in the sample collecting bucket suggest that something like 3000 ng of lead contamination from the helicopter were added to the 3.8 liters of water collected as an average sample by them. The average amount of lead in this sized sample reported by them was 1710 ng Pb or 1650 ng greater than the Caltech observed value of 57 ng/3.8 liters of water. It can be thus seen that helicopter contamination can account for all of their average lead values.

Bradford *et al.* also analyzed these same waters for K, Ca, and Sr. The

Caltech group also analyzed these waters for these three metals [3]. Although the data obtained by Bradford *et al.* for these metals were either at the limit of sensitivity of their method or were expressed in terms of upper limit concentrations for these metals, their approximate data nevertheless did not disagree with the more accurate results which were obtained by the Caltech groups using isotope dilution mass spectrometric techniques. Therefore, the only outstanding disagreement was in the lead values and this could be accounted for by artifact contamination.

The Caltech samples were collected in FEP Teflon screw cap bottles, cleaned by the methods outlined in the last section of this article. The bottles were brought to the field protected by three successively sealed polyethylene bags, the inner one of which had been cleaned with acid as described in the last section. In the field the investigator removed the bottle from the bags while wearing plastic gloves and collected the water sample with the mouth of the bottle pointing upstream. The bottle was resealed within its bags, brought back to the laboratory where a small amount of pure NBS acid was added, and the water was frozen and stored until the time of analysis. The lead blank for these 2- and 1-liter FEP sample bottles has been shown to be ~ 1 ng Pb/2-liter bottle.

B. Snow

There are very few reported measurements of lead in snow so that the general level of artifact metal contamination of snows cannot be judged by comparison of older literature values with recent determinations made by mass spectrometric isotope dilution techniques. On the other hand there is a lot of alkali and alkaline earth concentration data in polar snows available in the literature which can be compared with alkali and alkaline earth concentration data in polar snows determined in the Caltech clean laboratory by mass spectrometric isotope dilution techniques [4]. Data in table 3 show that isotope dilution concentrations for alkali and alkaline earths are 1 to 2 orders of magnitude lower than concentrations determined earlier by other methods. It is now generally agreed on the basis of subsequent investigations that this discrepancy can be ascribed to artifact metal contamination introduced into the snow samples as a consequence of a disregard of contamination control requirements by the earlier investigators.

It appears that the chemical compositions of snows at temperate latitudes have not been correctly determined even though the concentrations of dust and salts in this type of snow are higher. This is shown by the following. A recent and comprehensive study of the snows in the Califor-

TABLE 3. *Geochemical data for polar snows*

Investigators	Metal concentrations (μg/kg)			
	Na	Mg	Ca	K
(Greenland Interior)				
CIT [4]	20	5	6.4	2.7
Langway [5]	135	16	45	47
Rodriguez [6]	20	—	100	30
(Polar coastal regions)				
CIT [4]	350	45	17	16
Brocas and Delwiche [7]	1920	—	—	380
Matveev [8]	<170	90	170	—
(Antarctic Interior)				
CIT [4]	32	4	1.3	1.6
Matveev [8]	<347	185	593	—
Hanappe et al. [9]	30	4.9	8.0	5.1

TABLE 4. *Concentrations of potassium and calcium in accumulated snowpack*

	K (ppb)	Ca (ppb)
Whole pack, average by Hinkley [3]	9.7	18
Other Sierra snow analyses by Feth et al. [10]	400	500

nia High Sierra has been carried out by Feth *et al.* [10] and these same snows have also been studied in the Caltech clean laboratory using isotope dilution analytical techniques. A comparison of these two sets of data in Table 4 shows that concentrations measured by isotope dilution analytical techniques are more than an order of magnitude lower than the earlier values determined by other analytical methods. Part of this difference may be due to the fact that the samples in the earlier studies may have contained aged dirty surface snow, but it is believed that higher concentrations reported in the earlier study are also in part due to artifact metal contamination introduced during collection.

This artifact contamination was minimized in the Caltech study by using the following collection techniques. The entire 3-m section of the snow pack was sampled by first digging a trench such that the bottom of

the pack could be reached by means of a series of steps. The discarded snow was placed on the downwind side of the trench while the upwind side of the trench was kept virgin. After the trench had been dug with an acid cleaned shovel which had been brought to the field wrapped in polyethylene bags, the investigators brought the sample collection gear to the trench. They put on clean polyethylene gloves and polyethylene parkas. Sections of the upwind vertical face of the trench available from the different step levels were scraped clean with an FEP scraper that had been acid cleaned and packed for transportation as described in the previous section. The investigators then collected small horizontal cores with an FEP corer and placed them in FEP bottles. The corer and the bottles had been acid cleaned and packaged for transportation as described in the previous section. The samples were sealed in the bottles, brought back to the laboratory, acidified and stored frozen until analyzed. The lead content of the 3-m snow pack (which had density of 0.44 g/cm^3) averaged 0.59 ng/g Pb. Detailed isotope, chemical, and mass balance studies of these samples and related samples showed that 98 percent of this lead originated from industrial sources. This means that natural levels of lead in these snows should be about 0.01 ng/g. This concentration level is more than 2 orders of magnitude lower than the concentration levels which most investigators can reliably determine today using common analytical techniques.

C. Seawater

Recent investigations of the concentrations of lead in seawater show that most common concentration levels are less than 100 ng of Pb/kg seawater in surface waters near heavy industrialized coastal areas [11]. Most seawater sampling devices now being used do not fulfill the requirements needed to collect seawater samples containing lead at these low levels for two reasons. Materials used in constructing the collection devices such as neoprene, polyvinylchloride, and polystyrene, *etc.* will introduce contamination at these levels. The second cause of contamination error is that adequate cleaning procedures such as those outlined in the last section of this paper have not been used. New collectors constructed of Teflon which can be properly cleaned need to be built and used for sampling seawater. The aura of contamination surrounding a research vessel can be avoided while collecting surface samples by sampling ahead of the vessel as it moves into virgin waters.

Present values for the concentration of lead in deep ocean waters reported in the literature are about 30 ng Pb/kg [12]. It is believed that

these values may be erroneously high by more than an order of magnitude because the sampler used to collect the water which gave these values, although properly constructed and cleaned, was contaminated while being lowered through the aura of dirty water surrounding the research vessel. Consequently new types of deep water sampling devices are being developed which can be protected against contamination while being lowered through dirty, shallow water. A diagram of such a sampler is given in figure 1. In figure 1A the sample chamber of accordion pleated thin walled Teflon tubing is compressed to zero volume and the deep seawater entry port is protected by a bath of pure water containing less than 0.1 ng Pb/kg. All parts of this pure water bath are constructed of Teflon which has been cleaned according to the methods outlined at the end of this paper. At the deep water sampling depth a trigger is actuated which retracts the water bath shroud and ruptures the end diaphragm which retains the pure water. This is shown in figure 1B. The water sampler is being lowered continuously during this operation so that it is continuously dropping down into virgin water. After a short interval which permits the bath water to be washed away, a second trigger is actuated which expands the sample accordion bag and seals the entry port. This is shown in figure 1C. The sampler is lowered during the filling stage so that only virgin water is allowed to pass the entry port. The entire sampler is then brought to the surface. A small quantity of acid is introduced into the sample

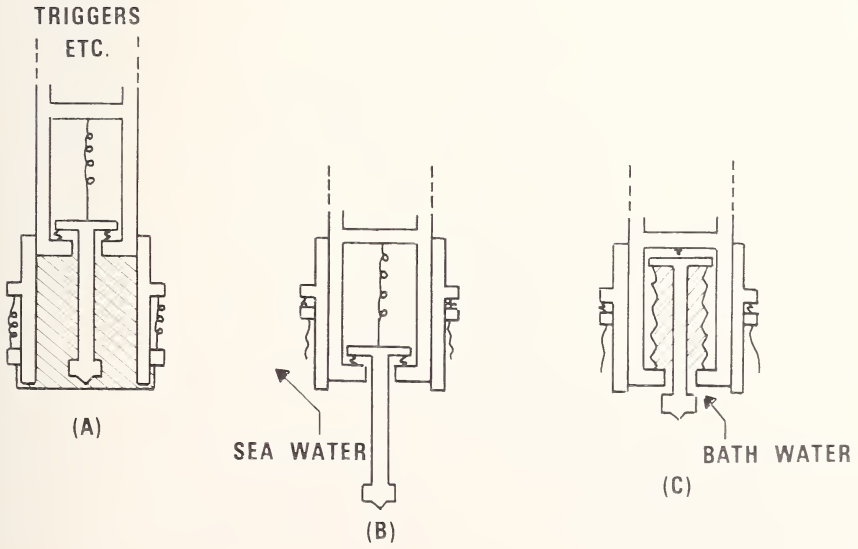

Figure 1. Deep ocean water sampler for collecting uncontaminated waters with Pb concentrations at 1 part per trillion.

chamber and mixed. This acid must be added to free adsorbed lead which collects on the inside surfaces of the sampler during the many hours required to bring the sampler to the surface. After standing an appropriate time, the acidified sample is then withdrawn through a series of protected ports located at the upper end of the sample chamber. By this means samples of 5 to 10 liters of seawater containing a few tenths to a few parts per trillion of lead can be collected for analysis without serious interference by artifact contamination.

D. Animal Tissue

Recent analyses of lead in tuna fish muscle in the CIT clean laboratories using mass spectrometric isotope dilution techniques have shown that lead concentrations in the muscle are about 0.0003 μg/g (wet weight) in fish dissected in a clean laboratory [13]. There is evidence that these initial concentrations of lead are elevated to much higher levels by industrial lead artifact contamination during the preparation of the fish muscle for human consumption. At the present time there is available only a single instance reported of an analysis of terrestrial mammalian muscle which was dissected in a clean laboratory and analyzed by mass spectrometric isotope dilution techniques [2]. In this case muscle from a wild mouse collected in a remote primitive area was found to contain 0.001 μg/g Pb. This animal was shown by related studies to be polluted with more than 83 percent industrial lead.

Samples of canned tuna muscle obtained from grocery stores have been found to contain 0.1 μg/g to 0.9 μg/g lead (wet weight).

A dried tuna muscle interlaboratory reference material was prepared by the National Bureau of Standards from fish which had been caught in exactly the same manner, time, and place as the tuna fish analyzed in the clean laboratory. High grade fillets of this latter tuna muscle were obtained in a commercial cannery and shipped to a commercial food processor where the meat was lyophilized, ground, and mixed. Aliquots of this mixture were placed in polyethylene bags and sealed in vacuum cans for distribution. An aliquot of this reference material analyzed in the CIT laboratory by isotope dilution methods was found to contain 0.4 μg/g Pb (dry weight) which is equivalent to 0.12 μg/g Pb (wet weight). This very large discrepancy which amounts to approximately 3 orders of magnitude between the concentrations of lead in tuna dissected in a clean laboratory and that processed by commercial food companies cannot be ascribed to lead soldered cans because the NBS reference material was packaged in polyethylene bags. Lead contamination of the NBS material must have

originated from lead containing seals, alloys, and coatings in the machinery of the food processing companies.

It has been shown that the above tissues cannot be reliably analyzed for lead by the techniques in common use today even though they have been seriously contaminated. Even though the lead concentrations in the NBS tuna material were elevated by more than 2 orders of magnitude above those existing in the fish when they were caught, most analysts in chemical oceanographic laboratories today cannot reliably measure lead in the tuna reference material [14]. This material was submitted to 11 university laboratories participating in the International Decade of Ocean Exploration Baseline Study of Heavy Metals Program and most analysts did not report lead values for the NBS tuna. A few reported erroneously high values and a few reported that its concentration was less than their measurement limit of < 0.5 μg/g.

It should be recognized that these incredibly low levels of lead in animal muscle are associated with enormously elevated lead concentrations in the skin and fur of these animals. For example, it has been observed in the CIT laboratory that tuna fish epidermis contains several μg/g of lead (wet weight), and that as much as 5 μg/g of Pb is contained in and on the fur of wild mice. This can pose serious problems of cross-contamination during dissection even though it is carried out in a clean laboratory. An explicit example of how this problem is handled in the Caltech laboratory is summarized below.

When the tuna is caught, it is handled with plastic gloves and sealed successively in three clean polyethylene bags at the collection site. It is frozen and kept frozen until the time of analysis. Before dissection the fish is allowed to partially thaw so that the tissues can be just barely cut, but they are still hard and firm. The work is carried out on benches covered with fresh plastic polyethylene sheets in the clean laboratory. To obtain muscle uncontaminated by epidermal slime one operator wearing plastic gloves holds the fish while another operator also wearing plastic gloves incises the skin with a stainless steel scalpel blade that has been cleaned in several different baths of concentrated HNO_3. Areas around initial incisions by the scalpel must be regarded as contaminated with epidermal slime. After the initial cut has been made and the edges of skin have been freed along these incisions, a freshly cleaned scalpel blade is used to cut back the skin flap about a cm away from the initial incision. A third operator also wearing plastic gloves pulls the skin away with acid-cleaned stainless steel tweezers as the skin is being cut. This area must be regarded as moderately contaminated with epidermal slime. The scalpel blade is changed again (the blade must be held directly in the fingers since com-

mercially available blade holders are made of unsuitable metals) and a large area of skin flap is then cut away while the third operator holds the flap away from the cutting area. After a large area of muscle is exposed, the operator makes an incision which completely circumscribes the cleaned, exposed muscle area which lies well within the slime-free region. The third operator now uses freshly cleaned tweezers to assist in removing the slab of muscle as the inside of it is cut free from bone rays. This partially frozen slab of muscle is then placed upon a chilled, acid-cleaned polyethylene block and all six sides of the slab are shaved in succession with single cuts of a large stainless steel butcher knife, and the shavings are discarded. Each side is cut using care to place freshly exposed surfaces of the muscle slab only on virgin areas of the polyethylene block and taking care that each shaving trimmed from the muscle slab does not contact either virgin areas of the polyethylene block or freshly exposed areas of the muscle slab. The stainless steel knife should be dipped in acid and rinsed with purest water and shaken dry several times during this operation. The knife is freshly acid-cleaned and then used to cut the trimmed, still barely frozen slab of muscle into aliquots for analysis. During these latter operations two investigators will usually be needed, one to manipulate the slab of muscle with stainless steel tweezers and the other to manipulate the knife. Tweezers which handle the discarded trimmings must not be used to touch virgin areas of the muscle so that a number of stainless steel tweezers must either be used or several tweezers must be repeatedly dipped in acid and rinsed with purest water for repeated use. It is convenient to hammer the back side of the stainless steel butcher knife with a small acid-cleaned stainless steel hammer for quick accurate cuts of the semi-frozen muscle. All of these operations must be preplanned and carried out quickly before the muscle thaws.

IV. The Need to Evaluate the Lead Pollution Hazard

Geochemical studies of the occurrences of lead in polar snow caps [4], oceans [12], the earth's troposphere [4,15], and other remote areas [2] show that industrial lead pollution is both worldwide and intense. The question is whether humans throughout the world are being subjected to the hazards of lead poisoning. It appears that the situation is serious and warrants evaluation through reliable analytical techniques. Mass spectrometric isotope dilution analyses combined with clean laboratory procedures have shown that the actual levels of lead in waters and animal tissues are very much lower than had been previously thought, that exten-

sive lead artifact contamination has been introduced during the collection and handling of samples because the necessity for contamination control has been ignored, and that most presently used analytical techniques for lead do not provide reliable and useful lead data. It is not possible to properly evaluate the lead pollution situation under these circumstances. The most crucial prerequisite to the solution of this problem is to institute on a much wider basis the use of clean laboratory practices.

V. Summary of Clean Laboratory Operations

A. Arrangement of Facilities

The layout of clean laboratory facilities involves consideration of the following areas of different activities:

1) The change room.
2) The laboratory ware cleaning hoods, sinks, and benches.
3) The stills (water, acids, and organics).
4) Instrument room (AA, ASV, drying ovens, high temperature furnaces, vacuum dryer, LT asher, desk area).
5) Chemical separations room with sinks, hoods, and benches.
6) Balance room with spikes and standards, AA source furnace loading area.

1. The Change Room

This is a small corridor-like area with a door at each end. It should be approximately 2 m to 3 m long and 2 m wide with a door opening into the building corridor and another door opening into the clean laboratory area. Seals at the sides and tops of doors should be neoprene held with stainless steel screws and strips. The bottom seal should be spring-loaded to retract up from the floor within a stainless steel shroud when the door is open, and forced down to the floor by a side lever when the door is closed. In this room shoes are changed, lab coats and head caps are put on. The floor area is divided into two parts; that next to the building access corridor is covered with disposable paper sheets, while the other half of the area next to the laboratory consists of a raised platform. The laboratory side of the room contains racks and shelves for laboratory coats and clean shoes and slippers that are to be worn in the laboratory. The corridor access side of this room should also contain a sink for washing the outsides of various

articles brought into the laboratory and it should also contain shelves and drawers for storing tools and other dirty articles which are needed occasionally inside the clean laboratory. The laboratory side of the clean room should contain shelves and storage space for expendable items that are used in large quantities such as boxes of Assembly Wipes, plastic gloves, *etc.* Assembly Wipes contain an order of magnitude less metals than do Kimwipes. Mops, squeegees, buckets, and other apparatus needed for periodic cleaning of the laboratory floors can be stored in this room. The electric fuse box access panel can be placed in this room.

The floor of this room must be watertight and covered with a continuous sheet of plastic (preferably a resin which sets gradually and which can be troweled) which rises up and covers the surfaces of the base boards for several inches so that the floor consists of a shallow basin that can be flooded and squeegeed with water. The raised floor shelf on the laboratory side of the room can be made movable so that both it and the floor beneath it can be cleaned. A floor drain need not be put in this room. The cleaning water can be squeegeed through the laboratory door and down drains in the laboratory floor.

2. The Laboratory Ware Cleaning Area

The laboratory ware cleaning area should contain a large hot acid bath hood, a clean air bench for final cleaning operations next to the end of the hood which contains the sink, and a plain bench for handling cleaned laboratory ware beside the clean air bench. The acid bath hood should provide at least 2 m face length of acid bath area and 2/3 m face length of sink area with a usable working shelf around two sides and the front of the sink. The sink should be at least 45 cm wide and 30 cm deep. It is important that the sink be situated within and at one end of the hood because cleaning operations require that articles be removed from hot acid baths with tongs and then rinsed over the sink. The sink should be provided with hot and cold tap water admitted through a swing spout that has been coated with acid resistant plastic. Intermediate grade distilled water derived from a high capacity still which supplies the entire building in which the clean laboratory is located should also be available at the sink through a polyethylene spigot. Off-On controls for tap water and intermediate distilled water should be accessible outside the hood at the front just below the level of the sink.

The acid bath portion of the hood should consist of a rectangular, shallow basin ($\sim 2 \times 1/2$ m) whose floor is at a lower level than the working shelf area around the sink. The bottom level of the shallow basin holding

the acid baths should be at a height such that when an electric hot plate plus an acid bath are set upon it the top of the acid bath should be at a height that is convenient for inserting and removing laboratory ware. The back of this shallow basin should spill into a narrow trough running along its entire length. The bottom of this narrow trough is in turn inclined to a single drain.

The hood should not be provided with sliding doors or windows because they are seldom required and they introduce serious problems regarding contamination and cleaning. All parts of the interior of the hood should be accessible for periodic cleaning by mechanical wiping. This means that the back panels should be removable. Regardless of what material the hood may be constructed of, the surface should not be exposed stainless steel. Instead, it should be an acid resistant plastic. These surfaces include the sink, and the outsides of the front and side panels. It is convenient to provide storage space beneath the hood for cleaning acids. Commercial wiring supplied with the electric hot plates should be replaced with heavy duty Teflon coated wires which are brought forward through ports in the lower face of the hood and attached to flush mounted electric outlets. Voltage or current regulating devices (which feed the flush mounted electric outlets) for the hot plates should be completely enclosed within spaces in the lower part of the hood and controls for these devices should be brought forward through ports to the front of the hood. A front and a back row of adjustable temperature hot plates can be placed in the shallow basin part of the hood. The hot plates should be housed within stainless steel sheeting and then covered with thin sheets of FEP Teflon plastic. Acid bath tanks can be made of 2-liter FEP bottles with the tops cut off, large polyethylene bottles with the tops cut off, and of Pyrex glass. The tops of the acid baths are covered either with Teflon watchglasses or with sheets of Teflon. Any commercial markings on the glass tanks must be removed by rubbing with cold concentrated HF followed by rinsing because such markings contain gross quantities of lead which readily decompose in acids.

A clean air bench should be placed next to the sink end of the hood. This clean air bench should contain hot plates and dilute acid soaking baths. Next to the clean air bench would be a bench for handling cleaned laboratory ware. It would hold wash bottles of purest water. Shelf space above it would hold both Saran and polyethylene wrap dispensers and it should have shelf space to hold sonic cleaner tanks.

During the laboratory ware cleaning process the articles are heated within at least three successive, different baths within the hood and are handled with stainless steel tongs by an operator wearing talc-free polyethylene gloves. Talc-free polyethylene gloves must be special-or-

dered from Hand Gards, Inc. Rinsing of the articles is carried out at the sink. In the final cleaning operation the articles are transferred to dilute acid soaking baths in the clean air bench outside the hood.

The articles are rinsed at the sink within the hood with purest water wash bottles and are either placed in sonic cleaner tanks or are placed in large FEP dishes loosely covered with aluminum foil and are then placed in a drying oven. The dried articles are brought back to the bench and wrapped with polyethylene film and Saran wrap and are then stored on shelves and in drawers.

3. Still Area

It is convenient to place the stills in an area adjacent to the laboratory cleaning hood. In particular the water still should feed a large reservoir which is adjacent to the cleaned laboratory ware handling bench. If there is a building supply of distilled water, the water still within the clean laboratory should consist of the following components: 1) the building distilled water should be passed through an ultra pure mixed bed resin column and then into either a nonboiling radiation still made of quartz or a cyclone scrubber boiling still made of quartz. The condensate should be stored in polyethylene, preferably a 55-gallon drum with a spigot. If building distilled water is not available, the tap water supply must be fed through high capacity ion exchange columns and then into one or two commercial quartz double distillation stills (in parallel) that are electrically heated and which have a combined output of two or more liters/hour. The water from these stills must then be fed by gravity through an ultra pure mixed bed resin column as before and then through the nonboiling radiation still or the cyclone scrubber boiling still. The final output rate of the still system should be at least 1 liter/hour.

The following factors are important in the purification of the water. If commercial quartz double distillation stills are used, it is important that the rate of overflow for a filling arm outside the pot be three to five times the distillation output rate. If the overflow is within the pot, the flush rate can be reduced. This also holds true for the nonboiling radiation still and the cyclone scrubber boiling still unless the latter two are equipped with automatic pot dumpers. Stills equipped with automatic pot dumpers should be set to dump the pot after no more than 10 volumes of pot water have been distilled. The nonboiling radiation still is available commercially; the cyclone scrubber boiling still was designed and built at the CIT laboratory. Both of these stills require automatic leveling devices if automatic pot dumpers are used. Leveling and dumper devices are not com-

mercially available, and must be custom made from quartz, Teflon, Teflon electromagnetic valves, and electric relays, timers, and pressure switches. They are expensive to design and construct. Stored high purity water is dispensed within the laboratory either by polyethylene squirt bottles of various sizes or by polyethylene Erlenmeyer flasks.

The investigator can prepare his own purified distilled acids but this operation entails considerable expense in labor and room. It would be advisable for the investigator to obtain double distilled acids from the U.S. National Bureau of Standards and to have available in the laboratory facilities for distilling acids if necessary. The most economical such device consists of two Teflon bottles screwed into a block of FEP at a right angle to each other. In operation the acid to be distilled is placed in one of the bottles and warmed mildly with a light bulb while the other bottle is placed in a cold water bath. The device is allowed to function for 2 to 4 weeks and the condensate in the cooled bottle is used. A quartz nonboiling radiation still or a cyclone scrubber boiling still should be available to prepare triple distilled methanol, chloroform, *etc.*

4. Instrument Room

There are gram quantities of contaminating metals within the equipment placed in the instrument room. It is desirable to place this instrument room upstream in the air flow through the laboratory from the laboratory ware cleaning hood area to reduce corrosion effects and subsequent dispersal of contaminating dusts from the contaminating metals in this equipment. This room should contain the atomic absorption unit, drying ovens, high temperature furnaces, vacuum dryers, low temperature ashers, desks with electric calculators, books, *etc.*

5. Chemical Separation Room

The air flow through this area should be upstream from the instrument room and the area should be provided with several sinks, clean air benches, reagent and laboratory ware storage facilities, working benches, and either of the following facilities for carrying out acid evaporations: (1) a hood within which are heated Teflon chambers (with easily removable lids) which contain the receptacles from which acids are being evaporated and which chambers are flushed with nitrogen gas purified by ultra filtration (these chambers are custom built, and details of their construction can be obtained from one of the investigators listed at the end of this section); or (2) a clean air evaporating hood which is similar to a clean

air bench except that the air is passed down through a Teflon grill which supports the hot plate and the acid evaporating dish, and the exhaust gases are boosted by a fan through an outlet up to the flue of a regular hood. These latter two devices are necessary to reduce air contamination during acid evaporations because it is not possible to purify ambient laboratory air sufficiently.

The sinks and bench surfaces in this area can be of stainless steel. Shelves and cupboard surfaces should be painted with acid resistant plastic.

6. Balance Room Area

The balance room area should contain a 10-kg capacity balance as well as a 150 g analytical balance. It should have bench space for pipetting spikes and standards and handling sample aliquots. It should also contain bench space for loading graphite furnaces. It should have shelf space for spikes and standard solutions, pipette apparatus, furnace apparatus, *etc.* This room should be located at the highest pressure end of the air purification train in the laboratory because the balance should be protected from acid fumes of the chemical separations room and the sample standardizing and AA source furnace loading operations are highly susceptible to contamination during manipulation.

B. AIR PURIFICATION

The following factors are involved in air purification:

1) Air supply to the laboratory.
2) Air supply to restricted bench areas.
3) Contamination from clothing and bodies.
4) Contamination from construction materials.
5) Dust removal operations from the laboratory.
6) Blank from air contamination.

1. Air Supply to the Laboratory

Air should be supplied through a series of four filters: a coarse fiber filter to remove the bulk of street dusts and insects; a medium fiber filter to remove most of the remaining dusts; an activated charcoal filter to remove the lead alkyl gases as well as other metallic gases; and then an ultra paper filter to remove traces of charcoal and fine particles down to

a few hundredths of a micrometer. Heating and cooling coils and humidity regulating sprays and air pumping fans should be inserted between the coarse and medium filters. The rate of the pumping fan should be adjusted to provide somewhat more air to the laboratory than is exhausted by all of the hood fans when the rooms are not pressurized by the pumping fan. As a temporary economy measure it is feasible to provide each laboratory room with a filter box which processes building air. Such filter boxes should contain a filter of medium porosity before the ultra filter so that the medium porosity filters can be renewed frequently at modest cost. A pumping fan may be required for each filter box. If so, it should be upstream from the filters.

The input to the various rooms of the laboratory must be adjusted to provide the highest pressure in the balance room, next highest pressure in the chemical procedures room, next highest pressure in the instrument room, and next highest pressure in the still and acid hood room, next highest pressure in the change room which in turn has a higher pressure than the building corridor, the interior crawl-ways of the building, and the exterior of the building. It is required that all differential pressures be monitored continuously by means of permanently installed manometers. When the laboratory air supply is shut down for maintenance, the hoods must be shut off first so that dirty air will not be sucked into the laboratory. At such time the hood exhaust exits must be sealed with plastic bags because the laboratory may become negative with respect to outside air, and dirty air will be sucked into the rooms through the hoods. Attempts should be made to bring corridor air pressures to equal outside pressure by opening outside windows to the corridor.

Doors should be eliminated from the entrances between rooms whenever possible. For example, the area between the acid cleaning hood and the still room need not require a door and the entrance between the balance room and chemical separation room need not require a door.

2. Air Supply to Restricted Bench Areas

If the entire laboratory is not pressurized with air filtered by ultra paper filters, then it is necessary to install at least two clean air benches, one next to the acid cleaning hood and one in the chemical processing room. In addition, if acid evaporations in the chemical processing room are not carried out in heated Teflon chambers flushed with filtered nitrogen then an additional clean air hood is required. These hoods are commercially available and are relatively inexpensive.

3. Contamination from Clothing and Bodies

A major fraction of the debris washed from the clean laboratory floors consists of human hair, skin, and clothing lint. This material is impregnated with large quantities of contaminated metals and its dispersal in chemical working areas at bench heights is minimized by wearing laboratory coats and operating room-style caps. The debris carried by street shoes into the dirty half of the change room contains extremely large concentrations of contamination metals and this dirt accumulates in such gross quantities on the floor of the change room that a disposable sheet of paper which can be changed every few days must be placed on the floor of that portion of the change room. It is convenient to have a large roll of brown wrapping paper and a sharp knife in the dirty half of the change room from which sections of floor paper may be cut and used several times a week.

The laboratory coats and operating room hair caps (if the caps are not disposable) are washed periodically in a regular washing machine but are transported to and from the laboratory in large sealed plastic bags. All investigators in the laboratory should, after donning clean shoes, laboratory coat and cap, wash their hands in a sink with soap and tap water followed by a distilled water rinse and a drying by Assembly Wipe. Generally the investigators wear disposable, non-talced polyethylene gloves for operations within the laboratory. This includes the touching and handling of all laboratory ware, wash bottles, cleaning tongs, balance room operations, and chemical procedures. There are very few activities in which the operators do not wear plastic gloves. These can be dispensed in three different sizes from rollers attached to the wall of the acid cleaning room. It is not convenient for investigators to change their clothing. Laboratory coats, hats, and shoes are never worn outside the laboratory. They are always worn in the laboratory and the hands are always washed upon each reentry into the laboratory from the outside. Some clean laboratories have a gelatin pad installed in the doorway leading from the change room into the acid hood room.

4. Contamination from Construction Materials

The walls of the laboratory should be painted with acid resistant plastic paints that are essentially free of metals being studied. It is convenient to use fluorescent lighting with the lights installed in acid resistant enameled recessed pans while the face of the light exposed to the laboratory is covered with a plastic shell which is either sealed to the ceiling of the

laboratory with silicone cement or held against a grommet seal in the ceiling by an internal bolt and nut. Air entry louvers are coated with acid resistant plastic paint. Before installation of these louvers the interiors of the ducts are wiped clean with damp Assembly Wipes up to the ultra paper filter. Benches, shelves, hoods, and parts constructed of wood and stainless steel are painted with acid resistant plastic paint. Windows should be double paned and sealed with silicone. Brass and pot metal parts of door knob assemblies should be removed; stainless steel parts should be substituted. The same is true for the hinges and screws. Plumbing installed behind the benches should be made of glass, plastic, and galvanized iron and should be sealed with plastic coverings after installation wherever feasible. Galvanized iron plumbing is necessary in many instances. All of this plumbing together with the electrical conduits beneath and behind the benches should be sealed with sheets of plastic before the benches are installed and then the final connections should be made. The benches then must fit flush against the wall and the join must be sealed with silicone cement. The floors of the laboratory must be covered with either troweled plastic or continuous sheets of plastic which continue up base boards for several inches along the fronts of the benches.

There should be one or two floor drains. All the laboratory floors should be so constructed that, if the floor drains were plugged, several inches of water could be contained by them without leaking under the benches. Distilled water pipes should be constructed of polyethylene and not polyvinylchloride. It is convenient to check each item of paint or construction material to be used in the laboratory by rough emission spectrographic analysis *before* it is permanently used or installed.

5. *Dust Removal Operations from the Laboratory*

At least once a week the floors of all the rooms of the laboratory, including the change room, are flooded with distilled water and this water is squeegeed down the drains, and the process is repeated once more with fresh distilled water. During this operation the soles of all the laboratory shoes are wiped with Assembly Wipes moistened with distilled water. Laboratory stools and other movable furniture items are removed from the floor area while it is cleaned. Three or four times a year the tops of all hoods, shelves, ventilating louvers, *etc.* are wiped before each successive chemical analysis with moistened Assembly Wipes and a sheet of fresh polyethylene film is laid on the surface. This film is disposed of after each chemical operation. In some instances it is necessary to handle with fingers the surfaces of apparatus that must come in contact with samples

(plant and animal dissections). Before such handling, the investigator wears three layers of polyethylene gloves and dips his gloved hands directly into cold concentrated nitric acid while a second investigator assists him to rinse the acid off with the purest water.

6. *Blank from Air Contamination*

In the CIT biogeochemical laboratory the contribution to the lead blank from air exposure is about 0.004 ng Pb/h of evaporating chamber exposure (flushed with filtered N_2 gas). Air contamination in a 1-liter beaker of water standing uncovered in the laboratory is about 30 ng Si/h exposure. The air contamination blank for potassium is believed to be negligible for most analytical determinations. There is positive evidence of potassium contamination of samples loaded on source filaments for the mass spectrometer upon standing for 5 to 10 days. It should be understood that 1/2 liter of air in an ordinary laboratory may contain 1 ng of Pb, and if this air is shaken with liquid in a bottle or separatory funnel, the 1 ng of Pb in the air will be added to the liquid.

C. CLEANING OF LABORATORY WARE AND OTHER ITEMS

Most of the CIT information regarding the cleaning of laboratory ware refers to lead. However, some information is available on alkali and alkaline earths. The following materials cannot be used to contain liquids that come in contact with the sample or to contact the sample itself: Pyrex, Kimax, polycarbonate, methacrylate, linear polyethylene, polypropylene, nylon, polyvinylchloride, Vycor, and platinum. Four materials are used to contact reagents and samples: FEP Teflon, TFE Teflon, ultra pure quartz, and conventional polyethylene. TFE Teflon is used wherever FEP cannot be used. It is less desirable than FEP Teflon because it is a sintered material filled with contamination which is difficult to remove, and it is susceptible to serious memory contamination. It is cleaned in the same manner as FEP Teflon except in special instances such as high temperature bombs, where it is cleaned with HF and HNO_3 at high temperatures and very high pressures. FEP Teflon, once it has been cleaned, can be reused many times. If it is used only for very low level samples, the reliability of its providing no metal contamination increases with each repeated cleaning step.

A summary of our knowledge of the relations between acid cleaning and contamination for FEP Teflon and conventional polyethylene is as follows: Experiments with isotope tracers show that large amounts of

lead from the commercial analytical reagent grade acids are not absorbed by FEP Teflon during hot acid treatment. Other experiments show that isotope tracer lead is not lost onto Teflon during acid treatment. We have not yet measured the amount of lead removed from Teflon or polyethylene containers by hot, concentrated acids. We have checked concentrated reagent acids standing cold for long periods in cleaned bottles and have observed no increase in lead concentrations within an error of 20 percent and if the contamination had been the same as that observed for cold, dilute acid, we would have observed a 100 percent increase in lead concentrations. All of our experiments with hot dilute acids refer to Teflon and polyethylene surfaces that have been previously treated with either hot (70 °C) concentrated HNO_3 or HCl respectively for 3 days. With hot (55 °C) dilute (0.5 to 0.05 wt %) acids acting on such surfaces we see 20-40 ng Pb coming from the walls of a 2-liter bottle over a period of 4 to 5 days. The rate of lead released decreases with time in a reproducible manner with the same or different bottles after the surfaces are retreated with hot concentrated acids. After about 5-days treatment with hot, dilute acids, the release of lead becomes approximately constant (to about 1 ng Pb/day/2-liter polyethylene bottle, and about 2.5 ng Pb/day/2-liter FEP bottle) and this can be reproduced with surprising accuracy (± 25%). Variations are observed in the amount of lead released among different bottles but such variations are within a factor of 5. If plastic surfaces are exposed to hot aqua regia and then treated with hot, dilute acids, the initial flood of lead leached is much greater than if the surface had been cleaned by either concentrated HNO_3 or HCl separately. Surfaces treated with hot aqua regia do not give lower lead contamination after soaking with hot, dilute acids. Several measurements have been made on the amount of lead contamination contributed by the bottle on long standing filled with dilute acids at room temperatures and the rates of lead contamination from the containers in these instances were observed to be 1/10 of those prevailing at 55 °C. This seems to contradict what has been observed for contamination rates caused by cold, concentrated acids. However, FEP on exposure to dilute HCl or HNO_3 will develop numerous, minute, subsurface bubbles. The rate of bubble formation by dilute acids is greatly accelerated by heat. No such bubble formation is observed after treatment with hot, concentrated acids. The bubble film can be eliminated by heating the plastic at 195 °C for 24 hours. We have not analyzed FEP Teflon or conventional polyethylene for lead. At the present time, the only way the CIT lab can be certain that a sample bottle will yield a blank that is reliable is to fill it completely with purest water after it has been cleaned, add a few ml of NBS acid and spike it with Pb^{208}.

then heat it for 1 day at 55 °C. The spike is extracted from the water with dithizone and analyzed, and the extra lead found beyond that expected from the water, the acid, and the dithizone extraction is assigned to the bottle.

It is recommended that bottles, beakers, resin columns, transfer pipettes, and separatory funnels be made of FEP Teflon and that evaporating dishes and transfer pipettes be made of quartz. High temperature bombs and other such items may be made of TFE Teflon whenever necessary. It is recommended that Pyrex bottles, beakers, and separatory funnels not be used at all. All reagent bottles should be FEP Teflon.

Quartz vessels must be used for acid decomposition of plant and amimal tissues because the acid mixtures cannot be brought to temperatures sufficiently high to completely oxidize some hydrocarbons in FEP Teflon vessels.

Hot plates and acid baths should be arranged in the hood to provide for the following cleaning procedures. FEP Teflon bottles are cleaned by first wiping the outside and rinsing the inside with $CHCl_3$ to remove grease. They are then rinsed with water and shaken with ~ 100 ml analytical reagent grade concentrated HNO_3 to remove surface salts. The bottles are rinsed and filled with A.R. concentrated HNO_3, capped loosely, and placed in a 70 °C bath of A.R. concentrated HNO_3 up to the neck for 3 days. The concentrated acid is poured out, the bottles are rinsed with pure water, filled with 0.05 percent purest HNO_3 (NBS or equivalent), set directly on a hot plate and heated loosely capped for 1 day. The dilute acid is poured out, the bottles are rinsed with purest water, filled with fresh 0.05 percent purest HNO_3 and heated as before for 5 days. The dilute rinse is then poured out, the bottles are rinsed with purest water and filled with 0.5 percent purest HNO_3. They are allowed to stand filled at room temperature until ready for sample collection. The filled bottles should be bagged in polyethylene to prevent surface dust collection.

FEP Teflon ware such as funnels, beakers, *etc.* is treated in the same way except that it is totally immersed in both the concentrated and diluted acids. The ware must be handled only with stainless steel tongs or with hands enclosed in non-talced polyethylene gloves. After soaking, the Teflon ware is rinsed, placed in large FEP dishes loosely covered with aluminum foil and dried at 110 °C. Both the FEP dish and the aluminum foil should be previously cleaned. After drying, the Teflon ware is wrapped in polyethylene film and then wrapped again with Saran film. The polyethylene film is dispensed from rollers 12 in wide and 1 mil thick. It is much cleaner than Saran wrap. The outer Saran wrap is used to hold the polyethylene film wrap in place. Some investigators place their cleaned ware unwrapped within clean plastic airtight boxes, and just be-

fore using, the ware is placed in FEP-lined sonic cleaning baths of purest water.

After initial acid cleaning TFE Teflon high pressure bombs are further cleaned with two or three successive heatings with mixtures of HF and HNO_3 to yield lead blanks less than or equal to 0.1 ng Pb.

All quartz beakers and evaporating dishes after each use are immersed in a solution of 10 percent analytical reagent grade HF in purest water for 10 minutes and then cleaned in the same manner as Teflon.

Once a piece of ware has been properly cleaned and it has been used only for low level quantities of metal, and if during use it has been handled with polyethylene gloves and its outside surface has not been exposed to contamination, but instead has only touched clean polyethylene film, the ware should be immediately rinsed with purest water and then soaked in the high purity dilute acid bath in preparation for its next use. If the ware has been exposed to more than 20 ng of Pb sample or more than several hundreds ng of lead spike then it must be recleaned in the low purity concentrated acid baths before soaking in the high purity dilute acid bath.

Polyethylene bottles and ware are cleaned in exactly the same way as Teflon except that HCl is substituted for both the concentrated and dilute acid treatments, and the drying temperature is 50 °C.

Aluminum foil is cleaned by dipping into cold concentrated HNO_3 and rinsing with purest water.

Polyethylene bags are cleaned by partially filling with cold concentrated analytical reagent grade HNO_3, folding the top shut, shaking, and rinsing three or four times with purest water. These bags can be dried by hanging them upside down from a plastic line with a plastic clip.

Millipore filters are cleaned by soaking in cold 6*N* HCl for 2 days, rinsing on a cleaned polyethylene Büchner funnel with purest water, soaking for 2 days at 55 °C in 1 wt % G. Frederick Smith HCl, rinsing on a Büchner funnel with purest water, soaking for 2 days at 55 °C with 1 wt % NH_4F (prepared by neutralizing high purity NH_4OH with NBS double distilled HF) followed by a final rinse with a very small quantity of purest water. The blank for these filters as determined by acid decomposition is less than 1 ng Pb/47 mm filter. Nucleopore filters are cleaned in the same manner as Millipore filters and lead blanks for these filters are the same.

In CIT blank calculations an assumed value of 0.1 ng Pb/analysis is used as originating from laboratory ware, exclusive of sample bottles. At the present time blank tracer experiments suggest that the total contribution from all properly cleaned ware exclusive of sample bottles is equal to or less than 0.1 ng Pb/analysis and the contribution from sample bottles is ~ 1 ng Pb/2-liter bottle (either FEP Teflon or conventional polyethylene). Some accidents have arisen from contaminated ware; some of them have

been associated with cross-contamination occurring during concurrent analysis of high level and low level samples.

D. PURIFICATION OF REAGENTS

All reagents used in the analysis of lead at CIT are either purified in the laboratory or, in the case of ultra pure acids, are obtained from NBS. All reagents are stored in FEP Teflon bottles which have been cleaned in the prescribed manner. Small volumes of reagents are dispensed by pouring from storage bottles into small FEP Teflon graduated cylinders. The latter are custom made and are cleaned in the prescribed manner.

Distilled water prepared in the CIT biogeochemical clean laboratory by distillation from a quartz-Teflon cyclone scrubber boiling still equipped with an automatic pot dumper, and stored in a 55-gal polyethylene drum contains less than 0.1 ng Pb/kg water.

Pure $CHCl_3$ is prepared from reagent grade Baker's Analyzed $CHCl_3$, first extracted with 2*N* HCl. This acid is made from G. Frederick Smith triple distilled HCl and purest water. The extraction is carried out in glass bottles that had previously contained A.R. HNO_3. The decantation separation is effected by using a 1-liter Teflon beaker. Six gallons of $CHCl_3$ are extracted at one time and the same acid is used to extract all 6 gallons. The acid extracted $CHCl_3$ is distilled in a cyclone scrubber quartz boiling still. The input $CHCl_3$ is stored in a Pyrex reservoir; the output $CHCl_3$ is collected in rinsed $CHCl_3$ glass A.R. acid reagent bottles. The boiling pot is emptied and refilled for each 6 volumes distilled. The loss in the cyclone scrubber is about 15 percent. The overall loss for each distillation is approximately one-third. The distillation is repeated twice more with the final distillate being received directly in the Teflon stock bottle. Six liters of purified $CHCl_3$ are obtained from 6 gallons of starting material. $CHCl_3$ prepared in this fashion contains about 0.002 ng Pb/ml. Triple distilled $CHCl_3$ not first extracted with acid contains about 0.012 ng Pb/ml. Reagent grade Baker's Analyzed $CHCl_3$ contains about 50 ng Pb/ml.

Pure dithizone is prepared from Eastman dithizone by adding 250 mg of Dz to 250 ml of 2 percent NH_4OH in a Teflon separatory funnel. Ten ml of purified $CHCl_3$ are added and the mixture is shaken for 1 minute and allowed to stand for 10 minutes. The $CHCl_3$ layer plus any film material in the inner layer is drained off and the extraction is repeated six times using fresh $CHCl_3$ for each extraction. On the sixth extraction the mixture is allowed to stand 2 hours. The $CHCl_3$ is drained off and 10 ml of purified $CHCl_3$ plus 4 ml concentrated HNO_3 are added to make the solution acid,

then the mixture is shaken for 30 seconds and allowed to stand for 10 minutes. The $CHCl_3$ layer is drained into a quartz dish and allowed to evaporate in a Teflon oven at room temperature. Stock solution Dz is prepared by dissolution of 20 mg of Dz in 250 ml purified $CHCl_3$. The lead isotope tracer exchange blank of this stock solution is 0.01 ng Pb/ml.

Twenty-five percent $(NH_4)_3$(citrate) is prepared from A.R. dibasic $(NH_4)_2H$(citrate) 75 g in 300 g of solution using purest water. Anhydrous NH_3 gas is bubbled into the solution from a lecture bottle using a millipore gas filter in the line and tubing made of Teflon. NH_3 is added until the pH is 8. The solution is extracted with 25 ml of stock Dz solution and the $CHCl_3$ layer together with any film at the solution interface is discarded. The citrate solution is washed twice with 10 ml portions of purified $CHCl_3$ each time. In the last wash the mixture is allowed to stand 1 1/2 hours before the $CHCl_3$ layer is discarded. Ten ml of purified Dz stock solution is shaken with the citrate solution and it is washed three times. The last wash is allowed to stand 2 to 10 hours before discarding the $CHCl_3$ layer.

One percent KCN is prepared by dissolving analytical reagent grade KCN in purest water to make 250 g of solution. This solution is extracted with 25 ml of a purified Dz solution containing 0.2 mg of Dz. The mixture is allowed to stand 10 minutes and the $CHCl_3$ layer is discarded together with any interface film. The KCN solution is washed five times with 5 ml of purified $CHCl_3$. The extraction is repeated and washed again five times, this time with 10 ml portions of purified $CHCl_3$. The fifth wash is allowed to stand 2 to 3 hours before draining.

The lead isotope tracer exchange blank at pH 7 to 8 for the citrate solution is 0.7 ng Pb/ml citrate. The exchange blank for the KCN solution at pH 8 is 0.2 ng Pb/ml KCN solution. These exchange blanks can be reduced for investigators using isotope dilution mass spectrometric analytical techniques by exchanging residual traces of common lead impurity in the solutions with nanogram amounts of a single isotope of lead (at lowered pH's upon long standing). The effective common lead exchange blanks at pH 7 to 8 for these two reagents have been reduced to 0.15 ng common Pb/ml of $(NH_4)_3$(citrate) and 0.064 ng common Pb/ml KCN solution by this exchange method using Pb^{206} spike. These blanks are caused mainly by isotope exchange between a fixed residue of common lead in the reagent and the isotope tracer being used to measure common lead in the dissolved sample. The extent of this exchange increases with decreasing pH; consequently, the magnitude of this isotope exchange blank will be determined by the lowest pH the mixture of spike and reagent is subjected to. The blanks and the procedures used by investigators employing nonisotope dilution methods should not be affected by these

large isotope exchange blanks from fixed residual impurities and it is probable that ordinary chemical method entrainment blanks from the two reagents purified as outlined above would be less than 0.1 of the larger figures quoted. The expected ordinary chemical method entrainment blanks might be approximately 0.07 ng Pb/ml citrate solution and 0.02 ng Pb/ml KCN solution.

The concentrated acids obtained from NBS are made by distillation from nonboiling radiation stills. Twice distilled acids are required in all cases. At the present time, expected blank for all NBS twice distilled acids is about 0.01 ng Pb/ml concentrated HCl, HNO_3, $HClO_4$, and HF. The FEP stock bottles should be cleaned by the rigorous methods outlined in the last section before they are returned to NBS for refilling. It is necessary to analyze a small portion of each stock bottle received from NBS for metals because occasional high levels of metals may be found (*i.e.*, greater than 0.04 ng Pb/ml acid) and in such instances the acid should be returned together with a new, empty, rigorously cleaned FEP stock bottle for a fresh refill. NBS keeps track of each user's bottles, and returns them accordingly. They do not mix bottles. After the initial receipt, each investigator is responsible for the cleanliness of his own bottles.

Dowex Ag–1 × 8 100-200 mesh ion exchange resin is prepared in 500 ml batches which is enough for about 100 determinations. The resin is placed in a 1-liter Teflon bottle with 8*N* GFS HCl and the mixture is shaken and the HCl is decanted. This is done four times and then enough 8*N* GFS HCl is added to make a thin slurry and this mixture is allowed to stand for 1 month being shaken about once a day. At the end of 1 month the mixture is transferred to a 1-liter Teflon beaker and sucked completely dry using a polyethylene filter wand pushed to the bottom of the beaker. Fresh 8*N* GFS HCl is added to make a thin slurry and the mixture is again allowed to stand for a month with periodic shaking. The acid is removed as before and fresh acid is added and the mixture is allowed to stand for a third month. This acid is removed by suction and the final slurry is made up with NBS 6*N* HCl. Fresh aliquots of this slurry are used for each analysis and the resin in the column is washed with 4 column volumes of 6*N* HCl before treatment with 1.5*N* HCl. Our blanks for this resin are about 0.1 ng Pb/ml resin.

E. Chemical Procedures

Outlines of analytical procedures used for the determination of trace amounts of metals in plant and animal tissues have been reported in the

literature and they will not be reviewed here. Details of the acid dissolution of tuna muscle will be summarized to illustrate the precautions taken to prevent contamination.

Frozen aliquots of dissected tuna muscle should be placed in quartz dissolution dishes and weighed immediately. They can be refrozen before vacuum drying so they will not froth. Vacuum drying is the method of choice for the determination of the water content, which is usually about 70 percent. The vacuum drying desiccator should be lined with cleaned aluminum foil and the quartz dissolution dishes must be handled with plastic gloves.

For large samples which froth upon the initial addition of acid, quartz dissolution dishes with conically outward-flared sides on the upper half of the vessel are used. These upper-angled sides of the dish prevent loss by frothing because rising films are thinned by extension until they break and fall back down. For 5 g of wet tuna muscle, 6 ml of HNO_3 and 2 ml of $HClO_4$ are used. The dish is covered loosely with a quartz watchglass and placed in a Teflon acid evaporating chamber swept out by filtered nitrogen gas and heated to 50-60 °C. After the initial rapid frothing is over and the sample is in liquid form, the temperature is increased over a period of several hours until the temperature reaches that necessary to fume $HClO_4$. At this time the solution may darken, indicating incipient charring and incomplete oxidation of the organic material. If this occurs, concentrated HNO_3 must be immediately added dropwise or a flash explosion may occur. It is convenient to displace the concave quartz cover slightly to one side and drop the HNO_3 onto the upper part of the angled side of the quartz dish. As the HNO_3 runs down the edge of the dish and causes a violent frothing in the hot $HClO_4$ mixture, the aerosols formed by the froth are retained by the quartz cover. HNO_3 is added dropwise until no further reaction takes place in the hot $HClO_4$. Afterwards the evaporation is continued until HNO_3 is completely gone and only fumes of $HClO_4$ remain. The cover is then removed and the evaporation of $HClO_4$ continued until the residue is bone dry. The dry residue should be white and crystalline with a possible tinge of yellow. No brown or black color is acceptable for this indicates incomplete oxidation. If the latter occurs, the dish must be cooled, small amounts of a 3:1 mixture of HNO_3 and $HClO_4$ must be added and heated again to fumes of $HClO_4$. It may be necessary to add HNO_3 dropwise again during this second treatment.

Occasionally two forms of precipitates give trouble. In one instance, the quantities of calcium and sulfur in the sample may be so large that the sulphate formed by the oxidation of the sulfur will precipitate calcium and in turn coprecipitate other metals such as barium and lead. This precipitate is difficult to redissolve once it has formed. To prevent its for-

mation it may be necessary to use smaller quantities of sample and large amounts of acids. In the case where seawater is evaporated to dryness with aqua regia to decompose all organic matter, the formation of calcium sulphate cannot be avoided, and in this instance the precipitate which is formed can be redissolved by prolonged subsequent heating in slightly acidified water at very large dilutions.

In other instances, silica gel precipitates form and these must be eliminated with HF. The silica gel precipitate will attach itself to the bottom of the quartz dish so that in those instances where this type of precipitate is found to occur the acid digestion must be started over with a fresh aliquot of sample in a Teflon evaporating dish. The dried residue is treated with HF and $HClO_4$ and heated gently to eliminate silica and then more strongly to decompose fluorides. The salts are transferred to quartz with HNO_3 and $HClO_4$ and heated to strong fumes of $HClO_4$ and then to dryness to insure complete oxidation of intractable proteins.

For lead analysis the dried residue is usually taken to dryness with HCl and then dissolved in 1.5*N* HCl and loaded on the resin column. During the acid dissolution procedure the dishes are handled with HNO_3-cleaned tongs by operators wearing plastic gloves. The lid of the Teflon acid evaporating chamber is opened only momentarily to observe the contents of the dish or to add reagents.

VI. Investigators Using Clean Laboratory Techniques

In addition to the authors at Caltech, the following persons carry out trace metal investigations in clean laboratories at different geographic locations (this is only a partial list):

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SAMPLING FOR CLINICAL CHEMISTRY

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Three requisites must be satisfied for a physiological sample to be acceptable for analysis in the clinical chemistry laboratory. If any of these three requisites is not met, the acquisition of a reliable and meaningful result is jeopardized.

To begin with, at the time the sample is collected, the patient must be in a physical state appropriate for the contemplated assay. Some of these physical requirements are reasonably apparent such as observance of being fasting, or supine, and avoidance of hyperventilation. An example that is less obvious concerns the interference by radioactive material already present in the patient's blood upon a radioimmunoassay.

The second requirement is for the blood drawn from the patient to be truly representative of that in the circulation. This implies circumvention of trauma to body tissues, avoidance of damage to the blood cells and alertness to the possible introduction of contaminants.

Finally, the specimen must be maintained in a manner that preserves its composition with regard to the parameter to be measured. While certain analytes remain stable for long periods, others require the use of chemical preservatives or that the sample be frozen. In some instances, no practical method of preservation has been developed.

Keywords: Accuracy and precision; analysis of body fluids; clinical analysis; clinical chemistry; clinical sampling; enzyme analysis; storage of clinical samples.

Physiological samples such as blood, urine, amniotic and cerebrospinal fluids are complex mixtures of inorganic and organic substances contained in an aqueous matrix. While still part of the organism, they are in dynamic equilibrium with the tissues to which they relate and their composition is a reflection of the overall metabolic status of the organism at any particular instant.

A large clinical laboratory capable of offering a truly comprehensive testing service may have a repertoire of 600 or more different analytical

procedures. These determinations are requested by physicians to be performed on samples from their patients for the purpose of making an initial diagnosis or for observing the effect of therapy. It is essential that the laboratory result be of sufficient reliability to guide the physician to a correct conclusion with regard to his patient. The majority, if not all of this large number of determinations, however, can be affected to some extent by the circumstances involving when and how the sample is drawn and its handling prior to the start of the analysis. The clinical laboratorian must consider sample quality and stability for every procedure in the test list. Since the health, and sometimes the life, of a patient depends upon the validity of the laboratory result, a thorough understanding of the problems of sampling is vital.

In the beginning—to quote a well-known reference—there is the patient. An alteration in posture will cause body water to shift from the extracellular fluid to the tissue compartment when the patient changes position from recumbency to sitting up or standing. This results in small but measurable alterations in the concentration of the serum protein-bound constituents. A person who bounds up the stairs to the doctor's laboratory to have blood drawn will very likely exhibit a creatine kinase level distinctly greater than normal. This finding also occurs in the practicing athlete when interpretation of the result may have to be quite different from that of a sedentary individual. Certain blood constituents are greatly altered within minutes or hours by eating, glucose being an obvious example, but there are others including phosphorus and triglycerides. In order to give a correct lipoproteinemia phenotype, the subject should have taken a "normal" diet for at least 2 weeks and have fasted for about 14 hours prior to the test. Failure to adhere to these instructions will almost certainly cause the lipoprotein electrophoretogram to misrepresent the true condition of the patient. It is common for pediatricians to need knowledge of the pH and gas partial pressure values in the blood of their young charges. Should the baby be crying lustily when the blood is drawn—either as a result of being pricked with a needle or for other reasons of which most parents have knowledge—the ensuing hyperventilation will nullify the fanciest expertise back in the laboratory.

It is becoming increasingly common for departments of radiology to carry out procedures in which the patient drinks, breathes or is injected with radioactive material. Isotopically labeled substances are employed for radiological scans of the organs such as brain, lungs and liver. Should it be that shortly thereafter blood is drawn for a laboratory measurement which happens to be carried out using a radioimmunoassay, serious error could occur.

What has been discussed so far are influences which originate within

the patient and which cause spurious results even though the sample is subsequently drawn and handled correctly. I would now like to consider some sources of error which have physical causes and are generated by the drawing of the sample. In general, it is almost always the case that a satisfactory sample can be drawn from the person with good veins. There are, however, those individuals whose veins are difficult to locate and a blood sample is obtained only after trauma to the tissues. When this occurs the blood may be unsuitable for those tests in which the intracellular concentration is markedly different—usually greater—than that of the plasma. Contamination with tissue fluid or even a small amount of red cell damage resulting in hemolysis will yield very misleading elevated results for serum potassium and certain enzymes including aldolase, isocitric dehydrogenase, argininosuccinate lyase, arginase and lactic dehydrogenase. The activity of these enzymes is 10 to 200 times as great in erythrocytes as in plasma, hence the need to avoid hemolysis. Pancreatic lipase, which is measurable in serum, is inhibited by the products of hemolysis so that a normal value obtained from a hemolyzed sample is therefore subject to question. There are many other substances in blood where there is a considerable difference in plasma and erythrocyte concentrations so that hemolyzed blood, in general, is unacceptable for clinical laboratory determinations. Not only may there be the physical effect of dilution with cellular contents, the contribution of fluid with concentrations of important substances vastly different from that of plasma, but hemoglobin can interfere with many colorimetric reactions which is difficult or impossible to correct by including a blank. Although the determination of acid phosphatase is usually of interest as a measure of prostatic pathology, the erythrocyte enzyme can interfere. In this case, however, judicious use of inhibitors of the red cell enzyme, or, more conveniently, selection of thymolphthalein phosphate as substrate gives a method virtually specific for the prostatic enzyme.

Immediately upon withdrawal from the organism, which for our purpose today is the human or animal body, the sample is no longer influenced by the many other metabolizing systems to which it was subjected previously. A change in environment has occurred. Blood is a metabolizing tissue and now no longer is there a constant replacement of substrates, a well-regulated pH, pO_2 and pCO_2 or a removal of reaction products. Instead, the sample in its sealed glass tube has become a closed system. An interesting example came to light a few years ago when it was found that blood for transfusion which had been drawn from donors and subsequently stored did not transport oxygen efficiently, and thus was virtually useless to the anemic recipient. The reason was finally tracked down and was found to be due to the disappearance of 2,3-

diphosphoglycerate (2,3-DPG), a metabolite occurring in the Embden-Meyerhof pathway. The role for this compound, which for many years was regarded as an oddity, was then discovered to be that of controlling the conformation of hemoglobin and thus its ability to transport oxygen. The simple step of including a sufficiency of glucose to the stored blood appears to have solved the problem by ensuring the continued functioning of the Embden-Meyerhof pathway and the continued presence of 2,3-DPG. This is just one example of the complexity of sample handling and the potentially far-reaching nature of the intricacies when dealing with physiological samples.

At the time of drawing the blood sample, it is necessary that all required apparatus be at hand for immediate use, owing to the unique property of blood of clotting. This implies that a prior decision has been taken—exactly what preparation is required for testing. In the medical field almost all blood is obtained by use of evacuated collecting tubes. These tubes are designed with a gum rubber stopper so that when used with a plastic needle holder unit, the stem of the double pointed needle punctures the vacuum tube stopper and the blood is drawn directly into the collection tube at a rate of about 1 ml per second, which results in gentle treatment of the blood and will not rupture the cells or give rise to hemolysis. A specimen collected in this manner has little chance of becoming contaminated, spilled or spoiled due to evaporation. The rubber stopper is self-sealing and is color coded to indicate the anticoagulant contained within the tube. The blood specimen may be centrifuged directly while still in this collection tube.

The decision, referred to above, lies in making a primary choice of whether or not to allow the blood to clot and, if not, what anticoagulant to select. Of course, the need for making this decision can and should be obviated by having the laboratory furnish a list of available tests together with instructions on specimen requirement in every case. Our concern today is to examine the various reasons for making a particular selection. The solution to one aspect of the problem appears obvious. If the test to be performed involves some property of the cellular portion of blood, use an anticoagulant. This applies to cell counts, blood typing and grouping, investigation of the hemoglobinopathies such as sickle cell disease, and of the intracellular enzymes such as the specific tests for the presence of a number of inherited diseases including galactosemia. The use of an anticoagulant is also an appropriate first step in the preparation of erythrocyte ghosts or stroma. Next, if the clotting mechanism is to be examined and quantitation of fibrinogen, prothrombin or any of the considerable number of clotting factors—with the possible exception of calcium—use

an anticoagulant.

The question now arises as to which of the several useful substances to select as the anticoagulant for the specific use in mind. Although not strictly within the purview of this discussion, I will mention blood counting since there is a close association with clinical chemistry for some pathological conditions. For the purpose of blood counting, not only should cells remain intact but internal structure and morphology must be faithfully preserved. Ethylene diaminetetraacetic acid, usually abbreviated to EDTA, is most frequently used at a concentration of 1 mg per ml blood because it produces only minimal alterations. Samples for abnormal hemoglobins and erythrocyte enzymes may be mixed with heparin, EDTA and even oxalate. Most workers prefer citrate when collecting blood for the clotting factors. This anticoagulant is added in solution rather than as the solid so that its effectiveness is immediate. The ratio of the volume of citrate solution to that of blood is also carefully observed so that the subsequent chemical reactions can be properly controlled with respect to reagent concentrations.

In order to use anticoagulants wisely, it is necessary to understand their modes of operation and also any other side effects which can occur. For example, EDTA, the chelator of divalent metal ions inhibits the coagulation cascade at the point at which calcium is required. The resulting plasma is quite suitable for the determination of calcium by atomic absorption spectrophotometry since the chelate is destroyed at the time the measurement is made. On the other hand, this plasma is not acceptable for dye binding methology as is used in the SMA-12/60 because of the successful competition by EDTA. Parallel arguments can be made for magnesium. There is an additional complication in this instance involving several magnesium-dependent enzymes, the phosphatases being of prime importance.

The fluorides, at a concentration of 10 mg per ml blood, also act as anticoagulants by precipitation of the serum calcium as CaF_2. However, magnesium is also removed, rendering the plasma unsuitable for determination of calcium, magnesium and the magnesium-dependent enzymes. Oxalates are still in very common use for their property of preventing the clotting of blood. The usual concentration is about 2 mg per ml blood. The serious and unavoidable limitations to the use of oxalates lie not only in the precipitation of calcium, magnesium and iron but also in the alteration of plasma components. The hematocrit using potassium oxalate is 8 to 13 percent less than that obtained with heparin. This shrinkage of erythrocytes results from a water shift from the cells to the plasma due to the osmotic effect caused by the addition of the salt to the plasma phase. Not

only is there a measurable dilution error for certain plasma constituents but also there may be an alteration in erythrocyte permeability and there is almost always some degree of hemolysis associated with the use of oxalates. In 1934, Heller and Paul introduced a so-called "balanced" oxalate preparation consisting of 3 parts ammonium oxalate to cause swelling of the erythrocytes balanced by 2 parts of potassium oxalate which causes shrinkage, 2 mg of the mixture being used per ml of blood. Opinions on the effectiveness of this combination are contradictory and in any case it cannot be used for any determination involving ammonia, nonprotein nitrogen, urea nitrogen by the urease method or total nitrogen. Whatever choice is made of anticoagulant, the sample should not be shaken roughly but tipped gently several times in order to avoid the production of hemolysis.

I would like to turn now to the problems of sample stability and to the various means available to preserve blood and its components for analysis. While preservation of a biological specimen is usually not a significant problem in the hospital clinical laboratory because reporting time is only a few hours, there are procedures not performed on a daily basis. In addition, the widespread use of reference laboratories by general practitioners, hospitals and research workers has made it necessary to establish without doubt whether it is possible to send a sample through the mail to a distant laboratory and then be able to rely upon the result obtained. The question of stability of a sample for a particular analysis requires that every situation be considered individually. As one example, there is still to be devised a way to stabilize blood for the quantitative determination of methemoglobin so that the analysis could be delayed for 1 to 2 days.

At the moment of shedding, blood is an actively metabolizing tissue, which in the collecting tube continues to utilize the available glucose of the plasma at a rate which is dependent upon the temperature. In order to obtain a reliable and meaningful glucose result it is essential to inhibit glycolysis immediately upon removal from the vein. If this is not done, a significant diminution of the glucose concentration of anticoagulated blood occurs within minutes. For many years either fluoride alone or a mixture of oxalate and fluoride was used to inhibit the rapidly metabolizing systems, particularly the magnesium-dependent enzyme, enolase. It is imperative to dissolve the fluoride rapidly for it to exert its effect and for this reason sodium fluoride, which is relatively insoluble, has been found not entirely satisfactory. The potassium salt is readily soluble, but has the disadvantage of being hygroscopic, although this does not pose a problem for the evacuated tube system. The glucose content of blood was found to be stable for as long as 10 days at room temperature in the presence of fluoride provided the sample was sterile. Fluoride, however, is not suffi-

ciently antibacterial to prevent microbial growth if the specimen contains microorganisms. In 1923, a combination of 10 mg fluoride + 1 mg thymol per ml blood was introduced. The presence of the thymol effectively controlled microbial growth so that nonsterile specimens were stable for at least 2 weeks. One of the better methods for the measurement of glucose which is now widely used in manual and automated versions relies upon reaction with o-toluidine in acetic acid. Unfortunately, thymol gives a positive interference in this method, 1 mg thymol per ml sample giving a bias equivalent to approximately 10 mg glucose per 100 ml. Following a thorough investigation, Bio-Science Laboratories is now using a heparin fluoride mixture for the preservation of whole blood for glucose determination. It is also our practice to centrifuge the sample and perform the determination on plasma, since it is in plasma that glucose is presented to the tissues. It now seems that the most effective and convenient process for obtaining an acceptable sample for glucose is to draw the blood into a plain tube and allow it to clot. Within 30 minutes centrifuge the sample and transfer the cell-free serum to another tube. Little or no glucose utilization appears to occur under these conditions, possibly due to the inaccessibility of substrate to the cells enmeshed in the clot. If the determination is to be delayed by more than a few hours, add 10 mg fluoride per ml serum when the sample should be stable for at least 1 week at normal environmental temperature.

While discussing glycolysis, the determination of lactate should be mentioned. This measurement is requested quite frequently and often on a stat basis. As soon as blood is withdrawn from the body, the lactic acid level begins to increase as a result of glycolysis. At 25 °C increases of 19 and 70 percent have been found at 3 minutes and 30 minutes, respectively. The method of sample stabilization requires not only that the lactate level be maintained as it existed in the patient, but that it not interfere with the subsequent assay procedure which uses the enzyme lactate dehydrogenase (LDH). This accounts for the rejection of enzyme inhibitors such as iodoacetate. The most widely used practice is to collect blood being careful to avoid a major source of contamination from lactic acid on the skin, and without stasis, which may cause a large positive error. If a tourniquet is necessary for venipuncture, the constriction time should be minimized, and after releasing the tourniquet there should be a wait of about 30 to 60 seconds before drawing the blood. One volume of whole blood is added immediately to an equal volume of 7 percent w/v perchloric acid, preferably ice-cold. The supernatant from this precipitation step is stable for at least 1 week at 30 °C.

In general terms, with the exception of investigation involving cellular constituents and the coagulation factors, serum is satisfactory for all other

clinical laboratory analyses. However, this is still too sweeping a statement since LDH and aldolase are liberated from thrombocytes during the clotting process and amino acids are present in serum in greater concentration than plasma from the same blood specimen. There is little doubt that serum should be separated from the clot as soon as possible to avoid contamination by leakage from the cells. Stability of samples is usually examined at normal ambient temperature, 25 °C; in the refrigerator at about 4 °C; at freezer temperature (−5 °C to −20 °C) and in dry ice at about −70 °C. While the majority of serum components are stable for an indefinitely long period while packed in dry ice, the use of solid CO_2 is an inconvenience; shipping samples packed in it is costly and it is still not necessarily available in the smaller communities. Our laboratory is now making a serious attempt to find alternatives to the use of dry ice for the purpose of stabilizing biological samples.

There is an abundance of conflicting reports concerning the stability of serum LDH at various temperatures. Most data suggest that LDH is reasonably stable at room temperature for about 10 days. However, there are a number of publications indicating loss of activity under a variety of differing temperatures. A possible explanation is that LDH has minimal stability at 0 °C and at temperatures above and below there is greater stability. It should be noted that the serum LDH is composed of five iso-enzymes, one of which is known to be more heat- and cold-labile than the others. This highlights one of the major problems of this complex field. Many workers establish optimum stability conditions using serum from healthy individuals such as laboratory technologists or medical students. In this way only one aspect of the problem is viewed. An appropriate number of abnormal cases always should be included for all too often, in the case of enzymes, the predominant activity is not identical with that in the normal individual.

Creatine kinase also has been the subject of much disagreement concerning stability. One publication indicates that the enzyme in serum from patients with muscular dystrophy may show very little loss of activity compared with that associated with myocardial infarction, which decreases to a variable extent overnight in the freezer.

One interesting development which came into general use with the advent of phenylketonuria screening programs is the collection of blood on pieces of a selected filter paper. The technique has a particular advantage in pediatrics when blood must be obtained from a heel or finger prick. Provided the blood is flowing freely from the puncture site and discrete drops fall on to the special filter paper, sufficient sample is available for the laboratory to conduct tests, the number of which is growing. In particular, the detection of certain red cell enzymes is possible and the quantitative

assay of phenylalanine and tyrosine is routine. The filter paper preparations appear stable for several weeks especially if they are stored under conditions of low humidity.

I have endeavored to indicate the complexity of the problems and that no assumptions should be made. I have by no means mentioned all of the problems associated with sampling that present themselves to the clinical laboratorian. These include the effect of drugs upon determinations, the folly of drawing blood from the arm into which an intravenous infusion is running, the contamination by the manufacturer of the stoppers of evacuated blood collection devices first with glycerol which interfered with triglyceride determinations, and now with a zinc compound which interferes with blood zinc and possibly other measurements. I have not drawn your attention to the need to thoroughly mix samples which have been taken from the freezer and thawed and now exhibit concentration gradients. There are the particular precautions associated with cerebrospinal fluid, amniotic fluid and, of course, urine. Sampling is an aspect of clinical chemistry in which much has already been achieved but there is still a great deal to accomplish.

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HIGH-PURITY REAGENTS FOR TRACE ANALYSIS

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Determination of ultratrace (<1 μg/g) metal content in diverse matrices requires high-purity reagents for dissolution, preconcentration and supporting electrolytes. Trace impurities in these reagents fluctuate not only with the degree of contamination during purification and containment by the manufacturer but also with the care exercised by the analyst.

Particulates are a prime source of ultratrace impurities in inorganic salts. Most of the Fe, Pb and Cu in water soluble sodium and potassium reagents can be removed by sub-micrometer filtration under pressure. These impurities can be further reduced by mercury cathode electrolysis.

Although liquids are more easily purified, they are not stored as easily as solids. Accelerated aging studies for liquids, particularly the mineral acids, in leached glass or plastic containers are necessary to insure the integrity of the product. After the chemical has been purified and stored, it must be equilibrated with its container and analyzed under contamination-free conditions. Until full, accurate disclosure of procedural details for ultratrace measurements are published by suppliers and the laboratory analyst, inaccuracies attributed to systematic errors can be expected. Improper handling of high-purity reagents is frequently observed. For example, there is no point to use an expensive reagent when the container is allowed to collect dust on an open shelf or in the fume hood of a heavily contaminated laboratory.

Keywords: Analytical blanks; analytical reagents; contamination control; high-purity reagents; membrane filtration; mercury cathode electrolysis; prepurification; purity definition; reagent contamination; ultrapurification.

I. Introduction

The inability to control the blank at levels insignificant in comparison with the constituent to be determined severely restricts the limits of detection for ultratrace (<1 μg/g) measurements. Primary causes of high blanks

have been recognized as impurities in reagents, particulates entering from air and leaching from containers and apparatus [1-9]. There is a dynamic interplay between these factors during all ultratrace analysis. If control of airborne and container contamination is included in the analytical methodology, then figure 1 lists the most important contamination parameters in the analytical laboratory.

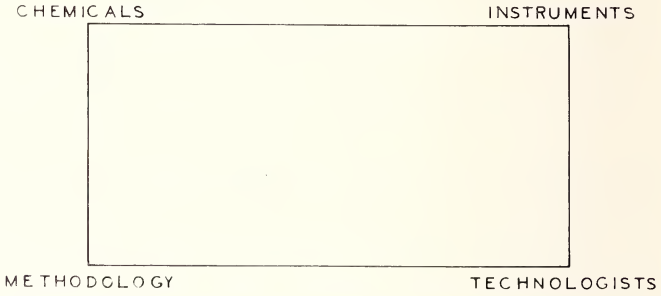

Figure 1. Contamination parameters in the analytical laboratory.

Rigid control of reagent purity is not sufficient if a deficiency in one of the other parameters exists. As laboratory personnel have variable education and experience, constant evaluation of professional competence is required in the training of a staff proficient in sophisticated techniques. Methodologies must be evaluated with certified standards to assure consistently reliable results. The importance of the calibration of instruments was vividly demonstrated in 1965 by the Standard Committee of the College of American Pathologists [10]. When samples of potassium nitrate and pyrene were distributed to 94 laboratories for absorbance measurement on a spectrophotometer, the results clustered about 11 values, each corresponding to a particular brand of instrument.

Some applications for high-purity reagents in ultratrace analysis are listed in table 1. In the analysis of high-purity materials or natural systems, ultrapure reagents are required for dissolution, preconcentration and supporting electrolytes. The new field of telecommunications is a good example. Here the maximum tolerable concentration of transition elements in chemical components for glass fiber waveguides ranges from 2 to 100 ng/g [11]. To date, the iron content in silicon dioxide suitable for waveguides is usually 2 to 20 ng/g.

The first step in the emission spectrographic analysis of this silica by our analytical group involves the dissolution of 1 gram in 4 ml of hydrofluoric acid [12]. Since ultrapure 48 percent hydrofluoric acid prepared by the Mattinson sub-boiling distillation [13] in our laborato-

TABLE 1. *Applications for high-purity reagents in trace analysis*

Application	Typical matrix
High-purity materials:	
Electronics	Metals, inorganic dopants, alloys, single crystals, thin films.
Telecommunications	SiO_2, $SiO_2 + B_2O_3$, $SiO_2 + TiO_2$, $SiO_2 + Na_2CO_3 + CaCO_3$, GaAs, GaP
Standards and reagents	Acids, bases, a variety of inorganic and organic chemicals.
Natural systems:	
Stratosphere	Lunar samples
Atmosphere	Air
Hydrosphere	Sea water, potable water
Lithosphere	Minerals, soils, agricultural commodities
Biosphere	Blood, urine, tissues

ries contains 2 to 5 ng/g of iron typically [14], 4 ml of the acid may contribute a blank up to 20 ng per gram of silicon dioxide. Usually a blank less than one-tenth of the element content undergoing measurement is desired for a trace determination. In this case the blank and sample present similar iron content. A significant blank is typical for iron which appears to be ubiquitous in all matrices.

Mattinson was concerned only with the lead content in HF distilled from, and collected in, Teflon FEP bottles. The iron concentration of the distillate was found to be substantially higher than that of lead in our laboratories [14]. Inasmuch as Fe, Zn, Al, Ni, Cu and Mn impurities were found to be imbedded in the walls of all Teflon FEP bottles [15], the higher Fe content can be ascribed in part to the leaching of Fe from the container walls. When ultralow Fe content of HF is the prime consideration, the acid should be stored preferentially in a well-leached polypropylene or high-pressure polyethylene bottle.

II. Designation of Purity

In the early 1950's solid state electronics research revealed that electrical properties of semiconductors are directly related to the impurity content. Electronics was the first, and continues to be, the major discipline to recognize the importance of trace impurities in ultrapure materials.

Definitions of purity that were originally devised for ultrapure metals have gradually been extended to inorganic and organic compounds.

In 1958 Melchior [16] defined the degree of purity, R (Reinheitgrad), by the equation: $R = -\log(100\text{-}g)$ where g is the weight percent of a pure element. For example, if a metal is 99.999 percent pure, $R = -\log 0.001 = 3$. In 1961 a British company suggested that the capital letter N denote nine; for example, 5N indicates 99.999 percent and 4N5 indicates 99.995 percent [17]. An overall assessment was obtained by adding the spectrographically detectable elements and subtracting from 100. Later the letter Z was added to the purity designation to show that zone melting was the preparative method: 5N (Z) corresponds to a zone-refined product 99.999 percent pure.

Recently the prefix "m" has been introduced to indicate metallic impurities whereas "t" designates purity based on total contaminants including oxygen, carbon and nitrogen [18]. In this system designed for metals, "m5N5;t4N" indicates that emission spectrographic analysis does not reflect the total impurities present.

In 1965 the IREA (All-Union Scientific Research Institute of Chemical Reagents and Ultrapure Chemical Substances) proposed a system based on the number and concentration of specific impurities. A designation of 10-5 means that 10 trace elements have a total concentration of 1×10^{-5} percent. The second number in this system is the negative of the logarithm of the total concentration of 10 trace elements (expressed as percent) [19]. The raw data for silica of 10-5 purity was the following:

	Concentration %
Al	2×10^{-6}
B	$<1 \times 10^{-7}$
Ca	5×10^{-6}
Fe	3×10^{-6}
Mg	$<1 \times 10^{-6}$
Na	$<5 \times 10^{-5}$
P	2×10^{-7}
Pb	$<5 \times 10^{-6}$
Sn	$<6 \times 10^{-6}$
Ti	$<4 \times 10^{-7}$

In this example the total of all impurities is considered to be less than 5×10^{-5} percent. Numbers from 5 and above are rounded off to 10. If the concentration of impurities totaled 6×10^{-5}, the value would be rounded off to 10×10^{-5} or 1×10^{-4} percent. The "purity index" would then be 4. Certainly such a logarithmic expression for the degree of purity would constitute a step forward in standardizing literature. In any case, the terms "ultrapure," "superpure," or "spectrographically pure" have no meaning unless accompanied by a detailed actual lot analysis, preferably with the method of analysis indicated.

At this conference last year, Barnard [20] presented the text of a certificate for ultrapure silicon dioxide prepared by the J. T. Baker Advanced Materials Team. The actual lot analysis included the state-of-the-art assessment of purity as well as the cation and anion impurity content. In addition, details for the dc-arc emission spectrographic procedures developed for analysis at the ng/g level were illuminated.

The unusually complete details reported for the analysis of high-purity EDTA [21] is another example of the minutiae demanded by the literature on ultrapurity. Only meticulous attention to all procedures that eliminate contamination will improve interlaboratory studies. Until detailed disclosure of procedural details for ultratrace measurements are provided by supplier and the consuming laboratory, the source of bias attributed to systematic errors cannot be identified. As the purity of reagents increases, the supplier relies more and more on the feedback provided by detailed characterization of these reagents in the literature.

III. Preparation of High-Purity Reagents

Since the preparation of high-purity chemicals is so intimately tied to analysis, trace analytical techniques must be highly developed by all teams working with high-purity materials. Monitoring of samples before and after purification by reliable analysis under "clean-room conditions" continues to be important. Too frequently the preparative chemist attempts to reduce impurities to the ng/g level when his analytical support is limited to the μg/g range. As a result, the discovery of the optimum purification scheme is endangered.

Preparative groups approach the purification of sodium salts by multistep schemes. For example, extraction of an aqueous solution with chloroform solutions of 8-quinolinol and ammonium pyrrolidinecarbodithioate is followed by passage of the aqueous solution through a column of a cation ion-exchange resin in the H+ form. After the resin is loaded with sodium, it is treated with high-purity Na_2EDTA (disodium salt of (ethylenedinitrilo) tetraacetate acid) to remove traces of polyvalent metals. Finally, the sodium is eluted from the column with high-purity HCl.

When such a purification sequence was repeated in our laboratory with exhaustive attention to contamination control, it was noted with surprise that the extraction with the chelating agents afforded an aqueous solution that met the target specifications. The ion-exchange step is, therefore, superfluous. This type of "operation overkill" can be avoided by appropriate analytical support.

Various high-purity reagents can be prepared in the laboratory by the methods listed in table 2. In many cases classical techniques such as crystallization, fractional distillation and solvent extraction are applicable. Frequently one method can serve for prepurification; a second, for ultrapurification. The assessment of purity for reagents fluctuates with the degree of contamination occurring during preparation, handling, containment and analysis [22-28].

TABLE 2. *Methods of purification for high-purity reagents*

Chromatography
Partition
Preparative gas-liquid chromatography
Liquid
Adsorption
Adsorptive filtration
Ion-Exchange
Crystallization
Distillation
Electrolysis
Extraction
Fractional Solidification
Progressive freezing
Zone melting
Ignition
Membrane filtration
Precipitation
Sublimation

A. INORGANIC SALTS

Membrane filtration under pressure is an excellent prepurification technique for water-soluble inorganic reagents. Filtration of aqueous solutions of sodium salts through a 0.2 μm cellulose acetate filter under 30 lb pressure can remove 80 to 90 percent of the iron and 50 to 60 percent of the lead and copper content from the dry salts [29]. These water-insoluble contaminants are invariably introduced into most industrial chemicals by electrostatic effects [26]. An extremely sensitive x-ray fluorescence method for the direct determination of cations in water-insoluble particulates provides a rapid evaluation of membrane filtration [30]. The prepurified solution can then be freed of additional trace elements by mercury cathode electrolysis [27].

Buffers and supporting electrolytes, often required in relatively large volumes with respect to a trace element being determined, are readily ultrapurified by mercury cathode electrolysis [31]. Sodium or potassium

salts of acetic, citric and boric acids, potassium acid phthalate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate are popular salts in buffer systems. In addition, potassium and sodium chloride are preferred supporting electrolytes that must be ultrapurified.

When electrolysis of these salts is carried out with a platinum anode and mercury cathode under an inert gas blanket (argon) at a potential of − 1.5 volts *versus* a standard calomel electrode, practically all of the elements reducible to the metallic state are removed by the mercury.

In anode stripping voltammetric procedures it is common to analyze for lead, cadmium or zinc at the micro-molar concentration in a 1 or 2 molar solution of a buffer or simple salt. In the determination of lead in blood, for example, the whole blood is refluxed with perchloric acid to oxidize organic matter, then 2 molar sodium acetate solution is added prior to anodic stripping analysis [32]. Since the lead content of normal whole blood is 0.3 to 0.4 μg/ml, roughly 1.5 micro-molar [33], the concentration of lead in the sodium acetate supporting electrolyte should be 0.15 micro-molar, one-tenth of the concentration being determined. The solid sodium acetate, therefore, should contain less than 0.0001 percent lead, a value not attained in most commercially available reagents.

Although mercury cathode electrolysis provides an excellent tool for the purification of ultrapure chemicals, unusually rigid handling procedures are required to preserve the integrity of the product. The electrolysis should be carried out in a laminar-flow hood in a class 100 clean area [34] with the application of clean-room techniques [35].

B. Organic Reagents

Organic liquids can be prepurified to ~ 99.8 percent by fractional distillation. Those that melt in the range − 25 to + 20 °C can be ultrapurified by fractional freezing of the distillate to 99.99 percent purity. A temperature of − 25 °C is the lowest temperature handled readily by most commercial cryostats. The limitation of temperature, of course, is determined by cryogenic equipment available. The following materials (M.P., °C in parenthesis) are candidates for this purification scheme: cyclohexane (6.5), cyclohexanone (− 16), dioxane (12), anilinel (− 16), *p*-xylene (14). The boiling points (144, 139 and 138 °C) and the melting points (− 25, − 47 and + 13 °C) of the ortho, meta and para-isomeric xylenes clearly demonstrate that freezing should be a powerful purification tool. Actually, *p*-xylene (99.99% assay by GLC chromatography) has been obtained after partially freezing 99.86 percent distilled material [36].

C. Acids

Inorganic acids find extensive applications in all types of trace analysis. In the laboratory sub-boiling distillation of HCl, HNO_3 and H_2SO_4 in vitreous silica and HF in Teflon TFE stills affords distillates with unusually low cation content, when stored in Teflon FEP bottles [37]. These acids can be obtained commercially with most cations controlled at the low ng/g level [38]. Although Teflon FEP containers are ideal for the in-house transport of acids, current Department of Transportation regulations do not permit the shipment of acids, other than HF, in free-standing plastic containers. Vitreous silica and high-silica (Vycor, Corning) glass have desirable chemical resistance, but economics eliminates them from general consideration.

Comparison of acid purification in the literature is difficult to interpret as fragmentary data on essential parameters relating to leaching from glass and plastics are usually reported. A complete description of the leaching action of a chemical on glass should record the composition of the glass, the upper temperature of glass working and the manufacturer. The pretreatment of the surface with water, detergent, hydrofluoric or other acid is also important. A description of the removal of surface contamination from polyethylene or polypropylene by abrasive action prior to acid cleaning may be the key to low desorption. Access to radiotracer methods is frequently required to interpret correctly the movement of traces in closed systems.

Analysts all too frequently dismiss borosilicate glass as an inferior container for acids. Accelerated aging tests of acids in leached containers along with analysis of the acid before and after aging in our laboratory, however, indicate that for high-purity acids packaged in pretreated borosilicate glass 12 elements [39] remain unchanged. Emission spectrographic analysis of concentrated hydrochloric acid stored in acid-leached, sealed 100-ml ampoules at 50 °C for 13 days showed that the following elements are unchanged: Ca, Co, Cr, Fe, Pb, Mn, Hg, Ni, K, Sn, Sr, Zn. The only elements that were leached were sodium, boron, silicon, aluminum, copper and magnesium. The increase in the sodium content far surpassed that of every other element. The original sodium content (<50 ng/g) reached a level of 1200 ng/g when the ampoules were filled without pretreatment whatever. After acid leaching the sodium never exceeded 90 ng/g. Special acid pretreatment affords borosilicate containers that do not add to the original impurity content significantly, except for sodium and silicon. Accelerated aging studies for 2.5 days at 50 °C approximate levels reached at room temperature in approximately 6 months. Storage of commercial high-purity acids in a cold room until shipment was thought to be

necessary to preserve the integrity of the acid, but room temperature storage is adequate. In a recent evaluation of a high-purity, commercial nitric acid, one group reported discrepancies with a supplier's values [30]. The differences were ascribed to the deterioration in the quality of the reagent upon storage. Expanded studies on leaching and storage are required to reconcile these differences.

D. Bases

Isopiestic distillation from reagent-grade ammonium hydroxide into high-purity water affords 10N NH_4OH within 3 days [40]. Uptake of the ammonium hydroxide by acidic samples rather than water is a good way to perform a neutralization with a high-purity reagent. If cylinder ammonia is first bubbled through an ammonical solution of EDTA and then through high-purity water with cooling, high-quality aqueous ammonia is available. Gassing water with ammonia directly from a cylinder is not recommended as surprisingly high values for Cu and Ni, 100 ng/g respectively, were found in 10 percent NH_4OH solution prepared in this way. When an EDTA solution bubbler was introduced, Cu and Ni values were reduced to 4 and 1 ng/g [29].

E. Water

Water is the major volume reagent for the trace analyst; its purity can support or undermine his entire analytical operation. According to revised ASTM specifications, Type I, "ultrapure" water is recommended for the preparation of solutions for trace metal analysis [41]. Specifications include a minimum electrical resistivity of 16.66 megohm-cm and a pH of 6.8 to 7.2 at 25 °C. Resistance measurements afford qualitative evaluation of small dissociated ions. Particulate matter, nonionized materials and charged molecules with low solution mobilities are not detected. Quantitative elemental analyses of high-purity water have been reported by several investigators [28,37,42]. Tap water purified by a train of prefilter, carbon, mixed-bed resins and a 0.2 μm cellulose acetate filter delivers water with common cations below the ng/g level. Airborne organisms, usually belonging to the family pseudomonadaceae, can enter the outlet end of the deionizer (or stills) and multiply in water of the highest purity. Amino acids and protein contamination in water has been ascribed to these organisms [43]. Excessive handling of water containers should also be avoided since amino acids from bare hands are an ever-present contamination hazard [44].

Table 3 lists the particulate matter content found in five water samples. The most striking observation is that water contains 5 and 10 μm particles after filtration through a 0.2 μm cellulose acetate filter. This finding has been checked by several pharmaceutical laboratories for all membrane filters commercially available. With the shift to a 0.45 μm filter, the number of 5 μm particles in the filtrate increases. Some laboratories use cartridges of mixed-bed resins without proper filtration of the effluent water. Resins are not designed for efficient particulate matter removal; in fact, fine resin particles may wash out of the resin bed.

TABLE 3. *Particulate matter in water*[a]

	Number of particles/10 ml			
	(5 μm)	(10 μm)	(15 μm)	(20 μm)
A. Tap Water	4180	800	263	109
B. Tap water (purified with carbon, mixed bed resin and 0.2 μm cellulose acetate filter)	12	5	1	0
C. B stirred with magnetic bar	39	12	5	2
D. B stirred with forefinger	6350	1002	337	75
E. B-plus distillation (0.45 in place of 0.2 μm filter)	45	12	4	3

[a] PC-305 unit, High Accuracy Products Corp., Claremont, Calif. 91711

The increase in 5 μm particles from 12 to 6350 per 10 ml by inelegant finger-stirring demonstrates that the hands are indeed a prime source of contamination in trace analysis. This particular experiment emphasizes the need to eliminate excessive handling of solutions in ultrapurity work.

Analysts frequently have access to central distillation or deionization systems in the analytical laboratory. Personal experience, however, has shown that filters, ion-exchange resin or carbon beds are not properly maintained in this equipment. Furthermore, water obtained through improperly constructed distribution lines can add 50 to 100 ng of lead per ml to effluent water when the source water from public supplies contained less than 5 ng of lead per ml [24]. Since the complete reliability of a central system is beyond the control of the trace analyst, he should not jeopardize his results by faith in the distilled or deionized water faucet conveniently located above his sink. Instead, he should install a unit so that the effluent water is available within a laminar-flow hood, thus eliminating airborne contamination at the point of use.

IV. Storage

The conditions of storage and distribution are pertinent factors in the final purity of reagents. Experience indicates that it is incumbent upon the analyst to determine the suitability of commercially available products for critical applications. Unfortunately, high-purity reagents are frequently mishandled by the analyst. All too frequently high-purity chemicals in original containers collect dust on an open shelf or in the fume hood of the laboratory. The fume hood is a particularly unfortunate storage area inasmuch as large volumes of contaminated air are drawn over the surface of the container. Unless the outside of the container is properly cleaned, the integrity of the contents may be compromised. There is no point to pay a premium for sodium acetate containing 10 ng/g of iron if this reagent is dissolved in water of unknown quality in a heavily contaminated laboratory.

High-purity reagents and standard solutions are frequently stored at ambient temperatures inside a laminar-flow hood. To free work area in the hood, containers can be placed in an inexpensive outer polyethylene container on a laboratory shelf [14]. Storage of standard solutions in outer containers in a refrigerator is recommended; storage in the frozen state is a further refinement. Frozen solutions should be removed from the freezer well in advance of use so that complete solution is attained when the temperature of standardization is reached.

V. Summary

Reagents, ultrapure with respect to many elements, are needed in survey ultratrace analysis. For single-element analysis the control of only one element in a reagent is essential. Improved limits of detection in survey analysis depend on contamination-free water, mineral acids, organic solvents, chelating agents and inorganic salts required in routine methodology.

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CONTAMINATION OF ATMOSPHERIC PARTICULATE MATTER COLLECTED AT REMOTE SHIPBOARD AND ISLAND LOCATIONS

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As part of a study of the chemistry of marine aerosols, atmospheric particulate samples have been collected simultaneously on the bow and on the stern of the University of Rhode Island oceanographic vessel TRIDENT to determine the degree to which particulates generated or modified by the ship itself can influence the chemistry of ambient air particulates. Samples have been analyzed for a variety of trace elements, including Na, Mg, Ca, K, Fe, Cu, Mn, Al, and Pb. The results indicate that certain elements are most subject to this form of contamination.

It is possible to use meteorological parameters to determine whether or not samples are contaminated by local sources. Atmospheric particulate samples have been collected from coastal towers in Hawaii and Bermuda. The composition of the particulates at the Hawaii tower is dependent not only on meteorological conditions at the time of collection, but also on conditions *(e.g.,* wind direction, rainfall, *etc.)* 24 hours previous to sample collection. Detailed meteorological information is thus critical in remote locations to determine whether or not the sample collected is representative of the ambient atmosphere.

Keywords: Analytical blanks; atmospheric sampling; coastal sampling; contamination of atmospheric particulate samples; sample handling; shipboard atmospheric sampling.

I. Introduction

For the past several years the University of Rhode Island has participated in several programs involving the collection and chemical analysis of atmospheric particulate matter from remote regions. This paper will

examine a few of the problems associated with the collection of representative samples from these areas.

II. Shipboard Collection of Atmospheric Samples

A. Collection of Representative Samples

There are several studies of the chemistry of atmospheric particulates which have relied on samples collected from ships [1–9]. However, there are indications that samples collected from ships may not always be representative of the composition of the ambient marine atmosphere, since the samples may also contain ship stack effluents, dirt, paint, rust, bow spray, and corrosion and wear products of the sampling apparatus itself [10]. For the past 5 years, atmospheric samples have been collected onboard the University of Rhode Island's R/V TRIDENT using a system designed to prevent collection of these additional constituents. This system has been described in detail by Moyers *et al.* [10] and Duce *et al.* [11].

The TRIDENT's atmospheric collection system is mounted on a specially constructed 8-meter-high A-frame tower mounted on the bow of the ship. The pump is separated from the filter holder by 3 meters of flexible tubing to prevent contamination of the filter by the pump [12]. The sampler is controlled automatically by relative wind direction as indicated by an anemometer mounted directly above the sampler. The sampler is operated only when the relative wind is from the bow. When the relative wind is from any other direction, the sampler automatically turns off and a door closes to protect the filter.

The following experiment was designed to determine which elements were most susceptible to contamination by particles from the ship itself. A second filtering apparatus was mounted on the stern of the ship, 10 meters behind the stack (also using flexible tubing to prevent contamination by the sampling pump). The relative locations of the two samplers are shown in figure 1. The stern sampler had no automatic controls and operated regardless of wind direction. Atmospheric particulate samples were collected simultaneously on the bow and stern using double $8'' \times 10''$ Whatman 41 filters. The filters were ashed in an LTA-500 low temperature asher, dissolved in Suprapur® HNO_3 and HF and analyzed by atomic absorption spectrophotometry for several trace metals. A comparison of the analyses of samples from the two locations on the ship is given in table 1.

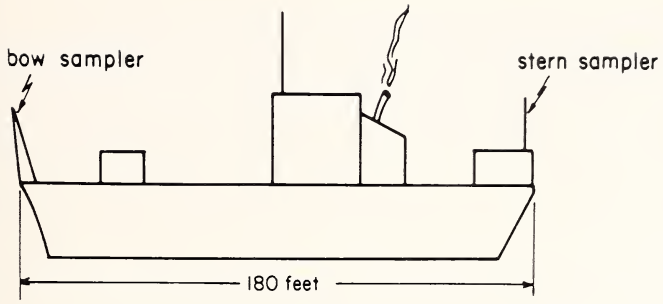

Figure 1. Basic profile of R.V. TRIDENT showing locations of air samplers.

TABLE 1. *Ratio of stern to bow values of trace metal concentrations and air volumes sampled*

Element	Sample pair						
	1	2	3	4	5	6	Mean
Cd	22.0	1.5	1.8	2.5	0.2	1.8	5.0
Cr	2.0	1.0	0.1	0.2	3.6	0.8	1.3
Al	0.9	2.2	.9	.6	1.8	13.9	3.4
Pb	1.1	0.3	.3	.4	0.9	7.9	1.8
Fe	1.1	1.9	.7	1.6	1.1	2.0	1.4
Cu	4.2	6.1	.9	1.8	2.1	4.4	3.2
Mn	4.7	0.9	.6	0.3	1.3	14.4	3.7
Zn	2.9	1.4	2.4	5.6	3.6	2.7	3.1
m^3	1.8	1.0	2.4	1.3	1.2	2.9	

Rigorous comparison of the bow samples with the stern samples is not possible for two reasons: 1. the samples were not strictly simultaneous, since the bow sampler turned off during obviously unfavorable collecting conditions at the bow. An indication of this is seen in the cubic meter ratios given in table 1. During the time when the bow sampler is off, the stern sampler is collecting ambient concentrations, the stern sampler being upwind of the ship; 2. even with the precautions used with the bow sampler, collection of particles originating from the ship is still possible at the bow location, especially after the system turns on after prolonged periods of unfavorable collecting conditions. This effect will be discussed later.

Three observations about the data in table 1 can be made: 1. some elements show random excesses (*i.e.*, Cr, Al, Pb, and Mn). That is to say, excesses at the bow location appeared as frequently as excesses at the stern; 2. Fe shows no significant differences at either location considering the distance between the samplers; 3. even considering the limitations of the

experiment, three elements, Cu, Cd, and Zn, were routinely higher at the stern location indicating a strong likelihood that these elements are subject to shipboard contamination more seriously than the others.

In order to determine if substantial increases of alkali and alkaline earth metals are found at the stern location, the concentrations of these elements were normalized to Na. The mean values of the element/Na ratios are presented in table 2. There is a statistically significant increase in the Ca/Na ratio at the stern location compared to the bow. Regression analysis of Ca *vs.* Na at the stern yielded the following:

$$\text{Ca}\ (\mu g/m^3) = 0.028\ \text{Na}\ (\mu g/m^3) + 0.080\ (\mu g/m^3)$$
$$\sigma\ \text{slope} = 0.006$$
$$\sigma\ \text{intercept} = 0.017\ (\mu g/m^3)$$

This suggests that 80 ± 17 ng/m³ of the Ca is from a source other than seawater. In contrast, the Ca-Na regression equation for bow samples is

$$\text{Ca}\ (\mu g/m^3) = 0.036\ \text{Na}\ (\mu g/m^3) + 0.0025\ (\mu g/m^3)$$
$$\sigma\ \text{slope} = 0.006$$
$$\sigma\ \text{intercept} = 0.024\ (\text{Mg}/m^3)$$

Thus, at the bow there is no significant intercept or excess Ca.

It should be pointed out that portions of the cruise track were fairly close to land causing the samples collected there to have much higher concentrations of trace metals than are usual in marine areas. It is quite likely that contamination problems are much more serious in remote oceanic areas. Several factors should be considered in the planning of programs relying on atmospheric samples collected from ships. It is obvious that the collection site on board ship is critical to minimize contamination

TABLE 2. *Comparison of the mean ratios of alkali and alkaline earth concentrations to* Na *concentrations as a function of location on the ship*

Ratio	Bow	Stern	Different at 95% significance level?
Mg/Na	0.142 ± 0.023	0.133 ± 0.011	no
Ca/Na	.035 ± .011	.071 ± .020	yes
K/Na	.053 ± .011	.051 ± .006	no

problems. Moreover, the automated wind-direction controlled sampler is helpful in reducing gross contamination from ship stack materials emitted seconds before collection. In addition, collection of samples only while underway lessens the possibility that the ambient air previously contaminated by the ship will be collected. This condition is likely to occur when the ship is stopped, the ship effectively becoming a stationary

source. The ship then "pollutes" the air surrounding the ship and collection of uncontaminated marine air is virtually impossible until the ship moves away from the area. These procedures have been found to minimize contamination problems of this sort on board ship.

B. Contamination by Handling

Filter blank corrections in this remote area sampling program are accomplished using what we term "comprehensive" blanks. Comprehensive blanks are processed in the same manner as the samples collected on the ship with the exception that no air is drawn through the filters. Comprehensive blank filters are cut and stored individually in polyethylene bags, opened and mounted in the filter holder below deck in a laminar flow clean bench, mounted and dismounted on the sampling tower at the bow, dismounted from the filter holder and stored in a polyethylene container for later analysis. By contrast, a "filter" blank, when referred to in this paper, is a filter removed from its plastic bag under laboratory conditions just prior to analysis. Although from the same lot of filters as the "comprehensive" blanks, these filters are never taken on the ship.

Comparison of the filter blanks with comprehensive blanks taken on two different cruises by two different individuals is presented in table 3. This table shows that there are significant increases in the levels of Mg, K, and Cd exhibited in the comprehensive blanks of both cruises. Statistically significant (95% level) increases of Pb, Fe, and Zn were observed in the comprehensive blanks on one cruise (TR-134). There is no statistical difference between the comprehensive blanks of TR-132 and TR-134.

In conclusion, there are significant differences between comprehensive blanks and filter blanks for several elements. If filter blanks alone are used for blank correction, erroneously high quantities of atmospheric Mg, K, Cd, Pb, Fe, and Zn may be reported. This effect is especially critical when the blank correction represents a substantial part of the total filter plus sample quantity for any element.

III. Collection of Samples Representative of Marine Air from Coastal Locations

Approximately 200 atmospheric particulate samples were collected over a 1 year period from a 20-meter-high tower located on the windward coast of Oahu, Hawaii. These samples were analyzed for trace metals, and some of the results of this study have been reported by Hoffman *et al.*

TABLE 3. *Comparison of laboratory filter blanks with comprehensive blanks*
μ/g8″ × 10″ Whatman 41 filter

Element	Comprehensive blanks		Filter blanks (10)[a] mean ± 1σ	Comprehensive blanks different from the filter blanks at 95% significance level?	
	TR-132 (4)[a] mean ± 1σ	TR-134 (4)[a] mean ± 1σ		TR-132	TR-134
Na	68 ± 5	87 ± 18	69 ± 19	no	no
Mg	5.2 ± 0.5	8.0 ± 2.2	4.4 ± 0.7	yes	yes
Ca	38 ± 14	32 ± 3	32 ± 9	no	no
K	2.0 ± 0.3	2.7 ± 1.1	0.41 ± 0.55	yes	yes
Cd	0.071 ± .030	0.053 ± 0.006	.034 ± .007	yes	yes
Cr	.59 ± .18	0.49 ± 0.21	.52 ± .18	no	no
Al	15.5 ± 3.0	15.8 ± 2.3	14.8 ± 2.9	no	no
Pb	0.79 ± 0.68	0.72 ± 0.34	0.26 ± 0.07	no	yes
Fe	9.2 ± 3.4	11.4 ± 3.1	7.7 ± 2.5	no	yes
Cu	0.99 ± 0.53	1.1 ± 0.5	0.7 ± 0.12	no	no
Mn	.16 ± .03	0.16 ± 0.02	.20 ± .02	no	no
Zn	3.3 ± 2.2	2.2 ± 0.4	1.2 ± .5	no	yes

[a] Number of filters analyzed.

[13]. Only a portion of the samples that were collected and analyzed were considered representative of marine air. Representativeness was based solely on wind direction. If the sample was collected during a period when the winds were consistently on-shore (from the sea) during the period of collection and at least 24 hours prior to the collection, the sample was considered to be representative of marine air. If, however, the wind was from the land during a portion of the collection period or any time up to 24 hours before sampling was initiated, the samples were not considered to be representative of marine air and were not reported by Hoffman *et al.* [13]. Figures 2 to 4 illustrate the effect of local wind direction on the trace element composition of samples collected at this

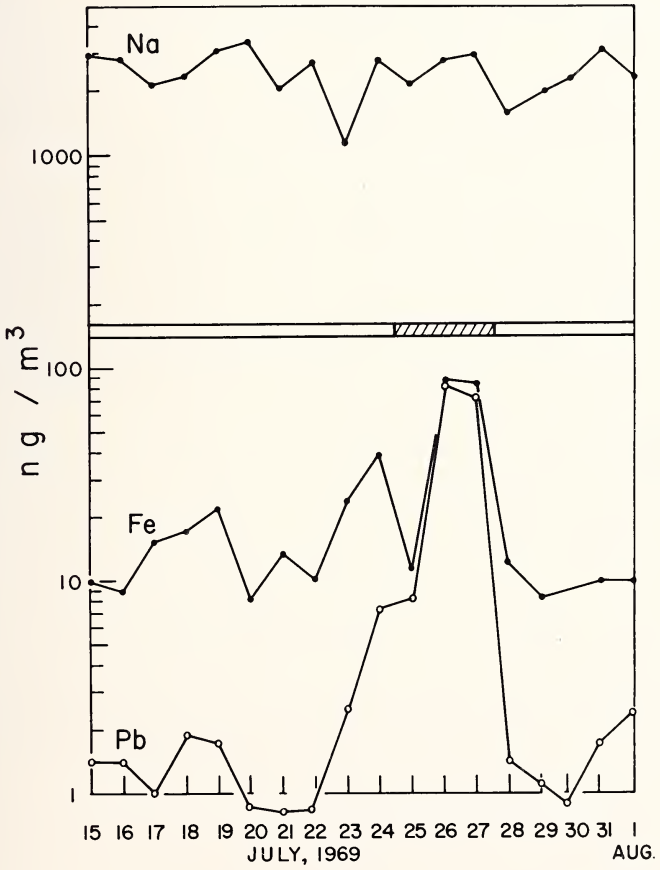

Figure 2. Concentrations of Na, Fe, and Pb in atmospheric particulate samples collected daily on windward Oahu. Sampling period is from July 15-August 1, 1969. Open bar indicates on-shore winds; solid bar indicates off-shore winds.

coastal location. Figure 2 shows the average daily atmospheric concentrations of Na, Fe, and Pb from July 15 to August 1, 1969 at the windward Oahu location. The open bar indicates periods of on-shore winds recorded by an anemometer on the tower, while the slashed bar indicates off-shore winds. On July 25 the winds at the sampling tower shifted from on-shore to off-shore. Accompanying this wind shift was an increase of atmospheric Pb by more than an order of magnitude and an increase of Fe by over a factor of 2. The concentrations of Na were not appreciably affected by the wind shift. Sodium is a primary constituent in ocean derived sea salt particles whose production rate is closely related to wind speed [14]. Since the Na concentrations from this source are at least 1 or 2 orders of magnitude higher than the trace element concentrations, locally derived particulate matter containing a small amount of Na is not likely to significantly affect the marine Na concentrations. Note that the Pb increase appears to start 2 days before the local wind shift. This indicates that local wind direction alone may not be sufficient to determine the representativeness of the sample. In this case, the local surface wind direction changes may have been preceded by upper level wind changes.

Figure 3 illustrates, as in figure 2, the effect which surface wind direction changes have on the composition of the samples. Again there are dramatic increases in both the Fe and Pb concentrations during the period of off-shore winds. Na concentrations are not affected by the change. (The drop in Na concentrations on October 4 and October 5 are due to very low wind speeds resulting in low production rates of sea salt particles at the sea surface during those days.) Again the Fe and Pb increase began a day or two before the local surface wind shift.

Figure 4 illustrates the sampling period of December 13 to December 22, 1969. During the winter months the trade winds are much less persistent in Hawaii than during the summer. Again, the concentrations of Fe and Pb increase markedly when the winds come from the land. However, note that the concentrations of Fe and Pb during the on-shore wind periods, especially when these periods last only a day to two, do not generally approach the low concentrations found during the summer when the on-shore winds are quite persistent. It appears that during periods when the winds are not consistently on-shore, particles originating from local land sources may prevent collection of representative samples of marine air even during the times when the wind is from the sea. Locally derived particulate matter is likely transported out to sea during obviously unfavorable sampling conditions and then returns and is collected for several days when the wind shifts to on-shore. Depending on how often the wind shifts, an entire season may be unsuitable for collection of marine air from coastal locations.

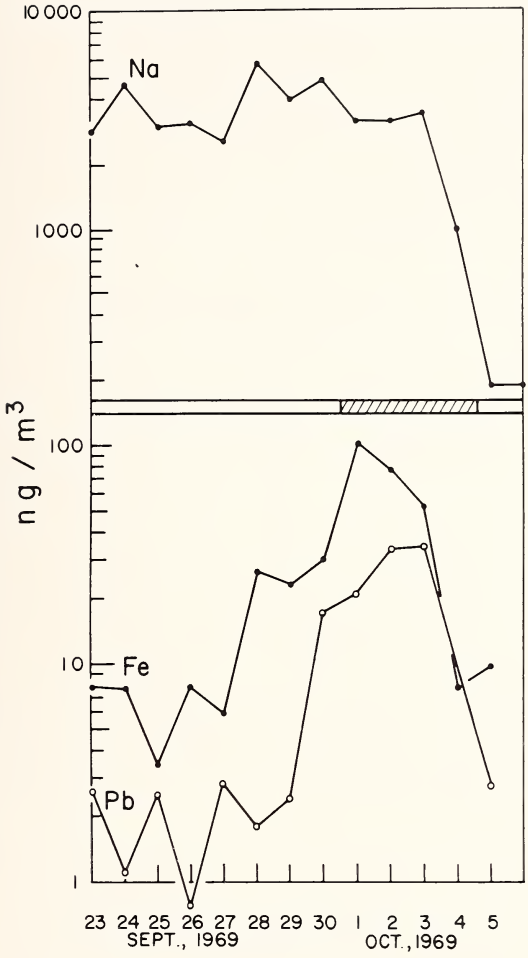

Figure 3. Concentration of Na, Fe, and Pb in atmospheric particulate samples collected daily on windward Oahu. Sampling period is from September 23-October 5, 1969. Open bar indicates on-shore winds; solid bar indicates off-shore winds.

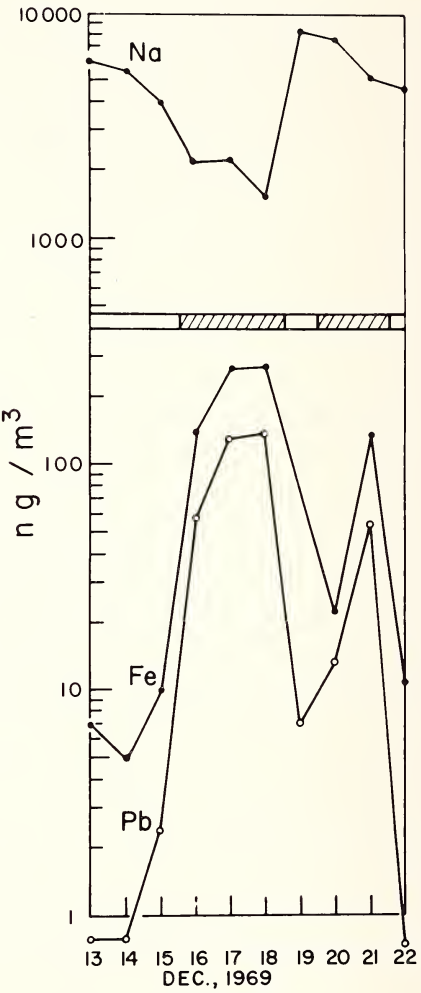

Figure 4. Concentrations of Na, Fe, and Pb in atmospheric particulate samples collected daily on windward Oahu. Sampling period is from December 13-December 23, 1969. Open bar indicates on-shore winds; solid bar indicates off-shore winds.

IV. Conclusions

The collection of representative atmospheric particulate samples in remote marine areas requires special care to avoid the collection of locally derived particulate matter. Without such care, samples collected on ships may contain particles originating from the ship itself. It was found that samples collected at coastal locations will be contaminated with local dust and pollution products if the winds are not consistently on-shore. In most cases, the representativeness of a coastal sample can be determined by examination of the local wind records. The excess particles will cause the samples to contain anomalously high concentrations of Ca and Zn and perhaps excesses of Cd, Fe, Cu, Al, and Pb.

Appropriate blank corrections are also critical especially in remote locations where some elements are present in extremely low concentrations. Routine filter handling processes during the collection procedure were found to add significant quantities of Mg, K, Cd, Pb, Fe, and Zn to the samples. The blank correction procedure must include this handling factor to avoid erroneously high values for these elements in atmospheric samples from remote areas.

V. Acknowledgements

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SAMPLING AND ANALYSIS FOR SULFUR COMPOUNDS IN AUTOMOBILE EXHAUST

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Fuel sulfur is oxidized to the dioxide and trioxide in automobile engines. Adding an oxidation catalyst for emission control may alter the SO_3/SO_2 ratio in the exhaust.

An engine, a current production V-8 equipped with 1975 emission controls including an oxidizing catalytic converter (but without exhaust-gas recirculation), was run at steady operating conditions on an engine dynamometer. The exhaust was passed through a conventional exhaust system to a 23-in-diameter, 36-ft-long dilution tunnel.

Samples were withdrawn from before and after the catalytic converter, from the tailpipe, and from before and after filters sampling the diluted exhaust in the tunnel. Material balances for the fuel sulfur were carefully checked to ensure that valid data were being obtained.

Experiments were conducted with several catalysts, at two levels of fuel sulfur content. The results indicate less than 1 percent conversion to sulfate when the catalyst is not used, but substantial conversion to sulfate with the catalyst in use at the operating conditions of the experiments. A large increase in particulate mass is observed with the catalyst; the increase has been shown to be largely sulfuric acid and associated water.

Considerable care must be exercised in the sampling and analysis of exhaust sample if errors are to be avoided. Details of the overall method, and reasons for the precautions taken will be presented, with data supporting the conclusions.

Keywords: Automobile exhaust; catalytic oxidation converter; dilution tunnel; isopropanol; particulate filtration; sulfate; sulfur dioxide; sulfur trioxide; sulfur trioxide condensation.

I. Objective

The overall objective of this program was to determine the extent to which fuel sulfur is oxidized to S^{+6} (SO_3, H_2SO_4, $SO_3 \cdot xH_2O$, or $SO_4^=$) when automobile exhaust is vented to the atmosphere *via* 1975 catalytic oxidation converters [1]. Subordinate objectives were to determine any storage or release of sulfur compounds within the exhaust system (sulfur material balance) and to compare the degree of sulfur oxidation in exhaust systems having no catalyst with systems having two different types of catalyst.

These overall objectives could be met only if the analytical steps were reliable. The analytical task was the classic one: collect a representative sample, and analyze it. In this work, the collection aspect proved to be the more difficult of the two. This paper deals principally with the collection rather than the analysis of the samples; a discussion of the experimental work and data from the viewpoint of the stated program objectives can be found in references [1,2].

II. Experimental Design

A. Collection Strategy

Unleaded gasoline of average sulfur content, *i.e.*, 300 ppm, burned in an automobile engine produces primarily gaseous combustion products. Without a catalyst, the particulate matter is typically 0.01 g per mile (6. mg/km) while the fuel consumption is around 150 g per mile (90 g/km) plus about 2000 g air; with a catalyst, the particulate emissions at the tailpipe are about 15 times greater. Fuel sulfur oxidation products can be collected as (1) SO_2 and $SO_3 \cdot xH_2O$ in the gas phase, or (2) upon dilution as particulate $SO_3 \cdot xH_2O$ and $SO_4^=$, plus SO_2 which remains in the gas phase.

We deliberately designed the collection system to be redundant; redundancy proved valuable in cases when a spurious analysis was obtained, and was indispensable in checking the data for internal consistency and material balances. Five units sampled the gas phase SO_3 and SO_2 at various points in the exhaust system and after exhaust quenching or dilution (fig. 1). In addition, five filters were used to sample and collect the diluted exhaust for particulate matter. In this manner, knowing the total amount of fuel sulfur consumed and the various sampling and dilution

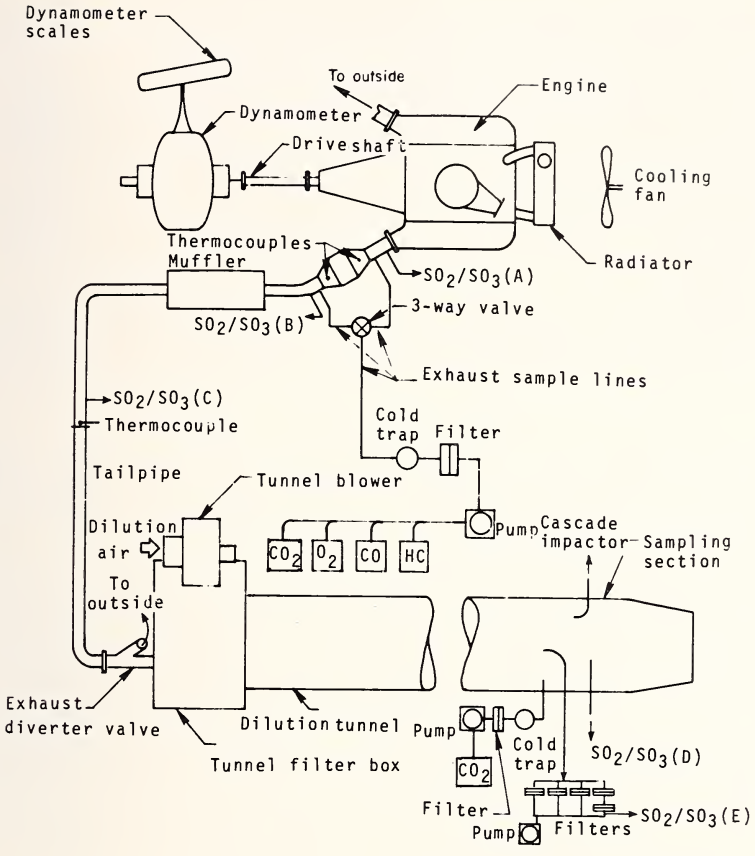

Figure 1. Layout of experimental facility.

ratios, it was possible to reconstruct the total sulfur balance at several points and to establish the SO_2-SO_3 oxidation ratio at these points.

Each gas phase sampling unit utilized the condensation method, commonly called the Shell coil or Goksøyr-Ross technique, to trap the gas phase SO_3 (as condensed $SO_3 \cdot xH_2O$) while passing the SO_2 [3]. The latter was trapped in H_2O_2 bubblers as H_2SO_4 [4]; particulate matter entrained in the hot exhaust stream (and not SO_2 or SO_3; see Discussion) was trapped on a quartz wool plug filter preceding the condenser and bubblers (fig. 2). This method was selected over other possible methods for several reasons. First, SO_2 and SO_3 are collected separately, permitting the oxidation ratio to be determined at each sampling point. Second, the likelihood of spurious oxidation of SO_2 to SO_3 *within the sampling and collection system* is minimized; an alternative method, based upon SO_3

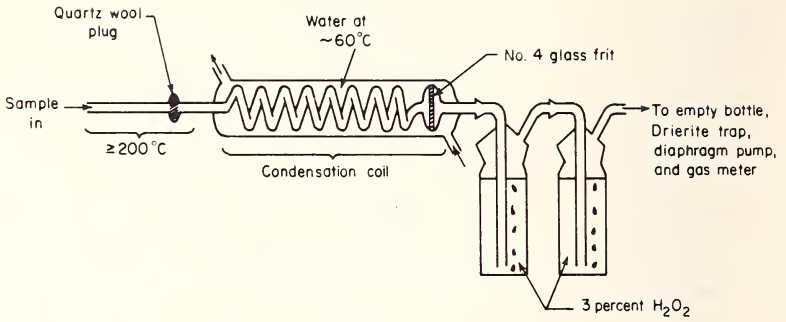

Figure 2. Sampling system for SO_2 and SO_3.

absorption in aqueous isopropyl alcohol (in which SO_2 is only slightly soluble), can and does give high SO_3 values [5]. Third, the collected solution is readily analyzed for $SO_4^=$ by the sensitive and reliable barium perchlorate-thorin titrimetric method [6].

Particulate filtration of the diluted exhaust is a confirmatory or backup method for SO_3 measurement, and probably is more reliable than the gas phase SO_3 condensation method *for diluted exhaust* because a larger volume can be sampled conveniently. In the absence of any cation to react with the SO_3 in the exhaust stream, the SO_3 when diluted and cooled by ambient air will form droplets of $SO_3 \cdot xH_2O$ that are filterable. In the diluted state, therefore, the SO_3 can be collected either as $SO_3 \cdot xH_2O$ in a heated condenser or as $SO_3 \cdot xH_2O$ on a filter at approximately ambient temperature. In either case the SO_2 passes through the collector owing either to virtual insolubility (condenser) or to displacement (filter).

Insuring a chemically valid collection is only part of the collection problem. Obviously, if one wishes (as we did) to compute material balances throughout the system one must know that the collected sample is a definite, representative fraction of the total sample. A representative sample was assured by the highly turbulent flow through the exhaust and dilution system, and by prior profile measurements in the dilution tunnel. The known fraction requirement was more difficult to establish; for this presentation it is sufficient to note that this one aspect caused the most trouble and posed the largest single source of uncertainty in the data, specifically in the diluted exhaust data.

B. Analysis

From the sampling points described above several different samples were collected during each run. These were:

Gas Phase

Particulate $SO_4^=$ (quartz wool plug filter)
$SO_3 \cdot xH_2O$ (heated condenser)
SO_2 (H_2O_2 bubblers)

Particulate Phase

$SO_4^=$ ($MSO_4 + SO_3 \cdot xH_2O$).

As shown in figure 3, each sample was treated so that the final sample was essentially pure dilute H_2SO_4. The actual $SO_4^=$ determination, therefore, was identical for all the samples.

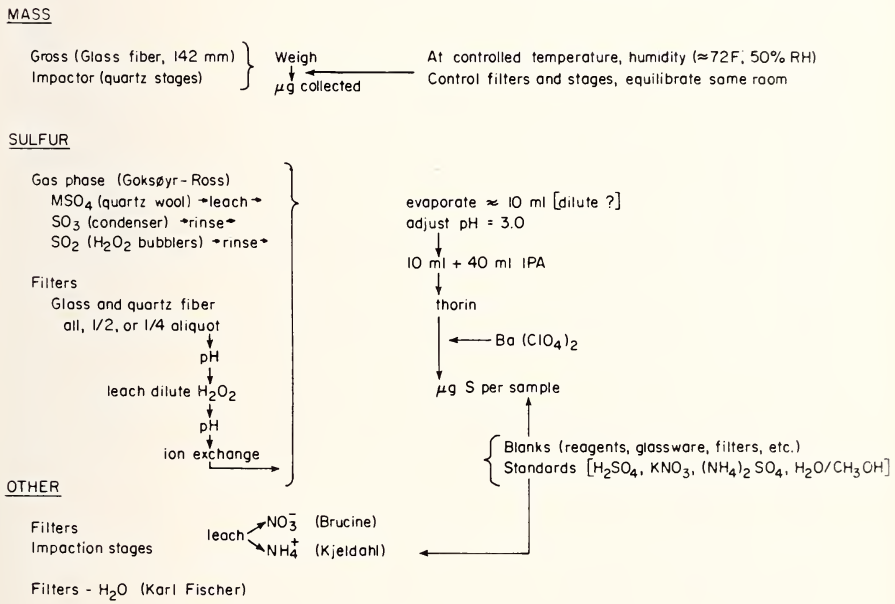

Figure 3. Analysis techniques for exhaust collections.

Hundreds of analyses were performed in the authors' laboratories during these experiments. The results, presented in detail in table 1, could be used to determine precision, standard deviation repeatability or the like. These calculations were not made because statistical data treatment was not a program objective. One can note superficially, however, that replicate analyses usually agree to about 10 percent or better, whether between the two laboratories or among different filters from the same run.

TABLE 1. *Reduced Goksøyr-Ross and particulate filter data*

Run date	Speed, mph	Catalyst	Catalyst T°C	Cum. S,g	Fuel S, wt %	Fuel S, mg/mi		G-R samples[a] S, mg/mi A	B	C	D	E	Filter[b] S, mg/mi 1	2	3	4	Emission[c] S, mg/mi	
8/23	60	none	—	—	0.032	49.0	$SO_4^=$	0.2		0.1			0*	0*	0*	0**	as $SO_4^=$	0.1
							SO_2	71.2		40.4	50.3						as SO_2	50.3
							Total S	71.4		40.5							stored	−1.4
8/24	60	No. 1	627	39.4	0.032	49.9	$SO_4^=$	0.9	0.5		(6.2)		14.1*	14.3*	(5.0)*	15.4**	as $SO_4^=$	14.2
							SO_2	87.5	22.6		27.5						as SO_2	27.5
							Total S	88.4	27.6		33.7						stored	8.2
10/22A	60	No. 1	602	52.8	0.032	50.8	$SO_4^=$	0.2	18.5		(13.3)		18.5*	20.8	18.8	19.7	as $SO_4^=$	19.5
								0.3	19.8	7.8	18.6							
							SO_2	57.3	31.6	22.9	23.2	20.1					as SO_2	23.2
								61.3	35.3	23.8	26.4	23.1						
							Total S	57.5	50.1		36.5						stored	8.1
								61.6	55.1	31.6	45.0							
10/22P	60	none	—	—	0.032	50.8	$SO_4^=$	0.1	0.1	0.0	(0.8)		1.4*	0.3	0.3	0.4	as $SO_4^=$	0.3
								—	0.1	0.1	(0.3)							
							SO_2	59.9	50.9	44.7	45.4	50.3					as SO_2	49.0
								69.5	57.1	42.1	54.5	45.9						
							Total S	60.1	51.0	44.7	46.2						stored	1.5
									57.2	42.2	54.8							

TABLE 1. *Reduced Goksøyr-Ross and particulate filter data*—Continued

Run date	Speed, mph	Catalyst	Catalyst T°C	Cum-S, g	Fuel S, wt%	Fuel S, mg/mi		G-R samples[a] S, mg/mi A	B	C	D	E	Filter[b] S, mg/mi 1	2	3	4	Emission[c] S, mg/mi	
11/7A	60	No. 1	596	60.8	0.032	49.5	$SO_4^=$	0.3	29.3	24.0	22.0		23.9	20.7	22.2	19.2*	as $SO_4^=$	22.5
								0.2	29.0									
							SO_2	48.8	24.1	22.8	20.6						as SO_2	20.6
								52.4	25.6									
							Total S	49.1	53.4	46.8	42.6						stored	6.7
								52.6	54.6									
11/7P	60	none	—	—	0.059	88.5	$SO_4^=$	0.4	0.6	0.5	(0.7)		0.2	0	0	0.7*	as $SO_4^=$	0.2
								0.3	0.3									
							SO_2	87.2	103.0	87.9	89.4	78.5					as SO_2	84.0
								87.8	112.3									
							Total S	87.6	103.6	88.4	90.1						stored	4.3
								88.1	112.6									
11/28A	60	No. 2	580	17.0	0.059	100.1	$SO_4^=$	0.4	27.7	33.7	29.4		33.2	32.2	31.7	30.8*	as $SO_4^=$	31.6
							SO_2	89.6	47.6	58.3	56.3	54.9					as SO_2	55.6
							Total S	90.0	75.3	92.0	85.7						stored	12.9
11/28P	60	No. 2	610	32.9	.059	92.6	$SO_4^=$	0.5	39.8		35.1		44.7	38.1	40.3	37.6	as $SO_4^=$	39.6
							SO_2	72.9	39.3		42.9	41.7					as SO_2	42.3
							Total S	73.4	79.1		78.0						stored	10.7
12/12A	60	No. 2	618	84.5	.059	101.5	$SO_4^=$	0.4	35.4	31.6	33.1			38.3	37.5	31.7*	as $SO_4^=$	36.3
							SO_2	97.8	53.0	53.6	60.1	59.4					as SO_2	59.8
							Total S	98.2	88.4	85.2	93.1						stored	5.4

TABLE 1. *Reduced Goksøyr-Ross and particulate filter data*—Continued

Run date	Speed, mph	Cat-alyst	Cat-alyst T°C	Cum. S,g	Fuel S, wt %	Fuel S, mg/mi		G-R samples[a] S, mg/mi A	B	C	D	E	Filter[b] S, mg/mi 1	2	3	4	Emission[a] S, mg/mi	
12/12P	60	none	—	—	.059	102.0	$SO_4^=$	0.4	0.5	0.1	(1.4)			0.5	0.5	1.0*	as $SO_4^=$	0.5
							SO_2	97.7	88.5	87.9	98.7	95.3					as SO_2	97.0
							Total S	98.1	89.0	88.0	100.1						stored	4.5
1/9A	60	No. 2	640	92.8	.059	106.6	$SO_4^=$	0.6	35.2	(7.7)	29.5		28.4	32.7	29.9	27.6*	as $SO_4^=$	30.1
							SO_2	99.9	49.4	62.4	64.7	57.5					as SO_2	61.1
(NH_3 injection							Total S	100.5	84.6	(70.1)	94.2						stored	15.4
1/9P	60	none	—	—	.059	104.4	$SO_4^=$	0.3	0.5	0.4	(0.9)		1.0	0.9	0.8	0.7	as $SO_4^=$	0.9
							SO_2	94.6	97.6	83.1	108.6	95.3					as SO_2	102.0
							Total S	94.9	98.1	83.5	109.5						stored	1.5
2/12	60	No. 3	624	25.5	.059	107.0	$SO_4^=$	16.2	29.3	(6.9)	(17.7)		33.9	30.5	32.2	23.1*	as $SO_4^=$	32.2
								14.4	30.5	(11.2)	(13.1)							
							SO_2	84.4	39.3	38.7	(7.9)	(5.6)					as SO_2	42.7
								91.0	41.5	40.8	44.8	40.6						
							Total S	100.6	68.6	(45.6)							stored	32.1
								105.4	72.0	(52.0)	(57.9)							
2/14	60	No. 3	621	143.8	0.059	102.7	$SO_4^=$		37.8		34.5		37.3	36.8	37.9	29.3*	as $SO_4^=$	36.6
							SO_2		44.8		37.5						as SO_2	37.5
							Total S		82.6		72.0						stored	28.6

TABLE 1. *Reduced Goksøyr-Ross and particulate filter data*—Continued

Run date	Speed, mph	Catalyst	Catalyst T°C	Cum. S, G	Fuel S, wt %	Fuel S, mg/mi		G-R samples[a] S, mg/mi A	B	C	D	E	Filter[b] S, mg/mi 1	2	3	4	Emission[a] S, mg/mi	
2/21	30	No. 3	488	157.2	0.059	96.3	$SO_4^=$	0.9	29.0	28.3	31.8		34.2	32.7	33.2	25.0*	as $SO_4^=$	33.0
								0.3	28.9	29.7	(13.4)							
							SO_2	94.0	4.0	4.8	6.8						as SO_2	7.2
								97.2	4.4	4.4	7.6							
							Total S	94.9	33.0	33.1	38.6						stored	56.1
								97.5	33.3	34.1								
2/27	30	No. 3	488	257.5	0.062	100.8	$SO_4^=$	0.3	59.1		56.9		81.7	85.6	75.5		as $SO_4^=$	74.9
									61.8		(38.0)							
							SO_2	93.7	11.3		10.4						as SO_2	11.2
								97.6	11.6		11.5							
							Total S	94.0	70.4		67.3						stored	14.7
									73.4									

[a] Sampling positions: A—converter inlet
B—converter exit
C—tailpipe (corresponding to normal tailpipe exit location, but several feet upstream of tunnel entrance)
D—dilution tunnel
E—dilution tunnel at exit of particulate filter.

Numbers in parentheses are believed to be grossly in error and are not used in computing emissions.

Underlined values from laboratory A; others from laboratory B.

[b] Values without asterisk are for Tissuquartz filters, * indicates Gelman A filter, ** indicates MSA filter.

[c] Values for $SO_4^=$ emission obtained by averaging $SO_4^=$ from G-R samples at position D and Tissuquartz filter values.
Exception: Gelman A and MSA filter values used for results of 8/23 and 8/24 runs.
Values for SO_2 emission obtained by averaging SO_2 from G-R samples at positions D and E.
Stored sulfur computed as the difference between fuel sulfur input rate and averaged $SO_4^=$ and SO_2 emission rates.

III. Discussion

The key to reaching this program's objectives was sampling. Unless proper precautions are taken and valid techniques used, the analyses will be useless. Two examples suffice to demonstrate the effect of incorrect approaches.

Example 1. Experimental Technique

The exhaust gas temperature through the tailpipe region is about 290 °C. In early experiments the exhaust gas was taken directly into the quartz wool filter-condenser coil with no insulation or additional heating around the 30-cm-long quartz tube sampling line. After a few runs the temperature drop in this line was examined. The actual gas temperature entering the condenser coil had dropped to about 50 °C. At 50 °C $SO_3 \cdot xH_2O$ condenses into droplets, while about 200 °C is necessary to keep $SO_3 \cdot xH_2O$ as a gas. Thus, premature $SO_3 \cdot xH_2O$ condensation occurred in the probe, the quartz wool filter and the associated glassware rather than in the condenser coil only as was intended. Premature condensation is uncontrolled and may lead to a loss of SO_3 or to SO_2 oxidation, depending on the quantities and temperatures involved. Table 2 summarizes the distribution of sulfate sulfur with and without auxiliary heating around the glassware preceding the condenser coil. Note that the amount of sulfate sulfur ($SO_3 \cdot xH_2O$) per liter of gas sampled is virtually the same in both cases; the distribution between the condenser coil and the glassware preceding the coil is the important point.

TABLE 2. *Effect of sampling temperature on sulfate distribution*

	Run No. 1 with oven	Run No. 2 no oven
Probe and quartz wool filters, mg SO_4	0.31 ($t \cong$ 190 °C)	10.42 ($t \cong$ 50 °C)
Coil, mg SO_4	9.43	1.13
Total, mg SO_4	9.74	11.55
Total, mg S/liter sampled	0.0337	0.0342

Example 2. Collection Method

Two methods are in general use to collect gas phase SO_2 and SO_3. One uses the isopropanol (IPA) bubbler (EPA Method 8) to collect SO_3, the other uses a condenser coil. In both methods SO_2 should be caught not in the SO_3 trap, but in subsequent H_2O_2 traps. In practice the IPA method tends to oxidize some of the SO_2 to SO_3 and therefore can give fictitiously high SO_3 results; the $SO_2 + SO_3$ total remains valid, however. In one experiment we collected two exhaust gas samples simultaneously from the same point in the exhaust pipe before the catalyst. Little conversion of SO_2 to SO_3 has occurred at that point, according to several investigators. However, as shown in table 3, the IPA method indicated about 8 percent SO_2 to SO_3 conversion while the condenser method showed only 0.3 percent conversion.

Although it has been argued that the condenser method may not be able to collect all the SO_3 and hence gives fictitiously low SO_3 values, the evidence rather is that the IPA method gives high SO_3 values. Table 1 has many points at which a comparison can be made between the filter sulfate value and the condenser sulfate value, both samples having been taken simultaneously from the same point within the dilution tunnel. Agreement generally is good, certainly much better than between the methods shown in table 3. Furthermore, in numerous experiments in which the condenser coil was followed by a second similar coil in series, the second coil had only traces of SO_3. These second-coil experiments do not prove unequivocally that the first coil removes essentially all the SO_3 in the gas stream; however, combined with the agreement between the condenser data and the filter data, the second coil experiments clearly show that the condenser method and not the IPA method is valid for SO_2-SO_3 collections in automobile exhaust collections.

TABLE 3. *Comparison of two* SO_3 *sampling methods*

	IPA	Condenser (Goksøyr-Ross)
SO_3, mg S/mi	7.4	0.3
SO_2, mg S/mi	84.9[a]	93.7[a]
Total S, mg S/mi	92.3	94.0
Fuel S, mg S/mi	100.8	100.8

[a] From H_2O_2 bubbler train.

IV. Summary

The problem of sampling and analyzing automobile exhaust is not so much one of complex methodologies but rather the recognition of potential problems before they occur. Frustration and dismay result when data from a long, tedious experiment are nullified by problems that are detected only after a critical evaluation later in the program. The exhaust studies, of which the sampling and analysis techniques reported here were but two aspects in the overall program, required a team of more than half a dozen professional persons of various disciplines. Each member had to assure the validity of his part of the experiment within the constraints of the program goals and limitations. As is true in virtually every research program, unforeseen difficulties arose. In hindsight they appear obvious but at the time they were not. The purpose of this paper will have been served if others working in similar fields are alerted to the many potential pitfalls that must be avoided before reliable data are produced.

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(In the present work the train consisted of a heated probe and quartz wool plug filter, two IPA bubblers and a dry bubbler in an ice bath; and two H_2O_2 bubblers and a dry bubbler followed by the usual drying tower, pump, and meter. These deviations from Method 8 as published were made to evaluate the IPA system. Each of the three bubblers in the IPA section was analyzed individually, and contained 77, 17, and 4 percent respectively of the total SO_3 found; the quartz wool plug filter had the remaining 2 percent. We believe that these modifications helped to demonstrate the undesirable oxidation of SO_2 to SO_3 and that the data can be extrapolated to the Method 8 train as published.)

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PREPARATION, ANALYSIS, AND SAMPLING CONSTANTS FOR A BIOTITE

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A 99.9+ percent pure 40-60 mesh biotite, intended primarily as a K-Ar dating standard, but useful in other applications, has been exhaustively analyzed. About 8 kilograms have been prepared for distribution.

Sampling constants K_s (*i.e.* the weights of samples necessary for 1% sampling error) have been determined for potassium (0.005 g), for sodium (1 g), for total iron (0.005 g), for calcium (2 g), for aluminum (0.001 g), and for some other elements. Nonuniformity with respect to potassium, sodium, and calcium may be partly due to diadochic substitution: nonuniformity with respect to calcium may be caused in part by 0.02 percent of apatite impurity. There is evidence that the biotite is geochronologically inhomogeneous; it behaves as a mixture of two biotites of different K-Ar ages. The sampling constant for radiogenic argon is about 0.05 gram.

Possibly the greatest value of this standard material will lie in its demonstration of the principles which ought to be observed during the preparation and distribution of geochemical standards. We hope that may be the first in a series of International Standard rocks and minerals which may be used with confidence to calibrate and control geochemical analysis.

Keywords: Biotite stoichiometry; geochemical standards; geochronological standards; K-Ar dating; sampling a biotite; sampling constants; standard biotite.

I. Introduction

In analytical geochemistry and geochronology, analyzed standards are essential for calibration and control. Few analyzed mineral standards are widely available. None have ever been fully certified with respect to

homogeneity and sampling characteristics. Biotite LP-6 Bio 40-60 mesh represents a first attempt to provide the geochemical community with a mineral standard of known composition and uniformity. While it was originally intended as a K-Ar dating reference sample, its usefulness in other applications has been demonstrated.

LP-6 is a biotite-pyroxenite from one of many biotite-rich zones in a pyroxenite mass peripheral to the Simalkameen composite pluton in the northern Okanogan Highlands of Washington State. It was found by C. D. Rinehart [1] during a geochronological study, and was chosen from among several other similar rocks for its richness in biotite, the quantity available, the predicted ease of mineral separation, and the approximate expected age. The biotite-pyroxenite is coarse-grained, xenomorphic granular, with grains up to 2 cm in linear dimension, and consists chiefly of augite and biotite. Minor amounts of microcline, albite, rutile, ferrohastingsite, apatite, calcite and magnetite are present. In thin section, the biotite is subhedral to anhedral and interstitial to augite, with local eutectic intergrowths in which augite encloses biotite grains poikilitically. Thus, the biotite is a primary constituent. Some bent cleavage traces show post-crystallization deformation in the biotite. Generally, the biotite is green, with minor brown biotite intergrown.

II. Collection and Purification

About 1760 kg of LP-6 were collected by Rinehart and shipped to Menlo Park in burlap sacks. The whole was crushed to about 20-mesh in the Palo Alto laboratories of the Utah Mining and Construction Company, using a 10″ Gy-roll reduction crusher. Preliminary separation of the biotite was accomplished in a 36″ × 6″ water column, using tap water. The overflow passed through a 30″ square 105-mesh stainless steel screen, carrying fine pyroxene through the screen and depositing 95-98 percent pure biotite. Additional biotite was washed from the coarse gangue using a 14″ × 4″ water column and a 60- or 80-mesh screen. The wet biotite was washed with distilled water, dried, and screened. The 24-32, 32-40, and 40-60 mesh material was further purified using deionized water in the 14″ × 4″ column, and washed with distilled water before drying. Each fraction was then passed through a Frantz Isodynamic Separator (setting: 15°, 15°, 0.56 amperes) at about 10 grams per hour. The total yield of the various fractions is shown in table 1.

TABLE 1. *Yield of LP-6 Biotite fractions*

Mesh size	Quantity (g)	Splits		Purity (%)
		No.	Wt (g)	
+24	2,150			99[a]
24–32	1,800	320	2	99.9
		256	5	99.9
32–40	1,000	172	5	99.9
40–60	7,500	960	8	99.9+
60–80 80–100	15,000	not split		95[a]

[a] Not passed through Frantz separator.

III. Impurities

The devastating effect of impurities in mineral separates used in K-Ar dating has been documented [2-4]. A mineral used to calibrate and control K-Ar age determinations must be of high purity if it is to be useful; therefore extensive investigation of the impurities in LP-6 Bio seemed to be necessary.

One hundred grams of LP-6 Bio 40-60 mesh were treated successively with 1:1 nitric acid, 6*N* sodium hydroxide solution, and dilute hydrochloric acid containing hydrogen peroxide. Biotite was completely dissolved, leaving 0.05 gram (0.05%) of unattacked pyroxene, amphibole, quartz, and feldspar. Some pyroxene grains were +60 mesh, but most of the residue was − 60 mesh, indicating that the foreign minerals existed as inclusions in the biotite. A few unidentified minerals were found; no zircons were detected.

Determinations of CO_2 and P_2O_5, using large samples, showed 0.00 percent CO_2 and 0.00−0.03 percent P_2O_5, indicating the absence of calcite and a trace of apatite. A formal microscopic inspection of 1000 grains revealed no mineral other than biotite, and no visible inclusions. However, during numerous other examinations of LP-6 Bio grains, we have occasionally found inclusions of rutile, apatite, *etc.*

IV. Splitting and Packaging

The vials in which LP-6 Bio 40-60 mesh, LP-6 Bio 32-40 mesh, and LP-6 Bio 24-32 mesh are distributed were cleaned by scrubbing with a brush in soap and water to remove the submicroscopic impurity which is always present on new glass [5]. They were then treated with concentrated sulfuric acid containing chromic acid (not potassium dichromate), rinsed with water, washed with dilute hydrochloric acid containing hydrogen peroxide, rinsed with distilled but not deionized water, and oven dried.

Splitting was done according to the scheme of figure 1, by coning and quartering on albanene paper, using a hard rubber spatula. No metallic splitting devices were used. Tramp iron introduced during the early stages of preparation was removed during the magnetic separation.

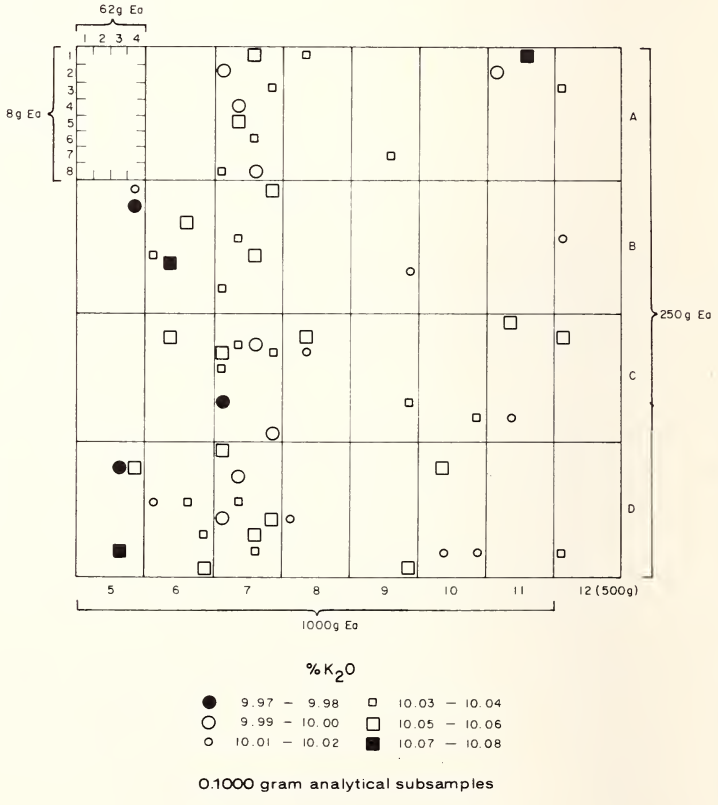

Figure 1. Splitting scheme.

V. Homogeneity

Table 2 shows results from contributing laboratories for potassium and for radiogenic argon. The scatter of these results is partly due to inhomogeneity. Without complete information on sample weights and methods of calibration employed by the several contributors, it is not possible to estimate how much of the scatter is due to analytical error, and how much is due to sampling error originating either in LP-6 Bio 40-60 mesh or in the investigators' calibrating standards.

Sampling constants [6] provide a convenient means of describing sampleability and nonuniformity with respect to individual constituents of a material. The sampling constant K_s is defined as the weight of material which must be taken to ensure a sampling error no greater than 1 percent with 68 percent confidence. The square root of the sampling constant, K_s, is numerically equal to the coefficient of variation to be expected in a series of analytical results on 1-gram subsamples using a precise method of determination. The sample weight needed to reduce sampling error to less than 1 percent at the 98 percent confidence level is designated w*. Table 3 compares sampling constants for potassium in several reference materials.

To test LP-6 Bio 40-60 mesh for inhomogeneity, deliberate efforts were made to fractionate portions of it. The only visible difference from grain to grain is that some grains are massive or "blocky" while others are typical thin biotite flakes. Therefore, 21 grams were passed over a vibrating mica table; six fractions were collected; these ranged in appearance from "blocky" to "flaky." Subsamples of these fractions were analyzed for potassium, sodium, calcium, and iron. In addition, eight 5-mg subsamples of fraction 1 ("blocky"), which showed no mineral other than biotite in a grain-by-grain optical count of 700 grains, were analyzed for calcium. Results appear in table 4. Inhomogeneity with respect to calcium is confirmed; it is evidently not due to isolated grains of calcium mineral, unless these grains are in the form of very small inclusions.

Whether the calcium inhomogeneity is due to diadochic substitution of calcium for potassium, or to minute inclusions of calcium mineral (which would have to behave like biotite in the impurity tests outlined above), is an open question. We are inclined to the inclusion hypothesis, although if this hypothesis is correct, the 99.9+ percent purity estimate may require some qualification. Submicroscopic inclusions may be thermodynamically stable in a two-phase system [7], but their possible effect on gross composition has traditionally been ignored by mineralogists.

LP-6 Bio 40-60 mesh is geochronologically inhomogeneous on the K-Ar scale. This type of inhomogeneity in an apparently unaltered mica

TABLE 2. *Submitted potassium and argon data, LP-6 Biotite 40-60 #*

POTASSIUM

Analyst				% K_2O
Armstrong (3 detns. on 6–II–B–4)				10.15
Baksi (7–IV–C–3)	10.06	10.09		10.01
	10.09	10.14		10.04
	10.07	10.14		
Delaloye	9.30	9.45		9.31
				9.42
Ferrara (6–II–A–2)				9.67
				9.62
Gittins (6–I–B–7)				10.14
Hebeda 7–I–D–5		10.09		10.12
7–IV–C–8		10.09		10.07
8–IV–C–3		10.08		10.01
Schlocker (see Table 1)		9.97	to	10.08
Katsura (6–III–C–1)				10.11
Krueger (6–II–A–8)	10.06	10.07		10.19
				10.20

Analyst			% K_2O
Leutwein	10.14	10.29	10.21
MacIntyre (7–II–B–1)		9.98	9.99
McBride (12–I–B–4)	10.167	10.099	10.128
	10.160	10.079	10.178
	10.016	10.045	
(5–III–A–6)		10.136	10.136
Rex (7–III–B–7)	9.84	9.92	9.89
	10.01	10.07	9.98
	9.97	9.94	9.95
Muysson 7–II–C–3			10.23
5–IV–D–5			10.09
Rijova			10.37
Smith (Gravimetric)			10.03
Turner (6 detns)			10.027
Webb 6–IV–B–1		10.022	10.019
		10.075	10.033
7–I–C–4		10.005	10.002
		10.144	10.102

TABLE 2. *Submitted potassium and argon data, LP-6 Biotite 40-60 #* —Continued

ARGON

Analyst		Ar, M/g × 10^{-9}
Armstrong (6–II–B–4)		1.935
Baksi (8 detns on 7–IV–C–3)		1.924
Hebeda 7–I–D–5		1.956
		1.933
		1.933
7–IV–C–8		1.902
		1.941
		1.936
8–IV–C–3		1.910
		1.922
		1.900
Krueger	1.927	1.952
	1.91	1.94
Leutwein	2.024	2.025
MacIntyre 7–II–B–1		1.870
		1.888
7–II–D–5		1.879
McBride 12–I–B–4		1.903
		1.914
Rex (7–III–B–7)		1.96
		1.89
		1.97
Turner	1.934	1.930
	1.926	1.923
Webb 6–IV–B–1		1.926
		1.922
		1.918
7–I–C–4		1.918
		1.942
		1.930
Giuliani	1.92	1.87
	1.85	1.85
	1.90	
Radicati		1.92

TABLE 3. *Sampling constants for potassium* [6][a]

Sample	%K_2O	K_s	$\sqrt{K_s}$	w*
LP-6 Bio 40–60#	10.03	0.005	0.07	0.045
USGS Muscovite P-207	10.2 (?)	.17	.41	1.5
Bern Biotite 4B	9.47	.012	.11	0.108
Nancy Biotite Mica-Fe	8.84	.01	.1	.09
Nancy Phlogopite Mica-Mg	10.18	.000	.00	.00[b]
PSU Orthoclase Or-1	14.92	.000	.00	.00[b]
PSU Biotite 5–110	10.00	.000	.00	.00[b]
USGS Granite G-1	5.52	.09	.3	.27

[a] K_s is the weight in grams needed for 1 percent sampling precision (68% confidence); $\sqrt{K_s}$ is numerically equal to the expected coefficient of variation using 1-gram samples; w* is the weight in grams required for 1 percent error with 98 percent confidence.

[b] Analytical methods are not precise enough to detect any nonuniformity in these samples, even at the microprobe level of sampling.

TABLE 4. *Analysis of fractions of LP-6 Biotite 40-60#* [a]

Fraction Number	Weight (g)	Subsample (g)	%K_2O	%Na_2O	%CaO	%Fe_2O_3
1. Blocky	0.4	0.100	9.80	0.07	0.36	11.55
2.	.8	.100	9.90	.06	.31	11.95
3.	2.7	.100	9.78	.06	.28	11.69
4.	1.9	.100	9.95	.07	.24	11.51
5.	6.9	.100	10.03	.06	.16	11.81
6. Flaky	8.4	.100	10.08	.12	.11	11.76
1. Blocky (eight determinations)		.005			.34 to .38	

[a] Analyses by L. B. Schlocker and C. O. Ingamells.

should be of some concern to geochronologists. Radiogenic argon determinations on the several mica-table fractions are reported in table 5. The apparent K-Ar age of these fractions varies from about 116 million years to about 132 million years. This inhomogeneity does not greatly impair the worth of the material as a K-Ar reference sample, provided care is taken in splitting out subsamples and provided adequate subsample weights are used. The need to mix the material before removing analytical subsamples should be obvious.

A source of apparent inhomogeneity which is often ignored is that of the sampling weight base line. Table 6 presents total hydrogen values, reported as percent H_2O, on three bottles of LP-6 Bio 40-60 mesh. A difference of 0.2 percent H_2O between samples will result in a difference of 0.02 percent K_2O and a difference of 0.08 percent SiO_2. These differences are large enough to have an appreciable effect on analytical reproducibility for all major constituents when highly precise methods are used. Note that drying the samples at 105 °C does not, in this case, suffice to remove the sampling weight base line difficulty. The H_2O loss in weight at 105 °C in splits 5-III-B-3 and 9-II-C-2 differ inappreciably, but the total hydrogen values are quite different. Experiment with other biotites leads to the hypothesis that oxidation of ferrous iron and loss of combined hydrogen are related.

While sampling weight base line effects will usually be of minor importance in using LP-6 Bio 40-60 mesh as a reference sample, there will certainly be instances where discrepancies due to these effects may lead to confusion if their causes are not recognized.

TABLE 5. *Geochronological inhomogeneity of LP-6 Biotite 40-60 #*

Fraction	%K_2O	^{40}Ar, M/g	Atmospheric argon
1-3 Blocky	9.86	1.78×10^{-9}	6.48×10^{-11} M/g[a]
1-3 Blocky	9.81	1.785×10^{-9}	[b]
	9.81	1.795×10^{-9}	[b]
4	9.95	1.830×10^{-9}	[b]
	9.95	1.839×10^{-9}	[b]
5	10.03	1.932×10^{-9}	[b]
	10.03	1.931×10^{-9}	[b]
6 Flaky	10.08	2.08×10^{-9}	6.14×10^{-11} M/g[a]
6 Flaky	10.08	2.029×10^{-9}	[b]
	10.08	2.037×10^{-9}	[b]

[a] Results by R. W. Kistler, U.S. Geological Survey.
[b] Results by D. L. Turner, University of Alaska.

TABLE 6. *Differences in total hydrogen content*

Sample number	Subsample weight (g)	Total hydrogen as H_2O (%)	H_2O (105°C)
5-III-B-3	0.05	3.58	0.12
		3.65	
		3.60	
		3.65	
		3.61	
		3.74	
		3.75	
		3.66	
		3.66	
	1.00	3.66	
9-II-C-2	0.05	3.82	0.13
		3.77	
		3.73	
		3.87	
		3.85	
		3.80	
		3.74	
		3.77	
		3.79	
		3.82	
		3.73	
8-II-B-1	1.00	3.61	0.11

Note: results on small samples by microcoulometry; on large samples by Penfield fusion. See: Cremer, M., Elsheimer, H. N., Escher, E. E., *Anal. Chim. Acta* **60,** 183 (1972).

The electron microprobe has been used to investigate the homogeneity of LP-6 Bio + 16 mesh. Two distinct biotites were found; these are individually homogeneous (except for calcium), as shown by the indices $\sigma/\sqrt{N}$ in table 7. The biotite PSU 5-110 [8] was used as a calibrating standard in these analyses; PSU 5-110 has never shown any measurable degree of inhomogeneity, even at the microprobe sampling level, and was analyzed by the most sophisticated primary methods available. The two LP-6 biotites are distinguishable visually in epoxy grain mounts, the high-Fe species occurring in more massive "blocky" grains. Some obvious anomalies in the data remain unexplained: while results on the +16 mesh material are not strictly comparable to those on the 40-60 mesh material, the need to consider the sampling characteristics of even the purest mineral separates is clearly demonstrated.

TABLE 7. *Microprobe analyses of LP-6 Biotite fractions*

	5–110[a]		LP–6 high Fe[b]		LP–6 low Fe[b]		LP–6 Bio 40–60 #	
	wt %	$\sigma/\sqrt{N}$	wt %	$\sigma/\sqrt{N}$	wt %	$\sigma/\sqrt{N}$	wt %[c]	wt %[d]
SiO_2	38.63	1.2	37.67	1.5	39.08	1.2	37.83	38.33
Al_2O_3	13.10	1.5	15.58	1.7	15.48	1.2	15.21	15.30
TiO_2	1.58	1.7	1.80	2.5	1.60	0.9	1.52	1.67
FeO[e]	10.93	1.7	11.17	1.2	9.54	1.0	11.10	10.58
MnO	.14	1.1	0.10	1.5	0.06	2.2	0.16	0.11
MgO	19.90	2.4	18.63	2.3	20.03	1.1	19.66	19.35
CaO			0.05	8.0	0.03	6.4	0.04	0.21
K_2O	10.00	1.1	10.21	1.1	10.24	1.8	10.14	10.03
Na_2O	0.26	1.2	0.09	2.1	0.13	3.2	0.14	0.09

[a] Used as a calibrating standard. Chemical analysis by C. O. Ingamells.
[b] Microprobe analyses by G. K. Czamanske.
[c] Microprobe analysis by J. Gittins.
[d] Most probable values for LP–6 Bio 40–60 # (see tables 8 and 9).
[e] Total iron calculated to FeO.

VI. Chemical Analysis and Stoichiometry

Our best estimate of the gross chemical composition of LP-6 Bio 40-60 mesh is given in table 8. Data accumulated here comes from many sources: contributors are listed at the end of this article. Values for K and for Ar* are listed by contributor in table 2. Less complete analyses of certain fractions of LP-6 Bio are presented in table 9. Calculated gross stoichiometry appears in table 10. The mineral is best described as eastonite, a trioctahedral mica.

TABLE 8. *Composition and homogeneity of LP-6 Biotite 40-60 #*

	Sample weight (g)	Wt %	K_s (g)
SiO_2[a]	0.7	38.33	0.001
Al_2O_3[a]	.7	15.20	.001
TiO_2[a]	.7	1.67	.05
Fe_2O_3[b]	3.7[c]	2.25	.005 (total Fe)
FeO[a]	0.5	8.55	K_s for FeO probably high
Cr_2O_3		0.04 (?)	K_s probably high
V_2O_5		0.02 (?)	
MnO[a]		.11	0.01
NiO		.05 (?)	
CoO		.01 (?)	
ZnO		.01	
MgO[a]	0.7	19.32	.005
CaO[a]	.7	.21	2.
BaO		.22	0.01
Li_2O		.005	
Na_2O[b]		.09	1.
K_2O[b]	10.0[d]	10.03[d]	0.005
Rb_2O[b]		0.025	
Cs_2O		.0006	
H_2O+[a]	1.0	3.53	see text, table 6
H_2O-[a]	0.7	0.13	see text, table 6
F[a]		.26	
P_2O_5[a]		.01	(0.00, 0.03) K_s probably very high
CO_2[a]	2.0	.00	
less 0 = F		0.11	
Total		99.97	

[a] Determination by primary methods.
[b] Determination by secondary methods, PSU 5–110 used as calibrating standard.
[c] Average of 37 results using 0.1 g subsamples.
[d] Average of 100 results using 0.1 g subsamples. Primary determination by V. C. Smith showed 10.03 percent K_2O.

TABLE 9. *Analyses of LP-6 Biotite 32-40#, 40-60#, + 16#*

	32–40# Split 20	40–60# 9–II–C–2	40–60# 8–II–B–1	40–60# Blocky	40–60# Flaky	+16#[a]
SiO_2	38.40	38.33		38.26	38.30	38.52
Al_2O_3	15.17[b]	15.30[b]	15.28[b]	15.26[b]	15.31[b]	15.52
TiO_2	1.66	1.67	1.67	1.54	1.69	1.68
Fe_2O_3	2.29	2.25	2.27	2.32	2.20	(2.16)
FeO	8.52	8.55	8.55		8.58	8.25
MnO	0.11	0.11	0.11	0.11	0.11	0.08
MgO	19.30[c]	19.35[c]	19.32[c]	19.28[c]	19.37[c]	19.47
CaO	0.41	0.21	0.19	0.34	0.14	0.04
BaO		.22	.22			
Na_2O	.09	.09	.09	.06	.12	.11
K_2O	10.03	10.03	10.03	9.85	10.08	10.23
Rb_2O	0.03	0.03	0.03	0.03	0.03	
H_2O+	3.45	3.66	3.50	3.75	3.48	
H_2O-	0.09	0.13	0.11	0.26	0.14	
F		.26	.26			

[a] Electron microprobe analysis by G. K. Czamanske.
[b] Uncorrected for Cr_2O_3, V_2O_5, etc.
[c] Uncorrected for NiO, ZnO, etc.

TABLE 10. *Gross stoichiometry of LP-6 Biotite 40-60#*

				Relative error
Na	0.026			
K	1.879			
Rb	0.004			
Ca	.033			
Ba	.011			
H_2O	.057			
	2.00	(Na, K, Rb, Ca, Ba, H_2O)	$^{+2.00}_{2.00}$	0.00
Mg	4.227			
Zn	0.001			
Mn	.014			
Ni	.006			
Co	.001			
Li	.004			
Fe''	.747			
	5.00	(Mg, Zn, Mn, Ni, Co, Li, Fe^{++})	$^{+10.00}_{5.00}$	0.00
Fe''	0.303			
Fe'''	.249			
Cr	.005			
V	.002			
Ti	.184			
Al	.257			
	1.00	(Fe^{++}, Fe^{+++}, Cr, V, Ti, Al)	$^{+2.89}_{1.00}$	−.11
Al	2.374			
Si	5.631			
	8.00	(Al, Si)	$^{+29.65}_{8.00}$	−.35
OH^-	3.399			
F	0.120			
	3.52	(OH^-, F)	$^{-3.52}_{3.52}$	+.48
O	20.48			
	20.48	(O)	$^{-40.96}_{20.48}$	
Total	40.00			−0.01

Note: There is a residual of 0.02 moles of H_2O, representing an uncertainty of about 0.02 percent in the dividing of total H_2O into H_2O+ and H_2O-. The inclusion of 0.057 moles of H_2O with the interlayer alkalies is arbitrary.

VII. Discussion

Although LP-6 Bio 40-60 mesh is better than 99.9 percent pure biotite, and contains far fewer impurities than most widely distributed mineral standards, it shows perceptible nonuniformity at the analytical subsampling level.

Figure 2 is a sampling diagram [9] for potassium in LP-6 Bio 40-60 mesh. The expected variability due to subsampling error in analytical results for potassium is found [6] from $R = \sqrt{K_s/w}$ percent, where R is the relative deviation due to subsampling error, K_s is the sampling constant for potassium (0.005 g for LP-6 Bio 40-60 mesh), and w is the analytical subsample weight in grams. Table 11 shows some corresponding values of R and w for potassium in LP-6 Bio 40-60 mesh.

Table 12 gives similar data for radiogenic argon. It is evident that subsample weight is an important parameter which must not be ignored in data evaluation, especially in standards programs. For example, if LP-6 Bio 40-60 mesh is used as a calibrating standard in ^{39}Ar—^{40}Ar dating, where small subsamples ($\approx$ 10 mg) are the rule, a large variance may be expected: in conventional K-Ar dating, where larger subsamples of up to 500 mg are taken for argon analysis, variance due to sampling

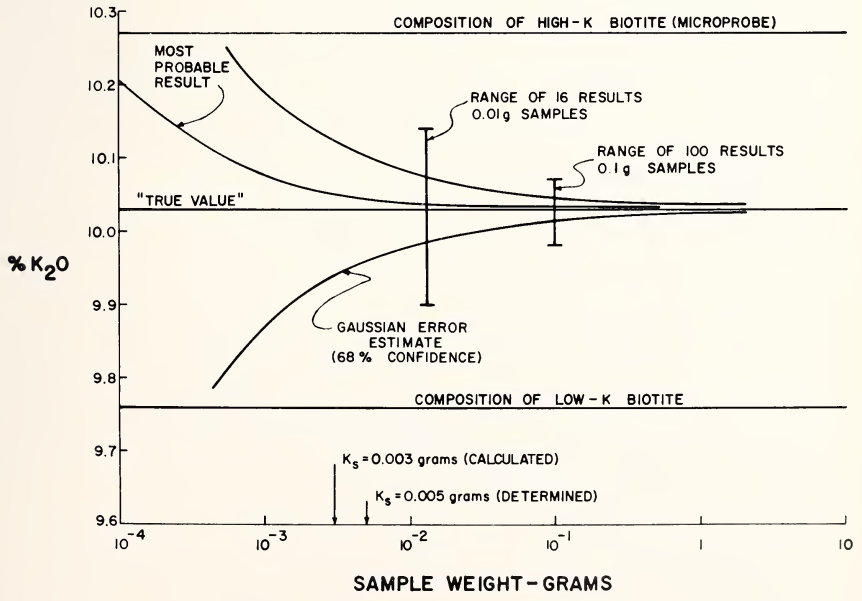

Figure 2. Sampling diagram for potassium in LP-6 Bio 40-60 mesh.

error may be tolerable. Obviously, certification should include a homogeneity statement, preferably in terms of a sampling constant, if misuse of the material is to be avoided.

The sampling constant for radiogenic argon in LP-6 Bio 40-60 mesh is estimated to be 0.05 g by calculation from the general sampling constant equation [6], and 0.04 g by the method of repetitive determination. Some other estimates of sampling constants are given in table 8.

One wonders how much unnecessary work and how much confusion have resulted through use of "standards" much less pure than LP-6 Bio 40-60 mesh which have never been thoroughly investigated for their subsampling characteristics.

During collaborative efforts to establish geochemical standards and reference samples, it is essential [10] for meaningful data evaluation that all participants report a description of their calibrating standards, a

TABLE 11. *Relative sampling error for potassium* (K_s = *0.005 g*)

Sample weight (g)	Relative error (%) (68% confidence)	Most probable result[a]	Error[b]	Expected range in 100 results (wt %)
1.	0.07	10.03	±0.007	10.01–10.05
0.1	.22	10.03	±.02	9.96–10.10
.01	.71	10.04	±.07	9.82–10.21
.001	2.2	10.08	(±.22)[c]	(9.76–10.27)[c]

[a] Ingamells and Switzer [6].
[b] Absolute error due to inhomogeneity, 68 percent confidence level.
[c] The range of results should not exceed these limits [9].

TABLE 12. *Relative sampling error for radiogenic argon* (K_s = *0.05 g*)

Sample weight (g)	Relative error (%) (68% confidence)	Absolute error[a] (68% confidence)	Range expected in 100 results[a]
10.	0.07	±0.0013	1.926–1.934
1.	.22	±.0042	1.917–1.943
0.1	.71	±.013	1.894–1.966

[a] Units are mol/g $\times 10^{-9}$.

description of their subsampling procedures, and the effective subsample weights. Rapid secondary methods which are ordinarily used in routine work, and which are often highly precise, are invaluable for determining sampling constants. Certificate values for elemental composition, however, should be based on primary methods so as to avoid systematic errors, the transfer of sampling and analytical error from existing standards to the new, and the consequent accumulation of inaccuracy.

LP-6 Bio 40-60 mesh should prove to be an adequate calibrating standard for potassium determination in biotites and similar materials if the analytical subsample weight is 50 mg or more. It should be adequate as a standard in Ar* determination if 500 mg are taken for analysis, and if the sample is thoroughly mixed before taking the analytical subsample. If a 1 percent subsampling error (68% confidence) is acceptable, as little as 5 mg may be taken for potassium determination, and as little as 50 mg for radiogenic argon analysis.

LP-6 Bio 40-60 mesh may be used as a microprobe standard for potassium if high-K grains are selected and they are taken as having 10.27 percent K_2O. It will probably be useful as a microprobe standard for iron, aluminum, silicon, and magnesium; this has not been thoroughly confirmed. Its worth as an emission spectrometric standard for major elements has been established. It is probably not desirable as a ^{39}Ar-^{40}Ar dating standard; in fact, its deficiency in this respect may draw attention to a hitherto unrecognized weakness of the ^{39}Ar-^{40}Ar method.

Possibly the greatest value of LP-6 Bio 40-60 mesh will lie in its demonstration of the principles which ought to be observed during the preparation and distribution of geochemical standards. We hope that it may be the first in a series of International standard rocks and minerals which may be used with confidence to calibrate and control geochemical analysis. Certainly it is the first such sample with which an attempt has been made to attain the highest possible degree of uniformity and also to quantitatively measure and report deviations from complete uniformity.

The selection, collection, preparation, and analysis for composition and degree of uniformity of a meaningful quantity of standard mineral is a long and complicated undertaking. LP-6 Bio was prepared in the face of severe difficulties and using only the simplest of equipment. It is reasonable to suppose that well-funded, properly supported, and competently organized projects of the same kind may do much better.

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AN APPROXIMATE METHOD OF COMPUTING ERRORS IN TRACE ANALYSIS DUE TO SAMPLING HETEROGENEOUS SOLIDS

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An approximate method of computing errors in the determination of trace elements in silicate rocks, errors that may be attributable to the size distribution at the sample grains, has been derived using the Poisson distribution and assuming no covariance between minerals and grain sizes. Standard deviations of trace-element analyses decrease as particle size decreases and as number of particles increases. Before a crushed rock is sampled, the analyst should ensure that the number of particles is so large that the relative error due to the number of grains in the portion for analysis will always be a magnitude or two less than other errors he may make in the analytical procedure.

Keywords: Analytical error; geochemical variance; particle size distribution; particle size errors; sampling errors; trace analysis.

A part of the variance of a set of determinations of a trace element in silicate rocks may be attributed to the variability of the portions to be analyzed. Although the analyst may use accurate, precise, and sensitive methods, imperfect sampling may cause error in portions weighed for analysis, and this error may be significant in the determination of trace elements.

Each ground rock sample is composed of grains. The bottle of ground sample and the portion weighed for analysis contain heterogeneous distributions of both minerals and size fractions, and the analyst usually must assume that the weighed portion is representative of the larger quantity in the bottle. The numbers and sizes of the grains in either portion may be described by such distributions as the multinomial, the binomial, or the Poisson, the last being most familiar to us as the distribution describing radioactivity counts. There is a practical lower limit to the size to which

the grains may be reduced; the variance attributable to the grains of this size may be used as the geochemical variance discussed by Shaw [1].

In sampling for analysis, the analyst takes a $(1/k)$th portion from the bottle as his sample. He can repeat his sample weight, but he can only approximate the distribution of the minerals and grain sizes in the sample he takes. For perfect sampling of a ground rock, defined as an operation which yields the same proportion of minerals and size fractions as in the larger portion sample, the number of grains, N_i, of the ith mineral on the jth sieve would occur in the same proportion existing in the bottle, and each mineral would have its own specific weight per grain, w_i.

As an example, assume that uranium is the element desired. Then each mineral contributes its own weight of uranium, U_i, to the total uranium, U_j, in the material on the jth sieve.

The weight of the first mineral on the sieve is N_1w_1, that of the second, N_2w_2, and that of the ith, N_iw_i. The weight fraction of the ith mineral would be the weight of that mineral divided by the sum of the weights of all minerals or $\Pi_i = N_iw_i \,/\, \Sigma N_iw_i$. The weight of uranium contributed by the first mineral on the sieve would be the weight fraction of the mineral times the uranium in the mineral, or $U = \Pi_1U_1$, and the total weight of uranium on the jth sieve would be the sum of the contributions of all minerals, or $U_j = \Sigma\Pi_iU_i$. These contributions to the weight of uranium are those expected under the assumption that the splitting from the larger to the smaller samples has been made without error. These expected values are shown in table 1.

TABLE 1. *Expected values in the jth sieve*

Mineral	Number of grains	Weight per grain	Mineral weight fraction	Uranium content of mineral	Uranium supplied by mineral
1	N_1	w_1	$\Pi_1 = \dfrac{N_1w_1}{\Sigma N_iw_i}$	U_1	Π_1U_1
2	N_2	w_2	$\Pi_2 = \dfrac{N_2w_2}{\Sigma N_iw_i}$	U_2	Π_2U_2
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
k	N_k	w_k	$\Pi_k = \dfrac{N_kw_k}{\Sigma N_iw_i}$	U_k	Π_kU_k
					$U_j = \Sigma\Pi_iU_i$

As all sampling operations are subject to errors of some magnitude, the splitting operation may not have given the expected number, N_1, of grains of the first mineral, but some other number of grains, n_1, and similarly for minerals 2 to k. The weights per grain, w_i, and the uranium content, U_i, of the minerals would remain the same, but because of the change of grains from N_i to n_i, the fraction Π_i of the mineral on the sieve would have some other value, p_i, and the uranium $\Pi_i U_i$ supplied by a mineral would change to some other value, $p_i U_i$. In the analytical sample, n_i can be estimated by its expected value, N_i, and W_j, the weight of the sample on the sieve, can be substituted for its equivalent, $\Sigma n_i w_i$, in the denominator of the sample weight fraction. The sample values for the uranium contribution of the individual minerals are shown in table 2, where the amount of uranium supplied by the minerals on the jth sieve is $u_j = \Sigma p_i U_i$.

TABLE 2. *Sample values for the jth sieve*

Mineral	Number of grains	Weight per grain	Mineral weight fraction	Uranium content of mineral	Uranium supplied by mineral
1	n_1	w_1	$p_1 = \frac{n_1 w_1}{W_j}$	U_1	$p_1 U_1$
2	n_2	w_2	$p_2 = \frac{n_2 w_2}{W_j}$	U_2	$p_2 U_2$
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
k	n_k	w_k	$p_k = \frac{n_k w_k}{W_j}$	U_k	$p_k U_k$
					$u_j = \Sigma p_i U_i$

The determined uranium, u, should be equal to the expected value, U, but because of sampling error these values may not be the same. At one extreme, the element of interest may be distributed homogeneously as small percentages in the grains of a major mineral, or at the other, the element may be contained in greater percentages in the relatively fewer grains of an accessory mineral. For all minerals, it will be assumed that each n_i follows a Poisson distribution, and it will be further assumed that the Poisson distributions of any two minerals or grain sizes are independent of each other.

If the analytical method is sufficiently precise so that its variance may be neglected, the precision of the determination of uranium in the ith mineral on the jth sieve will vary directly with the variation of the number of grains. The standard deviation of the number of grains, n_i, of a mineral would be σ_{n_i} whose units are grains. The standard deviation of the uranium content of the mineral could then be obtained by substituting σ_{n_i} for the number of grains, n_i, in the uranium fraction supplied by a mineral on a sieve, $w_i U_i n_i / W_j$. This yields $w_i U_i \sigma_{n_i} / W_j$, and if this expression is squared and the variance thus obtained summed over all minerals, there is obtained

$$\text{Var } (u) = \frac{\sum_i (w_i U_i \sigma_{n_i})^2}{W_j^2} \tag{1}$$

If the assumption that n_i follows the Poisson distribution is true, then

$$\text{Var } (n_i) = E(n_i) = N_i \tag{2}$$

where $E(n_i)$ is the expected value, or mean, of n_i. Rearranging the expression in table 1 for the proportion of the ith mineral, we obtain

$$N_i = \frac{\Pi_i \Sigma N_i w_i}{w_i} = \frac{\Pi_i W_j}{w_i} \tag{3}$$

as W_j is an estimate of $\Sigma N_i w_i$. Substituting the expected value, or the variance, N_i, for the variance $\sigma^2_{n_i}$ in (1), we obtain

$$\text{Var } (u) = \frac{\sum_i (w_i U_i)^2}{W_j^2} \cdot \frac{\Pi_i W_j}{w_i} = \frac{\sum_i w_i U_i^2 \Pi_i}{W_j} \tag{4}$$

By summing i minerals over j sieves, each mineral on each sieve having a different uranium content, and substituting W, the weight of the sample for analysis for its equivalent, ΣW_j, the sum of the weights of the minerals on j sieves, the variance of the uranium determination then becomes

$$\text{Var } (u) = \frac{\sum_i \sum_j w_{ij} U_{ij}^2 \Pi_{ij}}{W} \tag{5}$$

Behre and Hassialis [2] show a similar formula for calculating the weight of sample necessary for an analysis to be within a stated percentage of the true value at a specified probability. Unlike the present derivation in which there has been assumed no covariance, they use the binomial distribution with a covariance term to estimate the required sample weight. During the computational procedure shown by them in tabular form for several examples, an estimate of ns^2 is calculated and an estimate

of s^2, the variance, may be readily obtained by dividing by n, the number of grains in the analytical sample.

Application of equation 5 requires an estimation of the four unknowns. The weight of the analytical sample, W, is the choice of the analyst, and w_{ij}, the weight per grain of the ith mineral on the jth sieve, can be calculated. The particle shape may be assumed to be either cubical or spherical for ease of computation, but the effective diameter of the particle that passes the larger sieve and is caught on the next smaller one is a matter of personal preference. If one assumes that all particles just pass through the openings of the larger sieve, one obtains fewer particles per unit weight or volume and hence a slightly inflated estimate of variance that may be considered a safety factor.

The other two unknowns, Π_{ij} and U_{ij}, may be estimated after a sufficiently large sample of the crushed rock has been screened and mineral separations have been made. The amount of sample necessary to determine these estimates depends on the least abundant mineral species containing the trace element desired and for many samples in our laboratories, a kilogram of rock was the minimum required for screening and separating. The amount of uranium in the individual minerals for each size is determined to give estimates of U_{ij}, or if an insufficient amount of the least abundant mineral is present in each size, uranium may be determined in each unsized mineral and assumed to be constant over the size range.

As an example of the variances that might be expected, assume that 10 percent of a 1-gram sample of crushed granite occurred in the $-80+100$ mesh interval. The composition of this 1-gram sample is assumed the same regardless of mesh size and is expressed as number percent of the grains in column 1 of table 3 which shows the calculations for Π_i. The values of w_i in column 2 were computed under the assumption of spherical mineral particles of diameter slightly less than the wire opening of the larger of the two sieves for the interval.

TABLE 3. *Calculations for* Π_i

Mineral	(1) Percent of grains in interval	(2) Weight per grain g $10^5 \times W_i$	(3) Relative weights in interval	(4) Proportion in interval (3)/Σ(3)	(5) Proportion in sample 0.1 × (4)
Feldspar	70	1.002	38.12	0.669	0.0669
Quartz	24	1.042	13.59	.238	.0238
Allanite	5	1.566	4.26	.0747	.00747
Zircon	1	1.840	1.00	.0176	.00176

The variance components for the four minerals and the total variance for the material on the sieve are shown in table 4 for the −80 +100 mesh material. Similarly, assuming that 10 percent of the same material exists in the same proportions in the −200 +230 mesh size and that the uranium contents are constant over all size ranges, the variances that may result from such material are shown in table 5.

The data in table 4 show that quartz, feldspar, and allanite, which account for 99 percent of the material on the sieve, contribute a negligible amount to the error term due to the grains only, whereas the zircon, only 1 percent of the total material, could contribute practically all of the error term. The square root of the variance, a standard deviation of 2.9×10^{-6}, is sufficiently large to effect the precision of the determination of uranium in the material. The summation of $\Pi_i U_i$ for the four minerals in table 4 gives a uranium content of the material on the sieve of 7.06×10^{-6}, and the standard deviation is almost half this amount.

The calculations for the −200 +230 mesh material result in a variance of 9.07×10^{-15} and a standard deviation of 9.5×10^{-8}. As the amount of uranium is the same as in the example above, the standard deviation is now sufficiently small that it will not materially affect the precision of the determination in this size interval.

TABLE 4. *Variance components in sieve interval, − 80 + 100 mesh*

Mineral	w_i(g)	U_i(g/g)	Π_i	Variance
Feldspar	1.002×10^{-5}	2.1×10^{-6}	0.0669	2.96×10^{-18}
Quartz	1.042×10^{-5}	2.3×10^{-6}	.0238	1.31×10^{-18}
Allanite	1.566×10^{-5}	5.4×10^{-4}	.00747	3.41×10^{-14}
Zircon	1.840×10^{-5}	1.6×10^{-3}	.00176	8.29×10^{-12}
			Variance (U) = Σ =	8.32×10^{-12}

TABLE 5. *Variance components in sieve interval, − 200 + 230 mesh*

Mineral	w_i(g)	U_i(g/g)	Π_i	Variance
Feldspar	6.9×10^{-7}	2.1×10^{-6}	0.0669	2.04×10^{-19}
Quartz	7.2×10^{-7}	2.3×10^{-6}	.0238	9.06×10^{-20}
Allanite	10.8×10^{-7}	5.4×10^{-4}	.00747	2.35×10^{-15}
Zircon	12.7×10^{-7}	1.6×10^{-3}	.00176	5.72×10^{-15}
			Variance (U) = Σ =	9.07×10^{-15}

If we assume that the sample has 10 percent in the mesh interval, −8 +100, and 90 percent in the interval, −200 +230, similar calculations yield data below, which show that the standard deviation due to the −80 +100 mesh portion will have a noticeable effect on the determination, whereas the possible error due to the −200 +230 material is almost negligible. The standard deviation calculated for the larger grains of the 10 percent of the sample in the −80 +100 mesh portion is 10 times greater than the error due to the small grains of the remaining 90 percent.

As the standard deviation due to particle size in the determination of a trace element decreases as particle size decreases and as number of particles increases, it is important that a sample for analysis be ground as finely as possible. Before a crushed rock is sampled, the analyst should ensure that the number of particles is so large that the relative error due to the number of grains in the portion for analysis will always be a magnitude or two less than other errors he may make in the analytical procedure. For samples prepared so that the grains are larger than this optimum size, errors that may be due to the number and sizes of the particles may be partly avoided by an analyst using wet-chemical methods if he increases his sample size by a factor of k and takes a $(1/k)$th aliquot after the sample is in solution.

Our knowledge of the trace-element contents of minerals in rocks is constantly being refined, and we will soon be able to use average trace-element contents to calculate possible errors, provided modal analyses from grain counting of thin sections of rocks are available.

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SAMPLING AND ANALYSIS OF CARBON CONTAINED IN THE PRIMARY COOLANT OF PRESSURIZED WATER REACTORS

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A portable high pressure in-line filtration device, developed by the Westinghouse Analytical Laboratories, was installed in a pressurized water reactor (PWR) primary coolant sample line and the suspended solids removed by filtration.

The filtration medium was a 47 mm diameter silver filter having a porosity of 0.45 μm. Collection times of 8 hours with an average flow rate of 2.66 liters per minute (42.1 gal h^{-1}) were made. The total volume of primary coolant processed per run was 1,275 liters and resulted in the collection of 3.3 milligrams of suspended solids; more commonly known as crud. This corresponded to a concentration of 2.6 $\mu g\ \ell^{-1}$ or 2.6 ppb in the coolant.

After sufficient crud has been collected, the pre-tared silver filter is removed from the high pressure housing, dried, and reweighed. Eleven millimeter diameter discs are cut from the filter and placed in a preconditioned quartz combustion system and heated to 1150 °C within 30 minutes. An additional hour at 1150 °C is required to ensure complete reaction of the crud sample with the oxidizing atmosphere. The quantitative liberation of carbon dioxide is collected in a trap cooled with liquid nitrogen and subsequently released and measured by gas chromatography. Provisions are made for blank determinations on an unused silver filter with appropriate corrections and aliquot size determined by gamma spectroscopy.

The method developed for this analysis is applicable in the range of 1 to 200 micrograms of carbon. The accuracy and precision of the sampling method and the analytical procedure are discussed.

Keywords: Analysis; carbon; crud; filtration; pressurized water reactors; primary coolant; sampling; water.

In an operating pressurized water reactor (PWR), the corrosion rates of the containment and core materials are influenced by the selection of corrosion resistant alloys and by the mode of chemical treatment of the coo-

lant. During power generation, the circulating coolant contains soluble and insoluble impurities originating from fission products, activated corrosion products, and the make-up water. These impurities are circulated through the coolant system and are either deposited on or absorbed by the fuel clading and the system piping. The deposition and absorption of these impurities leads to reduction of heat transfer on the former and increased radioactivity on the latter. Thus, to maintain sufficiently low corrosion and deposition rates and activity levels, chemical treatment of the coolant, filtration, and ion-exchange processes are employed within a nuclear plant.

To better understand the nature of the various corrosion and transfer mechanisms and therefore the control of their corresponding rates, chemical, physical, and radiochemical analyses of activated corrosion products have been performed for many years. The results of these investigations have led to changes in the chemical treatment and the operating conditions, thus producing low particulate levels in the circulating coolant—on the order of several parts per billion. Nevertheless, it remains desirable and important to examine various properties of these suspended solids, more commonly called crud, in the circulating reactor coolant.

In the past, the usual method employed for the collection of crud samples has been the withdrawal of 10 liters of the coolant followed by filtration in the laboratory. This procedure required many hours and, since typical crud levels were 3 to 15 μg/l, only 30 to 150 μg of material were collected. The activity of the coolant, approximately 7 μCi/ml, limited the total volume that could be removed for filtration, and normal practice required a decay time of up to 1 week prior to laboratory filtration. The small amount of material thus collected could be assayed radiochemically but was usually insufficient for further detailed analyses.

In order to collect milligram quantities of crud, thousands of liters of circulating coolant must be processed. To accomplish this objective, a portable in-line filtration device was constructed utilizing a commercially available Millipore high pressure filter housing (fig. 1). The portable filtration unit is capable of removing suspended solids from PWR primary coolants by processing up to 300 liters per hour at system conditions. Depending on location of installation, the unit operates at 2300 psi (1.586×10^7 N/m^2) and either 65 °C or 350 °C. The unit is constructed of such materials that decontamination of radioactive residues can be readily accomplished at the reactor site, allowing it to be used elsewhere. The unit also meets the weight and size restrictions of commercial airlines thereby permitting air travel to and from reactor sites as common baggage, thus saving transportation cost and time.

The advantages of the in-line collection system are 1) it minimizes

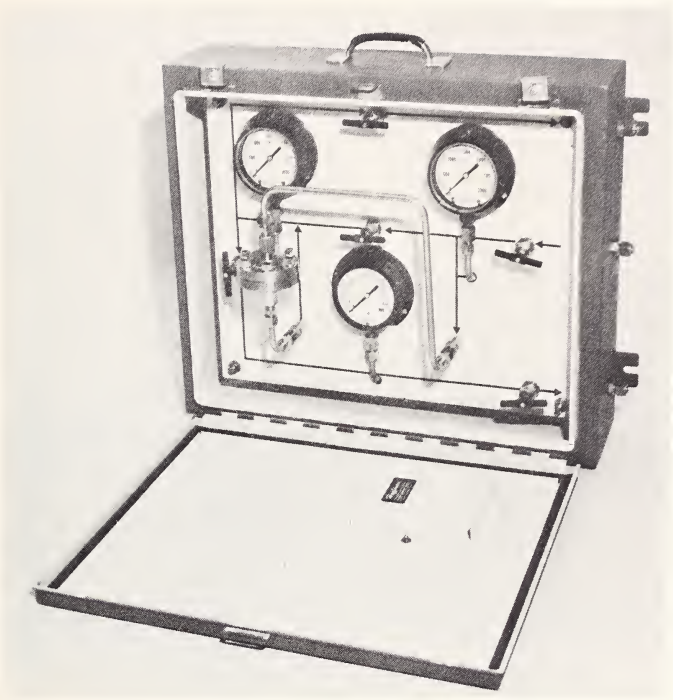

Figure 1. Photograph of the portable filtration unit used for collecting crud from circulating coolant.

laboratory handling of large radioactive volumes, 2) it requires less continuous monitoring than vacuum filtration, 3) it provides a more representative crud sample, and 4) it minimizes the chance of contamination of the sample. A schematic of the filtration system is shown in figure 2. With high safety integrity in mind, a commercial, high-pressure, filter housing was selected for installation in systems where inlet pressures are as high as 10,000 psi (6.8947×10^7 N/m^2) and the filtration medium, with appropriate support screens, can withstand a ΔP of 1,500 psi (1.034×10^7 N/m^2). The associated valving is rated at 6,500 psi (4.482×10^7 N/m^2). Normal operation of the filtration unit is downstream from the primary cooler and prior to the volume control tank where inlet pressures are 2,300 psi (1.586×10^7 N/m^2) but liquid temperatures are less than 95 °C. However, the filter unit can be used to remove suspended solids at 350 °C.

With the ability to collect large representative crud samples from various PWR's, interest in continuing surveillance of the chemical and physical condition of the primary coolant has prompted the need for sensitive

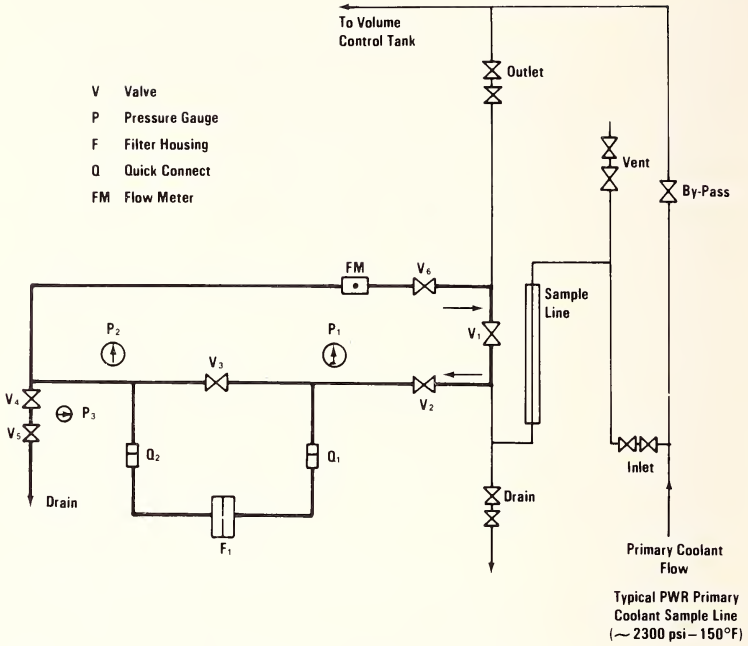

Figure 2. Schematic diagram of the filtration system.

analytical procedures. The measurement of the carbon contained within the circulating crud, obtained under various plant conditions, should help elucidate the origin of certain carbonaceous materials, such as metallic carbides or ion-exchange particulates.

The following method is based on the high-temperature oxidation of the total carbon contained within the suspended solids. The carbon dioxide formed is collected at liquid nitrogen temperature and subsequently released and measured utilizing gas chromatography. The analytical method is applicable in the range of 1 to 200 micrograms of carbon.

Basically, the in-line filtration unit is installed in the PWR primary coolant sampling line which is located in the nuclear sampling room. A silver filter having a porosity of 0.45 μm and a 47 mm diameter is used as the filtering medium. After sufficient crud has been collected, the pre-tared silver filter is removed from the high pressure housing, dried, reweighed, and a gross gamma count performed. Several 11-mm diameter discs are cut from the filter, gamma counted, and placed in a preconditioned quartz combustion system (fig. 3) and heated within 30 minutes to 1150 °C. An additional hour at 1150 °C is required to ensure complete reaction of the crud sample with the oxidizing atmosphere. The quantitative liberation of

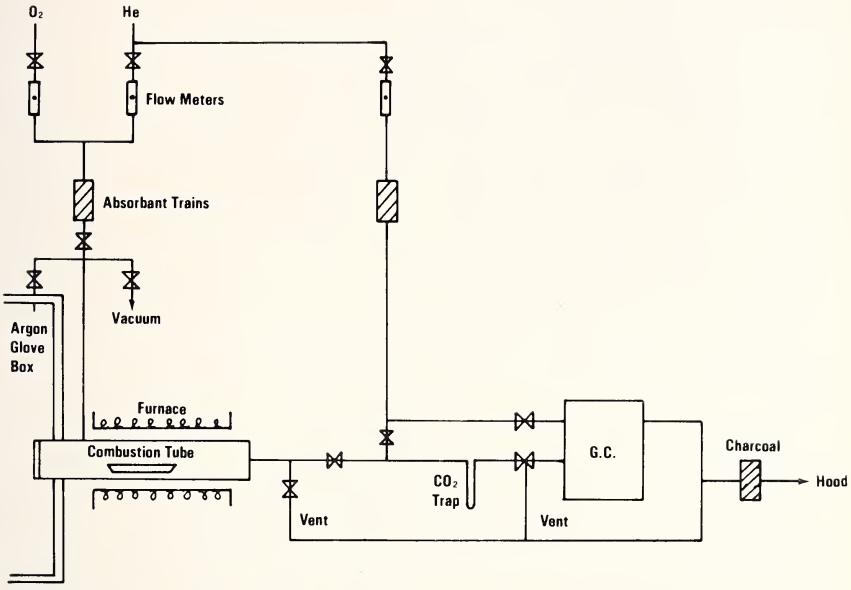

Figure 3. Schematic diagram of the combustion system used for converting carbon to carbon dioxide.

carbon dioxide is based on the thermal decomposition of carbonaceous materials contained within the crud. The carbon dioxide is collected in a trap cooled with liquid nitrogen and subsequently released and measured by gas chromatography. All gases released during the combustion period and the following analysis are passed through an activated charcoal trap and then vented into a hood equipped with absolute filters to ensure no release of volatile radioactive materials outside the controlled environment. The quartz combustion system contains a disposable quartz liner and boat and thereby minimizes the radioactive contamination of the overall system.

Prior to the actual analysis, a system blank is obtained; the critical assumption being that the blank will remain fairly constant during the ensuing analysis. The factors that contribute significantly to the blank are: 1) the initial purity of the helium and oxygen; 2) the effectiveness of the absorbants; 3) the effectiveness of the conditioning of the quartz components; 4) the integrity of the combustion system; and predominantly, 5) the physical handling of the quartz sleeve and its components undergo.

Since the stability and level of the system blank define the lower detection limit of the analytical procedure, it was necessary to evaluate the blank over a period of time. Each carbon value in table 1 represents the individual blank measurement obtained by analyzing 15 quartz sleeves and

TABLE 1. *Summary of system blank measurements for 15 runs performed over 5 days*

Run number	Carbon, μg	Run number	Carbon, μg
1	2.0	9	2.0
2	2.3	10	2.6
3	1.9	11	2.4
4	2.1	12	2.0
5	2.1	13	1.6
6	1.9	14	2.3
7	2.2	15	2.1
8	1.8	Overall average 2.1 ± 0.3 μgC	

boats over a 5-day period. Other factors were held constant so that the analytical technique of handling could be evaluated.

It was also determined that increasing the conditioning time of the quartz components to more than 4 hours did not appreciably reduce the system blank nor did repeating the residence time at system temperature (*i.e.*, a second analysis of the system blank without the quartz sleeve being removed from the system). It can be concluded that: 1) the carbon present in the system blank is the cumulative total of the overall system; 2) the blank is reproducible; and 3) the blank can be maintained at a low level with little day-to-day variation.

To ensure quantitative liberation of carbon dioxide, its cryogenic collection, and its subsequent release and chromatographic measurement, commercially available carbon standards were analyzed. Table 2 represents the carbon values obtained from three "Leco" standards containing 33 μg, 62 μg and 90 μg of carbon. The recovery values indicate quantitative release and measurement of these standards. Also, the measurement of two 90 μg carbon standards indicate that the system capacity is at least 180 μg of carbon. To assure satisfactory daily operation of the carbon analytical system, "Leco" standards are analyzed on a routine basis prior to carbon analysis of the crud samples.

Since the crud collected on the silver membrane is in such a state that physical removal cannot be quantitatively performed, total analysis of the membrane and crud is necessary. Thus, knowledge of the carbon content of the membrane is required. It should be noted that the homogeneity of the carbon impurity within each silver membrane had been assumed. Also, minimum variance of the carbon from membrane to membrane was required. The carbon values reported in table 3 were obtained from the measurement of each of three 11 mm diameter samples removed from four different silver membranes. (The silver membranes having a porosity

TABLE 2. *Carbon values and recoveries for three LECO standards*

	Total carbon recovered, μg	Net carbon recovered, μg[a]	Carbon recovery, %
33 μg C standards			
	33.9	31.9	96.7
	33.5	31.6	95.8
(32.1 ± 0.7 μg C)	34.6	32.8	99.4
	33.1	31.3	94.8
	35.0	32.7	99.1
62 μg C standards			
	64.7	61.8	99.6
(61.7 ± 0.8 μg C)	64.7	62.4	100.6
	63.0	30.9	98.2
90 μg C standards			
	91.8	89.8	99.8
(89.3 ± 1.6 μg C)	92.7	90.6	100.7
	89.6	87.6	97.3
	180.6[b]	178.5	99.2

[a] Values corrected for system blank.
[b] Two combined standards.

TABLE 3. *Measurements of carbon from three subsamples of each of four silver filters*

Filter and subsample number	Total Carbon measured, μg[a]	Subsample average, μg[a]
Ag—1a	10.5	
1b	9.0	9.6 ± 0.8
1c	9.4	
Ag—2a	9.3	
2b	7.5	8.8 ± 1.1
2c	9.5	
Ag—3a	6.9	8.5 ± 2.1
3b	7.7	
3c	10.8	
Ag—4a	6.9	
4b	7.1	7.5 ± 0.8
4c	8.4	
	Overall average 8.6 ± 1.4	

[a] Values include system blank of 2.1 ± 0.3 μg C

of 0.45 μm were obtained from Selas Flotronics, Inc., Spring House, Pennsylvania.) The micrograms of carbon reported represent the total amount of carbon present in an 11 mm sample and the system blank. Thus, approximately 9 μg of carbon represents the overall blank and is so applied to gross carbon values obtained from crud analysis. This overall blank raises the limit of reasonable detection of carbon to approximately 10 μg.

During the measurement of the carbon content of the silver membranes, it was observed that there was a 1 percent variance in weight between the 11 mm diameter subsamples (table 4). Based on a few milligrams expected crud deposition, this variance of weight (300 or 400 micrograms) would be of the same order of magnitude as that of the crud subsamples. Thus, weighing would lead to large errors in determining subsample size. To establish uniformity of deposition and the subsample weight, a gross gamma count of the entire crud sample was performed. The 11 millimeter subsamples were then gamma-counted under the same conditions and the ratio between the two measurements used to determine the fraction removed from the original sample. Table 5 represents the fraction of each subsample and the values reported are within 1 percent of the calculated surface area assuming uniform deposition.

With the establishment of the sampling and analytical procedures, crud was collected from the primary coolant of a PWR. The length of the collection period is dictated by the initial crud concentration and the level of radioactivity being deposited on the membrane and varies with each power plant. Also, the maximum flow rate through the filtration unit is dependent upon the minimum residence time of the primary coolant within containment. If the minimum residence time is exceeded then the activity of the nuclear sample room will be increased to unsafe levels due to the activity of the 7.5-second N^{16}. One hundred to three hundred liters per hour flow-rates have been established for various power plants with collection times of 3 to 16 hours. In this particular experiment, collection times of 8 hours with an average flow rate of 160 liters per hour were performed. The total volume of primary coolant processed per run was 1,275 liters and resulted in the collection of 3.30 milligrams of suspended solids. This corresponded to a crud concentration of 2.6 $\mu g\ \ell^{-1}$ or 2.6 ppb in the coolant.

The crud samples, confined within an appropriate container designed for the shipment of radioactive materials, were sent to the Westinghouse Analytical Laboratory. The crud sample collected on the silver membrane was vacuum dried, reweighed, and a gross gamma count performed. Three 11 mm subsamples were removed and gamma counted to determine the subsample size. Carbon analysis performed on each subsample

TABLE 4. *Variance in weight of subsamples of the silver membranes*

Filter number	Average subsample weight, mg[a]	Variance, ± mg
Ag—1	31.399	0.394
Ag—2	32.043	.340
Ag—3	32.358	.361
Ag—4	28.799	.407

[a] Average based on four measurements.

TABLE 5. *Measurement of subsamples by gamma spectroscopy*[a]

	Subsample		
	#1	#2	#3
$\frac{\text{Gross } \gamma \text{ count of subsample}}{\text{Gross } \gamma \text{ count of sample}} =$	0.103	0.101	0.108
$\frac{\text{Calculated subsample area}}{\text{Calculated sample area}} =$	0.1047		

[a] Integration of 2048 channels for 600 seconds

TABLE 6. *Results of the analysis of three separate subsamples from a single collection of crud*

Subsample number	Net carbon measured, μg[a]	Subsample weight, μg	Carbon concentration, %
1	54.2	340	15.9
2	53.1	333	16.0
3	55.2	356	15.5
		Overall average	15.8 ± 0.3

[a] Values corrected for average system and filter blank; 8.6 ± 1.4 μg C

resulted in the values reported in table 6. The precision of the three analyses is considered excellent relative to the overall sampling, handling, and analytical techniques. In this particular case, the carbon concentration in the PWR primary coolant sampled is on the order of 0.4 $\mu g\ \ell^{-1}$.

In summary, suspended solids, commonly called crud, have been collected from the primary coolant of a PWR using a portable in-line filtration unit and in an actual situation the crud level was determined to be 2.6 $\mu g\, \ell^{-1}$ of which the carbon concentration is 15.8 ± 0.3 percent which corresponds to a carbon level of about 400 ppt in the primary coolant.

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SAMPLING AND SAMPLE HANDLING FOR ACTIVATION ANALYSIS OF RIVER WATER

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A complete preanalysis scheme for determining trace elements in river and estuarine water by instrumental neutron activation analysis is described. The design, operation and evaluation of a new Teflon water sampler is included in the presentation of the preanalysis scheme. The evaluation of this water sampler consists of replicate sampling experiments and a comparison with a commercial sampling bottle (Van Dorn). The water sampler described allows for filtration of the sample as it is transferred from the sampler to a storage container. Lyophilization (freeze drying) is used as the preconcentration technique for the dissolved species, *i.e.*, liquid portion of the sample. Normalization of suspended particulate data to the element scandium is presented as a useful technique for locating man-made heavy metal input sources.

Keywords: Filtering water; river water; sampling water for trace elements; suspended particulates in water; Teflon water sampler; trace elements; trace elements in water; water.

I. Introduction

The current interest in sampling and sample handling for trace constituents is evidenced by the large attendance at this symposium. Previous speakers have discussed general problems associated with trace element sampling and sample handling so this point will not be belabored.

Sampling for trace elements is difficult in any matrix. However, if some component of the geochemical environment is to be investigated, the sam-

pling problems are exceedingly more difficult than for most synthetic materials, which may be homogeneous. In the environment, the system sampled is generally *not* homogeneous over the area of interest. The fluid systems, water and air, are in a state of continuous chemical and physical change, even during their sampling and storage. Therefore, obtaining and storing representative samples are not considered trivial problems.

In this work, an attempt was made to develop and evaluate a preanalysis scheme for trace elements in water. This includes the sampling protocol, as well as storage and handling techniques. If such a research technique can be evaluated thoroughly and quantitatively, it should be useful in studying less expensive and faster protocols which may be necessary for environmental surveillance work.

The preanalysis design presently used in this laboratory consists of an all Teflon, Teflon-coated metal, and nylon sampler to minimize contamination from construction materials. After sampling, immediate filtration is carried out in the field followed by fast freezing of the aqueous portion of the sample in liquid nitrogen. Upon return to the laboratory, samples are stored frozen and, prior to analysis by neutron activation, are preconcentrated by freeze drying.

When designing this scheme of sampling and sample handling it was felt that the total number of liquid sample transfers must be kept to a minimum to avoid unnecessary random sources of contamination. This goal was achieved by keeping the total number of sample transfers to one.

II. Procedure

The NBS water sampler is illustrated in figure 1. The sampler consists of a horizontal Teflon cylinder mounted on an "ice clamp action" type of frame, with flat Teflon end caps in place of what would be ice hooks. The frame, though made of metal (aluminum and stainless steel), has a baked-on Teflon coating. The Teflon cylinder has an interior thread on one end (fig. 2) to provide for attachment of a filtering mechanism, to be described below. A Teflon-coated rudder may be attached to the bottom of the cylinder to allow its proper orientation with the current flow. All parts of the sampler are assembled with nylon screws, bushings, washers, *etc.*

One of the unique features of the sampler is that it provides an option for sample filtration during the transfer to the storage container. A Teflon adaptor is threaded to mate the sampling cylinder with a polycarbonate filter holder. The components of this filtering mechanism are pictured in figure 3.

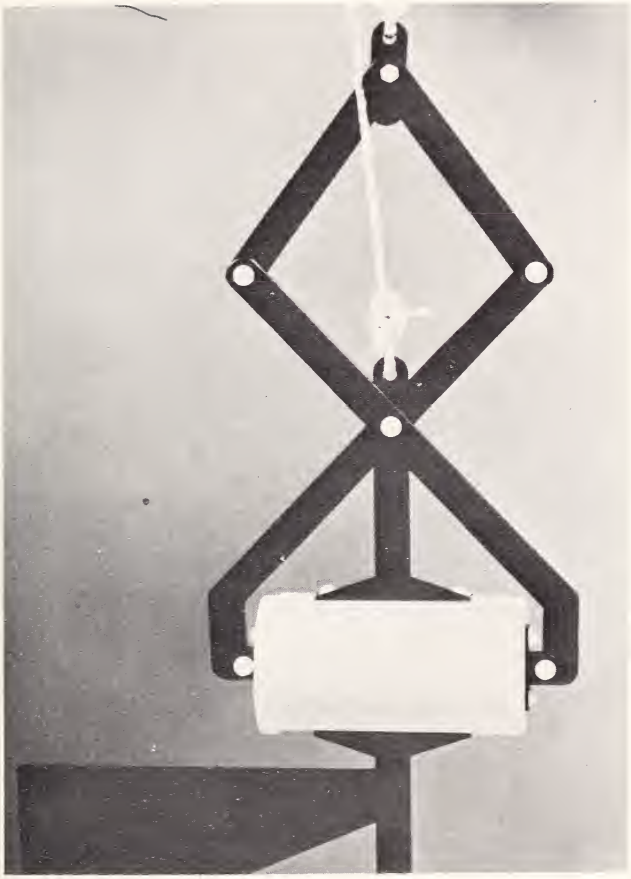


Figure 1. Teflon water sampler in vertical, closed position.

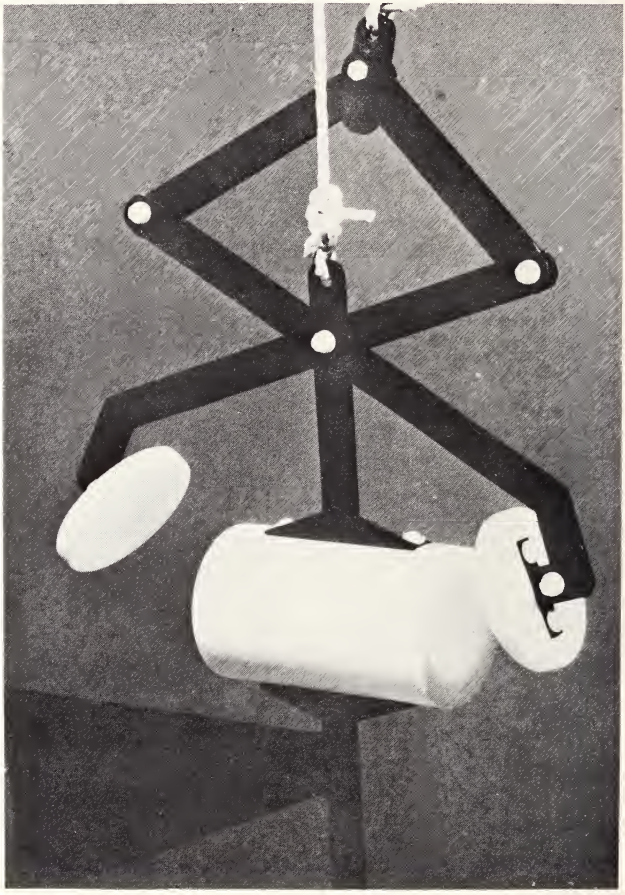


Figure 2. Teflon water sampler in vertical, partially open position, showing interior threads for attachment of filtering unit.

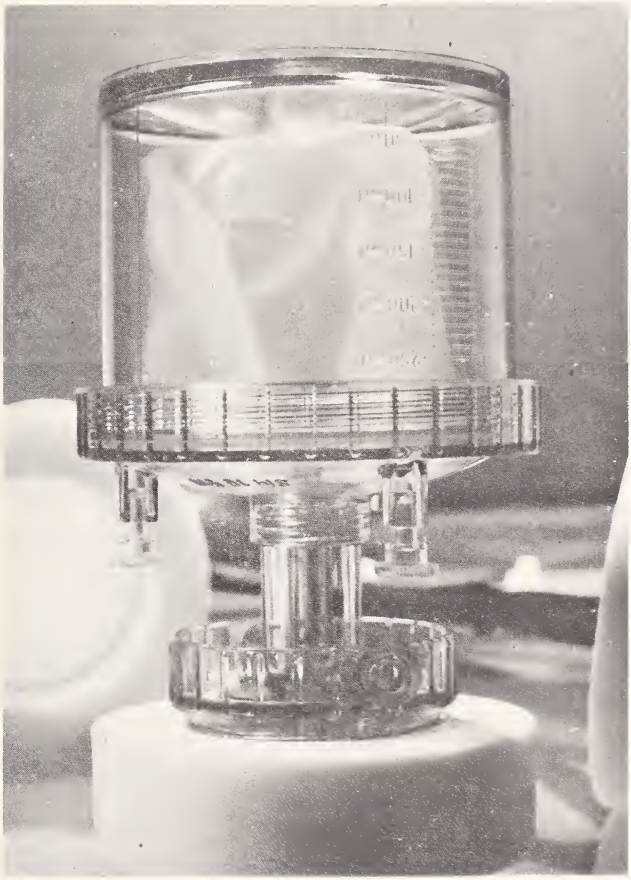


Figure 3. Attachment of polycarbonate filtering unit to filled water sampler through use of Teflon adaptor.

Generally, the filters used are a 47 mm diameter, 0.4 μm pore Nuclepore filter covered with a 47 mm diameter, 8 μm pore Nuclepore filter as a roughing filter. The filters are sealed between the Teflon adaptor and the filter support with a silicone O-ring. The receiver flask attached below the filter holds a polyethylene film bag. This bag serves as the sample container throughout the storage, freeze drying and analysis procedure. A hand pump is used to provide the vacuum for filtration in the field, *e.g.*, from a 14-ft rowboat. The complete sampling system assembled and ready for filtration is shown in figure 4.

One of the main advantages of the NBS water sampler is its capability of being lowered below the water surface in a closed configuration, avoiding contamination of the inner surface of the sampler with possible surface slicks or microlayers. After lowering the sampler to a depth of about 0.3 meters it may be opened by pulling a second rope.[1] The sampler is then lowered to the desired depth and allowed to equilibrate. The first rope is pulled to close the end caps and the water sample is brought back to the surface.

After attaching the filtering unit the sample may be filtered directly from the Teflon cylinder into the polyethylene storage bag. This is the only sample transfer in the entire procedure. The liquid sample contained in the polyethylene bag is frozen in the field in liquid nitrogen and stored in a cleaned plastic bag. The filters containing the suspended particulates are transferred to a cleaned plastic petri dish. Both the frozen liquid and filter portions of the sample are stored in dry ice until returning to the laboratory where they are transferred to a freezer.

The frozen liquid samples are prepared for analysis by preconcentrating using lyophilization (freeze drying). The technique for freeze drying has been described in detail elsewhere [1,2], however, a brief description may be appropriate here. The basic freeze drying unit, shown in figure 5, consists of a sample chamber, cold trap and source of vacuum. The frozen sample, still in the polyethylene film bag, is placed in the chamber which is then opened to the vacuum line. During the freeze drying process, water sublimes and a residue of solids accumulates at the bottom of the bag. After the process is finished the bag is folded up with the residue sealed inside, to make a small package for neutron irradiation.

Considerable work has been completed to evaluate the retention yields of trace elements during freeze drying. The results appear satisfactory for all elements investigated except for mercury and iodine (fig. 6). Recently, Filby, Shah and Funk [3] reported quantitative retention of mercury in a tracer study of the lyophilization of water.

[1]The NBS water sampler must be operated by two ropes, one supports the weight of the sampler while holding the end caps against the cylinder, the other supports this weight using it to pull the end caps up and away from the cylinder allowing for an uninterfered flow-through system.

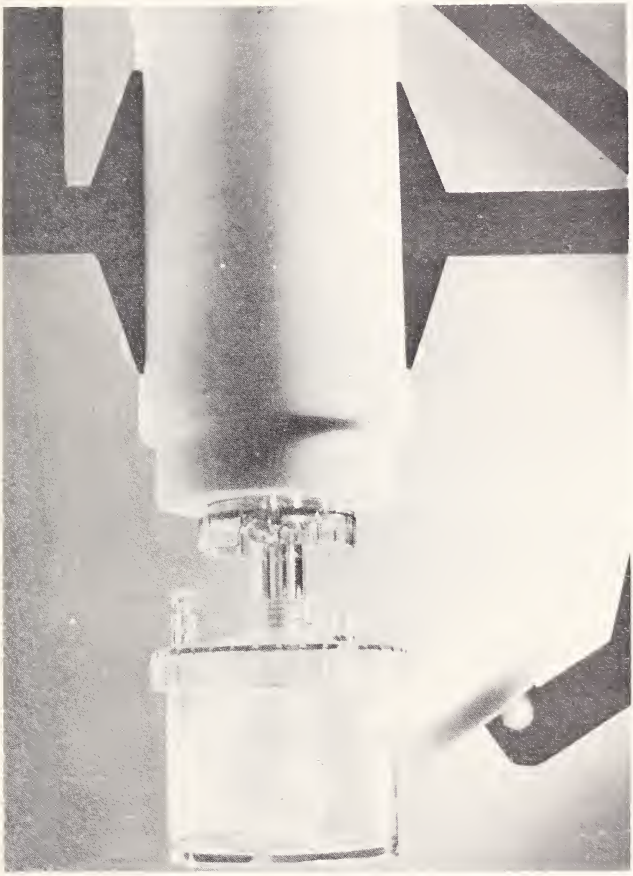
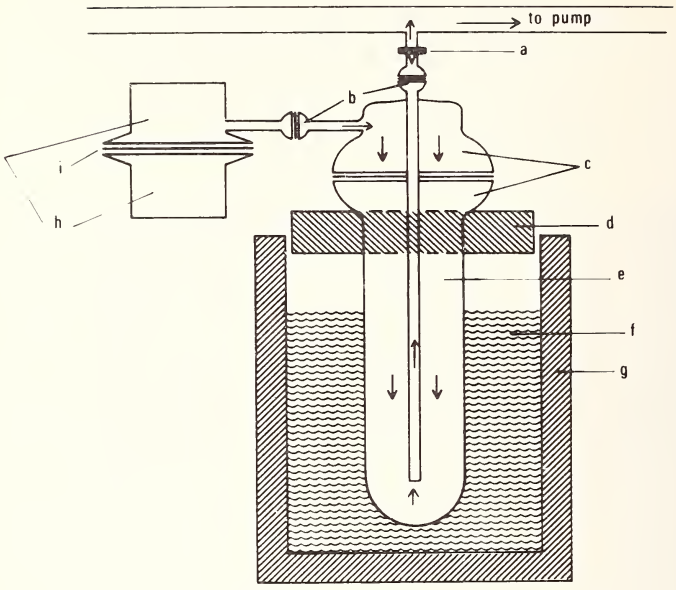


Figure 4. Teflon water sampler with filtering apparatus attached, ready for vacuum filtration into a clean polyethylene storage bag inside the filtering unit.



a teflon valve
b 15 mm o-ring joint
c 75 mm o-ring joint
d styrofoam flask lid
e cold trap
f liquid nitrogen
g stainless steel insulating flask
h freeze drying chamber
i gasket of polyethylene film

Figure 5. Schematic diagram showing one unit of the freeze drying system.

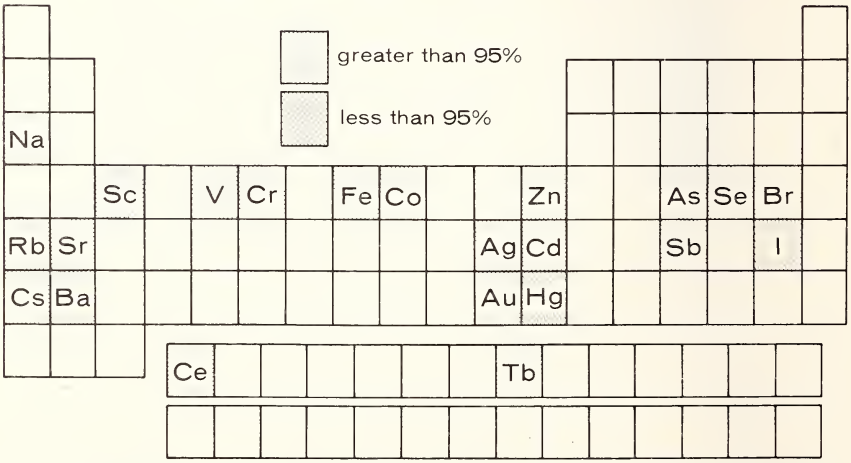


Figure 6. Retention yields of trace elements during freeze drying using radioactive tracers.

III. Results and Discussions

One of the main objectives of this work was to determine variability of data due to sampling. The approach used was to take replicate samples from a given location over as short a period of time as possible, usually 2 to 4 hours. It should be pointed out that in a field experiment it is difficult to distinguish between concentration variations due to time or position. In an estuarine or river system it is almost impossible to sample the same water mass over a 2- to 4-hour time interval.

The first station chosen for this kind of work was the Patuxent River at Laurel, Maryland. At this point the river is just a fresh water stream and it was necessary to wade to midcurrent to obtain the samples. Of course, bottom sediment is stirred up when wading but due to a rather swift current that day the disturbance created by wading subsided in several minutes and a sample could be taken upstream to minimize these effects.

At this location eight samples were taken. That is, eight separate wadings to midstream were made with processing of each sample on shore before the next one was initiated. Obviously, these replicate samples could not be considered to be taken from exactly the same body of water.

The results of instrumental neutron activation analysis (INAA) of the Laurel samples appear in tables 1 and 2. For the dissolved species, the coefficients of variation for the 13 elements determined in these "replicate samples" vary from a surprisingly low 3.0 percent for manganese to 80 percent for cobalt. A few outliers are obvious, cobalt in sample L-13 and chlorine in sample L-9. The high cobalt value in L-13 does not correlate with high values for scandium, iron, or thorium, elements which would be present in crustal particulate contamination. The low value for chlorine in sample L-9 is equally unexplainable. A low value of a halide in water might be explained by oxidation to the elemental form and volatilization during freeze drying. This idea is untenable since the bromide ion is more readily oxidized than chloride ion, and the value obtained for bromine in L-9 is just below one standard deviation from the average. Of these eight samples, originally weighing from 60 to 90 grams each, nine of the 13 elements determined exhibited coefficients of variation of 20 percent or less.

The results of INAA of the Laurel suspended particulates are shown in table 2. These samples were collected, freeze dried, irradiated and counted on polycarbonate film filters. Only the first six of the suspended particulate samples were analyzed for some of the elements which have long radioactive half-lives on irradiation. Technical difficulties prevented the analysis of the others. The elements measured in the suspended particulates have been normalized to scandium which is an element which

TABLE 1. *Concentration of dissolved species in Laurel samples*

Number	Elements												
	Ca (μg/g)	Na (μg/g)	Cl (μg/g)	Mn (μg/g)	Br (μg/g)	Mg (μg/g)	V (μg/g)	Sc (μg/g)	Fe (μg/g)	Co (μg/g)	La (μg/g)	Th (μg/g)	Sb (μg/g)
L–8	8.38	3.91	6.65	0.124	0.447	3.60	0.263	0.0251	174	0.907	0.214		0.0950
L–9	8.94	3.88	0.101	.119	.375	3.60	.358	.0326	239	1.03	.350		.0711
L–10	7.85	4.21	7.59	.115	.485	3.06	.507	.0415	215	.545	.303		.130
L–11	8.08	3.84	7.21	.113	.399	3.99	.274	.0272	173	.316	.260	0.0311	.0495
L–12	8.58	4.12	7.05	.116	.468	3.85	.215	.0293	171	.393	.305	.0421	.0703
L–13	8.46	4.31	6.82	.121	.465	4.28	.529	.0405	212	2.38	.348	.0408	.110
L–14	9.45	5.10	7.51	.117	.369	3.85	.615	.0676	232	.588	.224	.0370	.0879
L–15	8.61	4.28	7.69	.120	.560	4.03	.372	.0689	257	.514	.378	.0851	.0754
Average	8.54	4.21	6.33 7.21	.118 (Minus L–9)	.450	3.78	.392	.0416	209	.834	.298	.0472	.0861
Coefficient of Variation, %	5.7	9.7	40 5.5	3.9 (Minus L–9)	14	10	37	42	16	80	20	20	30

TABLE 2. *Concentration of suspended particulates in Laurel samples*

Number	Elements									
	Al (μg/g)	Mn (μg/g)	V (μg/g)	Cl (μg/g)	Na (μg/g)	Sc (μg/g)	Fe (μg/g)	Co (μg/g)	Th (μg/g)	Sb (μg/g)
L–8P	2.34	23.8	3.79	15.9	4.57	0.366	1.36	0.542	0.367	0.014
L–9P	2.20	20.9	2.80	18.3	6.17	.345	1.25	.552	.306	
L–10P	2.50	27.0	3.67	21.3	6.69	.405	1.38	.605	.354	.032
L–11P	2.55	23.9	2.76	14.7	10.8	.370	1.36	.534	.357	.016
L–12P	2.24	21.3	3.20	11.3	5.38	.359	1.27	.510	.409	.0093
L–13P		25.3		13.9	6.57	.360	1.25	.507	.392	.012
L–14P	2.30	24.6	3.53	14.7	7.95					
L–15P	2.66	25.0	3.64	30.2	8.61					
Average	2.40	24.0	3.34	17.5	7.09	.368	1.31	.542	.364	.017
CV[a], %	7.2	8.5	13	34	28	5.5	4.7	6.6	9.8	52
CV/[Sc][b], %	4.4	6.2	13	19	32		3.4	4.8	11	47

[a] Coefficient of variation.

[b] Coefficient of variation when the average elemental concentration is ratioed to the scandium concentration.

may be indicative of contributions due to clay particles and crustal weathering from natural sources. This is a procedure which has been used in transport studies of heavy metals in sediment [4]. The coefficient of variation is improved for nearly all elements when values are normalized to scandium, with only slight increases for the exceptions, thorium and sodium.

The fast moving fresh water stream at Laurel appears to be a well-mixed system and a single 50 to 100 gram sample under these conditions generally gives a relative standard deviation of 20 percent or less at concentrations down to the subnanogram/gram level for the elements analyzed.

A set of samples similar to those taken at Laurel were taken from the side of a boat anchored at the mouth of the Susquehanna River in the Chesapeake Bay (Turkey Point). In figure 7 are illustrated the results of the suspended particulates taken from this estuarine location. Again, for suspended particulates, the coefficient of variation for each element is significantly diminished (with the exception of manganese) when the data is normalized to scandium.

The importance of normalizing data obtained from suspended particulates using an element indicative of purely crustal weathering or natural sources is illustrated in figures 8, 9, and 10. In figure 8 is presented results for chromium in the suspended particulates of the Back River, just east of Baltimore, Maryland. A very large sewage treatment plant is located on the Back River. Station 1 is at the mouth of the river, which flows into the Chesapeake Bay. Station 6 is directly in the plume of the midstream effluent outfall of the sewage treatment plant. When the chromium concentration is plotted with respect to distance (as approximated by station number) from the effluent outfall a slight rise is observed as station 6 is approached. (Plotting the chromium data with respect to salinity instead of distance from effluent outfall gives an almost identical curve.) However, if the data is normalized to scandium a much more striking increase is seen as one approaches the plant effluent outfall. This indicated that there is an anthropogenic source of chromium in the particulate material coming from the sewage treatment plant or another upstream location.

Iron in the Back River exhibits a similar behavior and is shown in figure 9. However, in the case of iron no concentration gradient is observed in proceeding upstream from the mouth of the river. On the other hand, normalization against scandium shows a pronounced upstream gradient which apparently indicates a source of noncrustal iron upstream.

When the concentration of thorium and of thorium relative to scandium are plotted for the Back River suspended particulates (fig. 10), only a smoothing out of the data is obtained by normalizing to scandium, indicat-

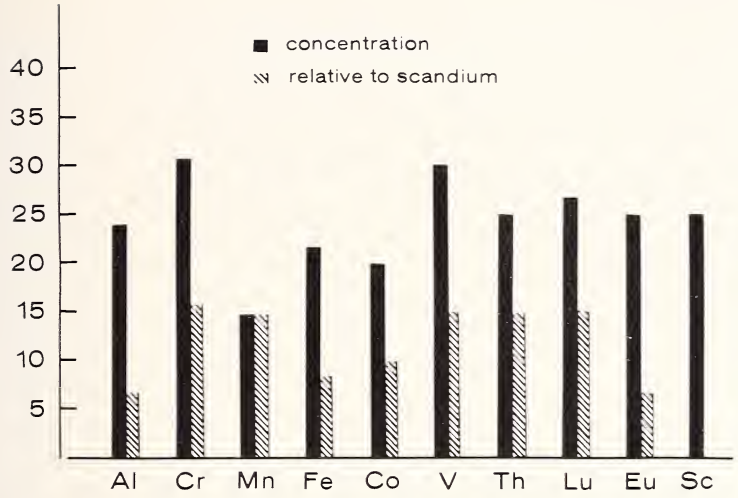


Figure 7. Coefficients of variation for trace elements from replicate samples of estuarine water, suspended particulates only.

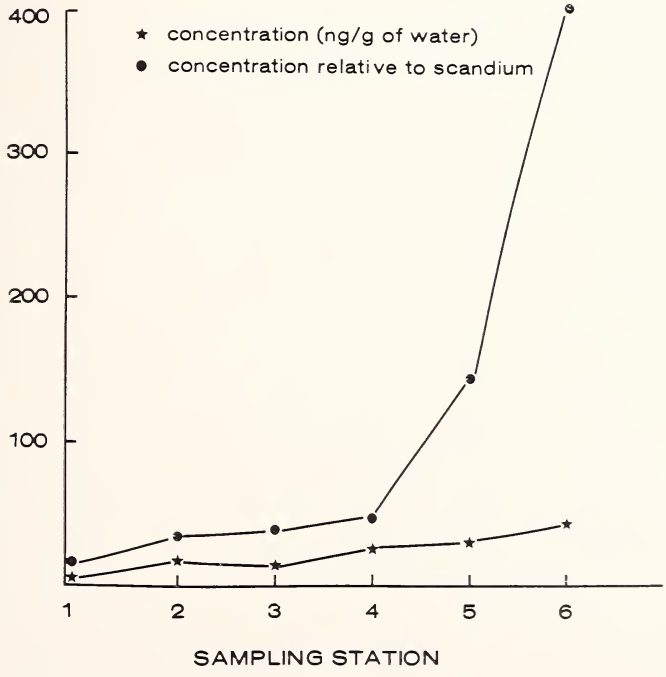


Figure 8. Chromium in back river suspended particulates.

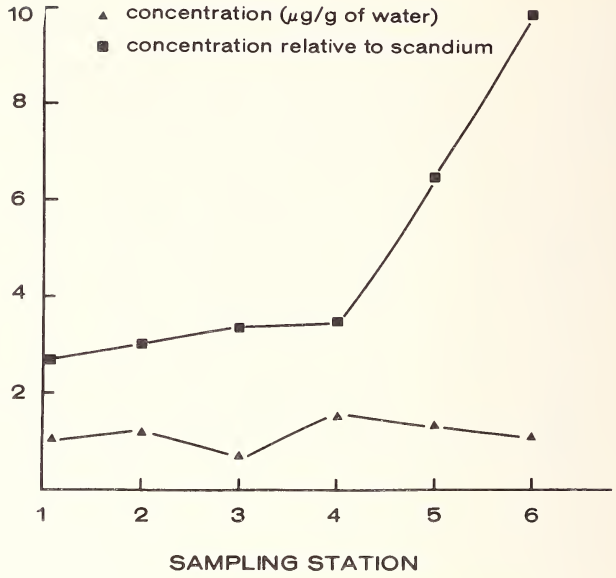


Figure 9. Iron in back river suspended particulates.

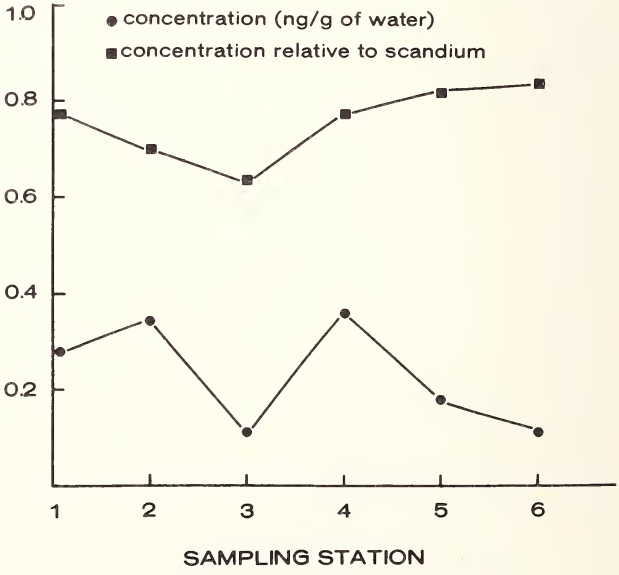


Figure 10. Thorium in back river suspended particulates.

ing no significant input of thorium from an anthropogenic source. It is felt that the added information obtained by this normalization technique should be useful in detecting sources of man-made heavy metal input.

In the Susquehanna River experiment, replicate samples taken with the NBS sampler were compared to samples taken with a Van Dorn commercial sampler. This particular Van Dorn sampler was part of the routine equipment on the research vessel used for this experiment and it is likely that the sampler has been used at least 3 days a week and up to 10 times a day for the past several years. The sampler was therefore expected to contaminate the samples. The results are found in table 3 in the chronological order in which they were taken. It has already been mentioned that it is very difficult to get a body of water to stay still during a replicate sampling experiment. This difficulty is demonstrated by noting the values for sodium in table 3. The sampling had begun at low tide, but the tide soon started coming in, leading to increasing salinity during the sampling period and possibly stirring up some of the bottom sediments.

The comparison of data for Van Dorn samples (VD1, VD2, VD3) to those for our Teflon sampler (NBS1, NBS2, NBS3, NBS4, NBS5) seems to indicate that the commercial sampler is contributing little if any contamination to the sample at the concentration levels found here. This may be explained in two ways: The sample from the Van Dorn was transferred immediately (<3 min) to the Teflon cylinder for filtration, leaving very little time for container-sample interactions. Alternatively, this particular Van Dorn sampler, which is made of PVC, rubber and surgical tubing, could have been used so often that all or most of the leachable contaminates had since been removed and it had reached an equilibrium with Chesapeake Bay water.

Other interesting features of the data in table 3 are the inconsistently high values for cobalt, iron and scandium in samples VD3 and NBS4. This could be suspended particulate contamination due to improper filtration or external contamination. These high values could also be real and caused by the disturbance of the sediments with the changing tide. In addition, the variability in samples from fresh water streams seems less than for samples from estuarine water, over a short timespan.

It is felt that the evaluation of the sampling of natural water using this system has just begun. Further work in evaluating the sampling of river and estuarine water is planned as well as initiating sampling studies in coastal ocean water.

TABLE 3. *Trace element concentrations in replicate samples of estuarine water*
(Dissolved Species Only)

Sample	Zn (μg/g)	Sr (μg/g)	Co (μg/g)	Fe (μg/g)	Se (μg/g)	Ag (μg/g)	Sc (μg/g)	Na (μg/g)
NBS[a]1	2.6	94	0.22	28	0.18	0.07	0.011	4.5
VD[b]1	8.9	108	.20	36	.14	.86	.008	6.6
NBS2	4.0	117	.27	44	.15	.39	.017	34
VD2	6.3	101	.24	38	.13	.27	.009	43
NBS3	3.9	121	.27	46	.22	.12	.024	44
VD3	7.1	121	.44	122	.14	.42	.034	45
NBS4	12	115	.67	266		.15	.28	48
NBS5	11	92	.26	44		.90	.024	36

[a] NBS = National Bureau of Standards samples.
[b] VD = Van Dorn samples.

IV. Acknowledgements

We are especially grateful to James Suddueth of the Analytical Chemistry Division at NBS for the design for this water sampler.

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HOMOGENEITY CONSIDERATIONS IN TRACE ANALYSES USING THE NUCLEAR TRACK TECHNIQUE

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Nuclear track results are compared to those obtained by other analytical methods using different quantities of homogeneous material.

Two different approaches are used to make the nuclear trace analysis of a sample more representative of the larger sample. Sample homogenization methods and detector manipulation are discussed.

Keywords: Detector techniques; nuclear track analysis; sample homogeneity.

Comparing nuclear track results to those obtained by other analytical methods using different quantities of material involves special considerations regarding homogeneity. The nuclear track technique utilizes the detection of charged particles from different nuclear reactions of specific isotopes (table 1) and the analyses are, in effect, on an atomic scale. The actual mass of the material analyzed is commonly less than 1 milligram and more typically about 1 to 10 micrograms. This quantity is defined by the total detector area counted and the range of the specific ionizing particle in the sample material involved. This range is highly dependent upon the energy of the ionizing particle and of the density of the sample material (table 2). For solids, particularly crystalline materials, certain elements are often concentrated in distinct phases that may have a scale larger than a single analysis.

There are basically two different approaches that can be used to make the nuclear track analysis of the sample more representative of a larger

TABLE 1. *Useful charged particles for nuclear track analysis*

Element determined	Isotope used	Incoming particle	Product isotope	Emitted charged particle
Lithium	^{6}Li	Thermal neutron	^{3}H	Alpha
Boron	^{10}B	Thermal neutron	^{7}Li	Alpha
Nitrogen	^{14}N	Thermal neutron	^{14}C	Proton
Oxygen	^{17}O	Thermal neutron	^{14}C	Alpha
Uranium	^{235}U	Thermal neutron	Fission products	Fission fragments
Uranium	^{238}U	Fast neutron	Fission products	Fission fragments
Thorium	^{232}Th	Fast neutron	Fission products	Fission fragments

TABLE 2. *Range of charged particles in materials of different densities*

Particle	Air	Particle range, cm	
		Aluminum	Uranium
Proton (0.51 MeV)	1.3	5.67×10^{-4}	3.07×10^{-4}
Alpha (1.79 MeV)	0.875	6.14×10^{-4}	3.11×10^{-4}
Fission fragment (96.6 MeV)	2.23	1.51×10^{-3}	6.04×10^{-4}

sample. One involves sample manipulation and the other involves detector (or track) manipulation.

Sample homogenization involves such actions as pulverizing, mixing and pelletizing, or melting and quenching. Detector manipulation is easier and avoids the possibility of introducing contamination or the loss of elements through volatilization. Spacing the detector some distance away from the sample utilizes the fact that the particles are released in random directions and are "mixed" before entering the detector. This has the additional advantage of averaging the depth parameter of the analysis. But perhaps the easiest way to improve homogeneity is to count random fields of view on the detector. This is valid as long as the elements of interest are not concentrated in small, sub-microscopic areas. Whichever method is selected, any standard should be prepared in the same manner.

In summary, then, the nuclear results are an expression of the homogeneity of the small amount of sample actually involved in the analysis. To realize the full value of the method, it is important to be aware of the amount of material involved, especially when making any comparison with results obtained by other analytical methods. An example of this is illustrated in table 3, where the sample size of the bovine liver is constant and the homogeneity of the nitrogen is reflected in the positions sampled. The average nitrogen value from the nuclear track technique and the Kjeldahl method is an indication of the nitrogen homogeneity based on different sample sizes used in the two analyses.

TABLE 3. *Nitrogen in bovine liver*

Sample position	Track density ($\times 10^{-5}$)[a,b] (tracks/cm^2)	Nitrogen found (%)
3	6.34 ± 0.65	10.39 ± 1.11
5	6.57 ± .68	10.77 ± 1.11
12	6.73 ± .72	11.03 ± 1.17
13	6.63 ± .69	10.89 ± 1.13
20	6.62 ± .80	10.84 ± 1.31
23	6.74 ± .86	11.00 ± 1.11
Average	6.61 ± .14	10.81 ± 0.24
Kjeldahl		10.59 ± .04

[a] Field of view, 2.83×10^{-5} cm^2.
[b] Number of random fields counted, 50.

Part III. SAMPLE HANDLING

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PROBLEMS IN SAMPLE TREATMENT IN TRACE ANALYSIS

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One of the most important—and most neglected—parts of any analytical procedure is the initial decomposition of the sample. Not only must the sample matrix be dissolved but the element sought must be converted reliably to the proper ionic form before chemical reactions characteristic of that element can be obtained. Complete dissolution is especially difficult with the quadrivalent and pentavalent elements which form very refractory oxides, carbides, *etc.*, particularly after having been heated. For example, the plutonium in the ash remaining from dry ashing large samples of soft tissue requires much more powerful treatment for its complete conversion to the ionic state than merely boiling with nitric acid. Fusion with molten potassium fluoride is one of the simplest and most effective methods for dissolving metallic as well as siliceous refractories because of the stability of the fluoride complexes and the high reaction rates available at its melting point of 846 °C. Inclusion of a small quantity of potassium nitrate even permits rapid and complete dissolution of refractory carbides such as Carborundum. The cake can then be transposed either with sulfuric acid to a pyrosulfate fusion with simultaneous volatilization of both hydrogen fluoride and silicon tetrafluoride, or with nitric and boric acids to give a solution free of sulfate. The procedure is neither difficult nor time-consuming as is generally supposed.

Extremely serious problems can be introduced by evaporating solutions of quadrivalent elements to dryness or by neutralizing them to too high a pH. Powerful sequestering agents can be produced from orthophosphates in the sample that will completely change the course of the analysis unless recognized and provided for. Standardizations are frequently carried out in a careless and inaccurate manner. Contamination is one of the greatest problems in trace analysis and needs to be avoided by good management and understanding of the causes. Preservation of both samples and standards can be vastly improved by application of a little more fundamental chemistry. Many other important "details" will be discussed which are frequently ignored or overlooked because of preoccupation with the more glamorous facets of the procedure, such as elegant separations or measurement techniques employed.

Keywords: Ashing (wet and dry); chemical errors; contamination; dissolution techniques; fusion dissolution; radiochemical analyses.

I. Introduction

An analytical procedure can be—and generally is—a very complicated combination of many individual unit operations. The number of different chemical reactions—both intended and inadvertent—that can occur from the time the sample is collected until the final measurement is made is enormous. While the analyst has his attention and interest focused on the unique separation or the exotic reagent or instrumental technique to be employed, millions of little chemical gremlins are busily at work, undermining the intended structure of the determination and, indeed, casting the deciding vote on its outcome. It seems fairly safe to say that there is probably more art involved in understanding and controlling the many secondary influences working against the desired course of events than in the primary chemical reactions desired. Chemical problems seem at times to increase almost exponentially with the charge on the metallic cation. While most monovalent and divalent elements are relatively free of problems due to solubility, hydrolysis, ion exchange, sorption, *etc.*, the tervalent and particularly quadrivalent and pentavalent elements require unrelenting care and attention to detail to achieve high and reproducible recoveries and accurate results. This paper will discuss a few of the basic chemical problems affecting the higher valent elements particularly, with some suggestions for their elimination or control.

II. Sample Preservation

A. Samples

It is self-evident that the best analysis cannot possibly be any better than the sample on which the determination is made. Certainly, the sample must be representative of the population from which it was taken, and to which it must relate in some known fashion. However, even the small analytical sample received in the laboratory must be treated properly or erroneous results will be obtained. One of the biggest questions involves acidification and/or filtration of aqueous samples before removing an aliquot for analysis. Some organizations officially require aqueous sam-

ples to be filtered as received, and the analysis performed on the filtrate. This philosophy will certainly obscure the real contribution of radioactive and chemically toxic elements on environmental pollution.

The great majority of natural waters has a pH between 6 and 8, including seawater, lakes and rivers, with some rivers and ponds being much more alkaline yet. It is well known that most metallic elements except the alkalis and the alkaline earths form extremely insoluble hydroxides, and in fact most tervalent and quadrivalent elements can be removed quantitatively from solution at pH's above 6, particularly if a little carrier is present. Consequently, filtration of natural water samples as received before analysis will certainly make pollution of the aquatic environment appear to be much less severe than it really is. In some tests on river water containing liquid effluents from mills processing uranium ore, nearly 95 percent of the thorium-230 but only about 25 percent of the radium-226 were found in the residue filtered from an unacidified sample. If the distribution of activity between the filterable and nonfilterable components is desired, filtration of samples as received can be done, but certainly both fractions should be analyzed. Unfortunately, in many cases known to the present author, the residues are discarded because of some very superficial and tenuous argument that the only interest was the content "in" the solution, meaning soluble or nonfilterable, rather than "in" the solution, meaning within the bounds of the container in whatever form. From the standpoint of evaluating environmental pollution, and excepting special interests, water samples should be acidified to redissolve as much hydrolyzed material as possible, including that deposited on the walls of the container, and then the sample shaken vigorously immediately before removing an aliquot for analysis to include a representative portion of the undissolved floc or sediment in the analysis.

Unless the residue is analyzed separately, water samples should not be filtered with or without acidification to avoid severe losses of components that should be included in the analysis. Many elements are known to sorb strongly on silica, cellulose fiber, *etc.*, at pH's above about 4 and some of which, like protactinium, do so even from strongly acid solutions. Barium occurs widely in the environment, and barium sulfate is very insoluble in acid and will carry down most of the large tervalent and quadrivalent ions quantitatively under certain conditions [1]. One of the most severe and surprising problems was encountered in the analysis of aqueous samples of radiological wastes from operating nuclear reactors [2]. Even from 10 percent nitric or hydrochloric acid solutions, and with vigorous shaking immediately before pouring the solution from the bottle, most of the activity remained inside the empty bottle from which it was removed only

very slowly and incompletely by further vigorous treatment with concentrated acids, including hydrofluoric, prolonged vigorous shaking, ultrasonic vibration, *etc.* Zirconium is well known for its tendency to hydrolyze even from fairly strong acid and deposit on the walls of the container. However, containers in which zirconium has deposited hydrolytically can be cleaned simply and rapidly by a brief wash with hydrofluoric acid. The most surprising aspect was the fact that a large part of the activity was manganese-54, cobalt-58 and -60, iron-59, *etc.*, which are not particularly hydrolytic and would certainly not be expected to be hydrolyzed or long remain insoluble in such strong acids. Autoradiography showed that the activity occurred in very finely-divided discrete particles with no evidence of the large aggregated patterns to be expected from flocculant, hydrolytic precipitates. Apparently, these are particles of the original fuel elements or cladding materials of zirconium, stainless steel, ceramics, *etc.* that never were dissolved but are in their original form. This view is substantiated by the fact that addition of paper pulp is of material assistance in keeping the particles off the container surface, so that the particles can be removed with the suspension of paper fibers, particularly from polyethylene bottles. A more difficult but completely unequivocal solution to the problem is to insert a small polyethylene bag in the sample container as a removable liner, and wet-ash the entire bag.

B. Standard Solutions

Undoubtedly, a large number of analytical problems and inaccurate results are caused by improperly prepared standard solutions on which the entire determination depends. Most solutions can be stabilized adequately by simply adding sufficient acid to prevent incipient hydrolysis. An acidity larger than about 0.01*N* will suffice for most monovalent and divalent ions. Most of the work reported in the literature shows that such solutions remain fairly stable over long periods of time until the pH is allowed to go higher than 2. In some cases, such as with silver, mercury and polonium, acid alone does not produce stable solutions, probably caused by reduction of the relatively noble elements to the metallic state or to a volatile lower state, either spontaneously or due to traces of reducing agents present. Addition of a suitable oxidant such as dichromate for silver and mercury [3], or quadrivalent cerium or bromate for polonium [4] produces solutions that are stable over long periods of time.

With the higher valent elements, progressively higher concentrations of acid and/or addition of complexing agents are required to produce adequately stable solutions. In addition, both the order of addition of

water and acid and the exact manner in which they are combined can make the difference between a homogeneous stable solution and an inhomogeneous erratic one. For example, if 250 mg of Nb_2O_5 is dissolved in a pyrosulfate fusion, the cake then redissolved in concentrated sulfuric acid and diluted to 250 ml with water, a slightly turbid solution will usually result depending on the quantity of acid used, that becomes increasingly turbid on standing. In contrast, if the sulfuric acid solution is diluted with dilute sulfuric acid instead of water, a completely clear solution is obtained that does not develop turbidity with time. One such solution prepared in this laboratory is still perfectly clear after standing for 11 years. Obviously, pure water has a hydroxyl ion concentration a million times higher than even 0.1*N* sulfuric acid, and is high enough to cause significant hydrolysis of the most hydrolytic elements.

The effect of manner of addition of reagents is exemplified by tin. If several milligrams of quadrivalent tin is fumed strongly with sulfuric acid, and the resulting solution is cooled to room temperature and diluted in an unparticular manner with water, the solution will exhibit a beautiful golden-yellow fluorescence when examined under short-wave ultraviolet light in a darkened room. The fluorescence becomes more intense with passage of time. If the fluorescent solution is reevaporated to fumes of sulfuric acid, and the cold solution is treated with individual drops of water with vigorous swirling and cooling between drops, so that the solution never warms up very much for very long, a completely nonfluorescent solution is obtained which develops the identical golden-yellow fluorescence on prolonged standing if cold, or more rapidly at higher temperatures. The transition apparently corresponds to the formation of a fluorescent hydrolytic polymer from the simpler sulfonated stannic ion.

Many elements require both high acidity and complexing agents to produce stable aqueous solutions. In particular, the sulfate ion is an excellent complexing agent in acid solutions that is yet mild enough to be relatively easily overridden by many extractants, *etc.* when necessary, and has not received the attention and use that it deserves. For example, protactinium has a reputation as being one of the most hydrolytic and capricious elements in the periodic table. The reputation is well-deserved because most investigators studiously avoid use of sulfate systems, preferring the extractions that are available from chloride or nitrate systems, and suffer the consequent hydrolytic depositions that inevitably occur with protactinium in such systems. In contrast, protactinium in a sodium sulfate-sulfuric acid solution will retain the same chemical characteristics for at least several years, showing not the slightest suggestion of the hydrolytic or capricious behavior so noticeable in other systems, due to the combination of high acidity and sulfate complexing [5,6]. Yet, the

sulfate complex can be easily converted to a chloride by addition of an equal volume of concentrated hydrochloric acid, and the protactinium can then be extracted quantitatively into disobutylcarbinol.

III. Sample Decomposition

One of the most important—and most neglected—parts of any analytical procedure is the initial decomposition of the sample. Not only must the sample matrix be dissolved, but the element sought must be converted reliably to the proper ionic form before chemical reactions characteristic of that element can be obtained. Complete dissolution is particularly difficult with the quadrivalent and pentavalent elements which form extremely refractory oxides, carbides, *etc.*, particularly after having been heated strongly. Many investigators attempt to leach the elements selectively from the sample matrix with various acids with varying degrees of success. The divalent and tervalent elements not bound up in acid-insoluble particles can frequently be leached fairly successfully; the higher valent elements more generally are not leachable, at least not quantitatively. If silica or some of the refractory silicates are involved, recovery of even the lower valent elements will be markedly incomplete unless hydrofluoric acid is employed. Consequently, methods based on such procedures are generally erratic and incapable of the highest accuracy and precision.

A. Alkaline Fusions

Although fusion with sodium peroxide is undoubtedly one of the most powerful methods available for dissolution of a wide variety of sample types, it has many disadvantages. It is a relatively dirty flux, and its attack on the fusion container is so severe, whether of silica, iron, nickel, zirconium, *etc.*, that large additional quantities of impurities are added to the sample. When applied to large samples of siliceous materials, the silica is solubilized but requires a great deal of effort subsequently to eliminate satisfactorily. Sodium carbonate fusions are relatively much cleaner and can be carried out in platinum. However, the same objection about disposition of the soluble silicate is present, and the fusion itself is relatively mild, leaving some considerable uncertainty as to the completeness of dissolution of refractory compounds.

B. PYROSULFATE FUSION

Fusion with an alkali pyrosulfate is undoubtedly one of the most powerful, clean and convenient methods available for the dissolution of nonsiliceous refractory compounds. The flux can be prepared simply by heating sodium or potassium sulfate with an equal molar quantity of concentrated sulfuric acid. This is particularly convenient when concentrated sulfuric acid remains from wet oxidation of organic compounds or elimination of other volatile acids. The flux is virtually a molten acid that can be used at temperatures up to nearly 800 °C. The melting points of the pure potassium and sodium salts are about 300 °C and 400 °C, respectively. However, on heating, the pyrosulfate is decomposed with loss of sulfur trioxide, and the melting point of the mixture of sulfate and pyrosulfate increases progressively. The high acid concentration, the high temperature available for reaction, the high concentration of sulfate ion as an inorganic complexing agent for metals, and the relatively high solubility of metallic sulfates at the high temperature used are all partly responsible for its efficiency in dissolving metallic oxides.

The flux is also a relatively powerful oxidizing agent. The last traces of very resistant organic matter are oxidized more rapidly and completely in a pyrosulfate fusion than by treatment with perchloric acid. Quadrivalent cerium is thermally decomposed in boiling sulfuric acid containing sodium sulfate to the tervalent state. However, after the excess sulfuric acid has been driven off and a pyrosulfate fusion obtained, the cerium is reoxidized to the quadrivalent state nearly halfway to completion, the oxidation undoubtedly being materially assisted by the strong complexing action of the high concentration of sulfate on the quadrivalent ion. Manganese is also oxidized to permanganate to the extent of only a few tenths percent, but even this small quantity is highly effective in producing oxidation of traces of other elements. For example, hexavalent plutonium is normally reduced quantitatively to the quadrivalent state in a pyrosulfate fusion due to thermal decomposition in a high sulfate system. In the presence of a few milligrams of either cerium or manganese, nearly half of the quadrivalent plutonium is reoxidized to the hexavalent state, apparently due to the oxidizing action of the oxidized forms of the two elements produced in the fusion. These examples show the complete reversal of chemical reactions that can be produced inadvertently by the presence of certain elements in the sample.

Although fusion with an alkali pyrosulfate has been widely used for many years, there are only scattered references to the consequences resulting from the choice of alkali metal used or to the effects of sulfate ion, both during the fusion and afterwards. The sodium salt is generally

recommended because "This salt melts more quickly and forms more soluble double salts with aluminum and some other metals" [7]. However, potassium is frequently present in the samples being analyzed, or its addition in other parts of the procedure might be necessary as in the potassium fluoride fusion in which the potassium salt has much greater solvent capacity and is generally much more effective than the other alkali fluorides. In some cases, the greater insolubility of the potassium salts can be exploited to the analysts' advantage. Consequently, some of the insoluble compounds need to be identified specifically, and some effects of sulfate ion described.

Most heavy metal sulfates except those of the alkali metals are extremely insoluble in concentrated sulfuric acid but become much more soluble in the presence of alkali metal sulfates, undoubtedly due to the formation of anionic sulfate complexes. For example, as little as 1 mg of tervalent iron, aluminum, or chromium is precipitated virtually quantitatively by fuming with 5 ml of concentrated sulfuric acid but remains completely in solution when 1 gram of sodium sulfate is present. The anhydrous sulfates of tervalent iron, aluminum, chromium, and quadrivalent vanadium are extremely difficult to redissolve in water or dilute acid even on prolonged boiling, probably due to the slowness of rehydration. The rapid dissolution of titanium dioxide in hot concentrated sulfuric acid containing ammonium sulfate is well known. Perhaps the most graphic demonstration of the effect of sulfate is provided by zirconium. If powdered zirconium metal is heated with concentrated sulfuric acid, evolution of hydrogen bubbles begins almost immediately, and the metal begins to dissolve. However, a white precipitate of zirconium sulfate soon forms, coating the unreacted metal, the evolution of hydrogen ceases, and the dissolution stops. If a few grams of sodium sulfate is added, the white precipitate clears up rapidly, evolution of hydrogen gas resumes, and the dissolution of the metallic zirconium proceeds to completion. Apparently, the sodium sulfate converts the insoluble zirconium sulfate to a sulfatozirconic acid, which is soluble in concentrated sulfuric acid, and dissolution of the uncovered metal proceeds. Heating with hot concentrated sulfuric acid containing sodium sulfate is a much simpler and convenient method of dissolving metallic zirconium than the more common one involving hydrofluoric acid.

A similar but much more subtle effect of sulfate and alkali metal ions is present during the fusion. If 50 mg of iron, aluminum or chromium is fused with 3 grams of anhydrous sodium sulfate and 2 ml of concentrated sulfuric acid, all three metal sulfates dissolve subsequently in water almost as fast as the cake itself disintegrates, giving completely clear solutions. In contrast, if the fusion is made with the potassium salt, the metal-

lic salts do not dissolve subsequently in dilute sulfuric or hydrochloric acids on prolonged boiling, even though the fusion itself was completely clear. Prolonged boiling with strong hydrochloric acid dissolves most, but frequently not all, of the insoluble anhydrous sulfates. Chromium is the most insoluble of the three elements, giving a very pale greenish white precipitate with very little discernible green color of the chromic ion in solution. However, if the fusion with the potassium salt is heated as hot as possible over the maximum heat from a blast burner for 15 or 20 minutes after the melt has cleared, the anhydrous sulfates will again dissolve in water almost as fast as the cake disintegrates. Apparently the prolonged high-temperature treatment decomposes much of the pyrosulfate to sulfate ion with loss of sulfur trioxide which increases formation of sulfate complexes that are more rapidly hydrated and solubilized during subsequent treatment with water. The same effect can be produced much more rapidly and conveniently by adding 1 or 2 grams of anhydrous sodium sulfate after most of the excess sulfuric acid has been driven off and the fusion has cleared. When presence of potassium is desirable or unavoidable, its insolubilizing effect can be largely overcome by adding two parts of sodium sulfate for each part of potassium sulfate present. For example, even 50 mg of chromium will dissolve in water giving a deep green solution almost as fast as the cake disintegrates if the fusion is made with a mixture of 6 grams sodium sulfate to 3 grams of potassium sulfate and the appropriate quantity of sulfuric acid. The exact cause of the differences caused by sodium or potassium is not clear. However, it is clear that a high concentration of sulfate ion is necessary to keep the metals present as water-soluble sulfato complexes and prevent formation of the very insoluble anhydrous sulfates.

The ability of sulfate ions to form anionic complexes with many metals that are soluble in hot concentrated sulfuric acid is of real practical importance in the complete dissolution of large samples of soil [8]. In samples containing large quantities of aluminum and iron, decomposition of the potassium fluoride cake with concentrated sulfuric acid causes precipitation of iron or aluminum sulfates in a form that retains sulfuric acid tenaciously. The resultant slurry is virtually impossible to transpose to a pyrosulfate fusion because it is not possible to heat the thick mud hot enough to volatilize sulfuric acid without causing prohibitive spattering. However, if sodium sulfate is added, the thick intractable mud changes rapidly to a thin fluid consistency, which permits the excess sulfuric acid to be volatilized rapidly and smoothly without significant spattering until the pyrosulfate fusion is obtained [8]. The quantity of sodium sulfate required clearly relates to the quantity of excess sulfuric acid present, and

the effect is reversible. For example, a 10-gram sample of soil fused with 30 grams of potassium fluoride can be transposed smoothly with 30 ml of concentrated sulfuric acid and 10 grams of anhydrous sodium sulfate in the presence of an average quantity of iron and aluminum. If an additional 5 ml of sulfuric acid is added, the thin fluid slurry will be reconverted to the thick intractable mud essentially identical to that produced without addition of sodium sulfate. An additional 10 grams of sodium sulfate will neutralize the additional sulfuric acid and restore the thin fluid characteristics.

Other differences resulting from the use of sodium or potassium salts are encountered after the pyrosulfate fusion cake is dissolved in water. The incompatibility of perchlorate ions in a system containing potassium is well known. Similarly, the potassium salts of the anionic sulfate complexes formed with most quadrivalent elements, particularly thorium, titanium, tin, *etc.*, are much less soluble in dilute sulfuric acid than the corresponding sodium salts or the free acids, and many more solubility problems result when potassium is present. For example, not more than 5 or 10 mg of quadrivalent titanium will remain dissolved in 30 ml of solution containing 5 grams of potassium acid sulfate when the solution is cooled [1]. Similar solutions containing only sodium salts remain clear with several times as much titanium.

Apparently, double potassium-barium salts of much decreased solubility are formed in the presence of barium because the ability of barium sulfate to carry all the large tervalent and quadrivalent ions from lead to at least californium is markedly enhanced by the presence of potassium. In fact, even aluminum and tervalent iron in high concentrations are partially precipitated with lead or barium sulfates in the presence of potassium. Also, the solubility of potassium sulfate itself in solutions of low acidity is much less than that of sodium sulfate. Consequently, use of potassium salts should be avoided unless their use is specifically indicated. In this case, special precautions must be observed to keep the quantity of the quadrivalent ions forming insoluble salts with potassium sulfate below the permissible limit.

C. Potassium Fluoride Fusion

Fusion with anhydrous potassium fluoride is probably the simplest, most effective and reliable method available for the complete dissolution of a wide variety of siliceous materials. The fusion can be carried out in platinum vessels with little or no attack on the container, keeping contamination with foreign materials at a minimum. The high melting point of

846 °C and the high concentration of fluoride ion, which forms some of the most stable inorganic complexes with many heavy metals, both contribute to high rates of chemical attack. Consequently, fusion with potassium fluoride is not only effective in dissolving silica and refractory silicates but most metallic oxides as well. In this respect, it is distinctly superior to pyrosulfate fusion, which is totally ineffective with siliceous materials. Other alkali metal fluorides are not as effective as the potassium salt because of either higher melting points and/or lower solvent capacity for metallic fluorides.

On heating potassium fluoride, the melt becomes progressively more alkaline due to slow but continuous loss of hydrogen fluoride produced by hydrolysis from water vapor from the air or from the combustion gases from the blast burner. Oxidation of organic materials in the slightly alkaline flux at the high temperature of fusion is very rapid, smooth and complete, particularly in the presence of a small quantity of nitrate. For example, simply pretreating soils or other basic materials with nitric acid will fix enough nitrate to provide for the subsequent smooth oxidation of organic matter. Similarly, addition of a small quantity of potassium nitrate to the potassium fluoride before or during fusion causes rapid oxidation and dissolution of very refractory carbides such as silicon carbide (Carborundum). Because of the increasing use of carbide refractories in the atomic energy industry, this method of sample decomposition will be most useful in the determination of various radionuclides in process and environmental samples. The increasing alkalinity becomes a disadvantage if the fusion is prolonged because insoluble hydroxides soon begin to precipitate and the dissolution action effectively ceases. However, this effect can be reversed or delayed by adding solid acids such as pyrosulfates or even silica, both of which can be added in solid form to the molten flux.

The effect of silica in a fluoride fusion is very interesting. In a procedure for the determination of the actinides in water, a ferric hydroxide scavenge is used to collect the precipitated hydroxides of the other elements. Because the precipitate also contains the siliceous sediments present, potassium fluoride fusion is employed for total decomposition of all materials present. Invariably, the molten flux is highly colored, turbid, and with almost black crystals of hydrolyzed iron compounds sticking to the sides of the platinum dish. The solvent capacity of the flux decreases rapidly, and the sample never does dissolve completely. If 0.5 gram of silica gel is added to 6 grams of potassium fluoride, at least 100 mg of iron will dissolve to give a completely clear, light yellow melt, identical to that obtained from siliceous samples. Apparently, the silica consumes alkali in being converted at least partly to metasilicate rather than fluosilicate and

keeps the iron and probably other metals in the form of soluble fluoride complexes.

After complete dissolution of the siliceous sample in the molten potassium fluoride, the cake can be transposed with concentrated sulfuric acid to a completely clear pyrosulfate fusion, with simultaneous elimination of both silicon tetrafluoride and hydrogen fluoride. This permits the remainder of the determination to be carried out in conventional glassware, completely demasked from the powerful complexing fluoride anion, and with complete elimination of silica by volatilization. This latter step eliminates the objection to alkaline fluxes mentioned earlier. Not only is the silica eliminated smoothly and simply, but it is done by volatilization so that no loss of other components of the sample occurs, and no retreatment of residues of any kind is required. About the only objection to this procedure is that the sample ends up in a sulfate system causing some additional problems in the presence of alkaline earths and the rare earths, and requiring that the rest of the procedure be worked out to be compatible with a sulfate system. However, this can be done very simply in most cases [8].

Many analytical chemists feel that there is something inherently difficult, dangerous or time consuming about procedures requiring fusions. The potassium fluoride fusion and pyrosulfate transposition mentioned above is described in detail elsewhere [8] and can be easily handled by technicians after one or two demonstrations. Figure 1a shows 10 grams of soil in a 250-ml platinum dish at the beginning of the analysis. The sample shown is a −200 mesh standard plutonium soil, but samples as coarse as −10 mesh have been dissolved successfully. Figure 1b shows the sample after a pretreatment with nitric and hydrofluoric acids and evaporation to near dryness. Figure 1c shows the potassium fluoride fusion after addition of 30 grams of anhydrous potassium fluoride. Most troubles encountered in fusions are due to use of too little flux or too little heat to do the job. By using a Fisher blast burner with the large grid to supply the amount of heat shown, the sample is completely dissolved giving a clear melt in 6 minutes, about 4.5 minutes of which is required to heat the sample, flux and dish to the fusion temperature. The clear melt is shown in figure 1d, with the wrinkles in the bottom of the platinum dish being clearly visible through the melt. After cooling to room temperature to permit association of all iron as the colorless ferric fluoride complex, the potassium fluoride cake generally has a light violet color, apparently due to manganese in the sample.

The potassium fluoride cake is then transposed to a sulfate system by addition of 35 ml of concentrated sulfuric acid and evaporation to dryness

on a hot plate as shown in figure 1e, with simultaneous elimination of silicon tetrafluoride and hydrogen fluoride. When heated over the blast burner, the salts melt but precipitation of the anhydrous sulfates of iron and aluminum produces a thick mud that retains the sulfuric acid so tenaciously that it is almost impossible to evaporate off the sulfuric acid to a pyrosulfate fusion without prohibitive spattering. On addition of sodium sulfate and further heating, the thick mud rapidly and very impressively clears to a thin suspension that is easily boiled without spattering. When most of the excess sulfuric acid has been volatilized, the insoluble salts begin to dissolve around the sides as shown in figure 1f. Further heating results in the completely clear pyrosulfate fusion shown in figure 1g, demonstrating conclusively the complete elimination of silica which would otherwise produce a turbidity because of its insolubility in sulfuric acid. On cooling, the pyrosulfate cake has a light yellow color due mainly to iron and is easily removed by flexing the sides of the platinum dish. Generally, the pyrosulfate cake dissolves completely in dilute hydrochloric acid as shown in figure 1h. (The upside-down thermometer is not indicative of the state of knowledge in this laboratory, but was done deliberately to get some calibration markings below the liquid level to demonstrate the clarity of the solution.) In the presence of barium or relatively high concentrations of calcium, a turbid solution due to precipitation of the insoluble alkaline earth sulfates results. This precipitate can be filtered off and retreated if necessary.

If a sulfate system is undesirable, as in analysis of samples containing high concentrations of alkaline earths or rare earths, the potassium fluoride cake can be transposed with nitric acid. However, the transposition is much more time consuming and certainly less neat when applied to the larger samples. Hydrofluoric acid cannot be removed efficiently by evaporation with nitric or even perchloric acids. Repeated evaporation is necessary followed by addition of boric acid or aluminum salts to reduce the concentration of fluoride ion sufficiently that the insoluble metallic fluorides will dissolve in nitric acid. Even then, the final aqueous solution will invariably still be slightly turbid. In addition, the original sample must be pretreated with acid to neutralize any alkaline components present or the alkaline cake will not even disintegrate completely on prolonged boiling with nitric acid. The potassium fluoride fusion has been used successfully with transposition by both sulfuric acid and nitric-boric acids on soil, soft tissue ash, vegetation ash, air dusts, water, *etc.*

Figure 1. Potassium fluoride fusion and pyrosulfate transposition. a. soil sample in a Pt dish, b. evaporated to near dryness after pretreatment with HNO_3 and HF, c. potassium fluoride fusion, d. clear melt obtained with blast burner, e. transposed to a sulfate

system by addition of concentrated H_2SO_4 and evaporated to dryness. f. excess H_2SO_4 volatilizes and salts begin to melt, g. further heating results in the completely clear pyrosulfate fusion, h. pyrosulfate cake dissolved in dilute HCl.

D. Phosphoric Acid

Orthophosphoric acid is known to form relatively stable complexes with a wide variety of elements. Mixtures with perchloric acid are remarkable solvents for iron and steels, including stainless steel, high-speed tungsten tool steels and even metallic tungsten. On dehydration, orthophosphoric acid forms a variety of more highly condensed chain and ring phosphates whose complexes with metals are generally even more stable. These very powerful sequestrants are produced from orthophosphates present in the sample during such common analytical operations as ignition of bone, evaporation of urine and feces to dryness, pyrosulfate fusion of phosphatic materials, and even fuming with perchloric acid in the presence of enough salts to give very high temperatures. Consequently, every sample that might conceivably contain any kind of phosphate compounds must be given deliberate treatment to prevent profound changes in the course of the desired chemical reactions caused by the inadvertent production of these powerful complexing agents. In most cases, the condensed phosphates can be converted to the less powerful orthophosphates by boiling, the hydrolysis being strongly catalyzed by acid.

The powerful complexing action of condensed phosphates can be utilized analytically to dissolve even refractory metallic oxides in such highly phosphatic materials as bone ash, urine, feces, *etc.* This is very fortunate because those are the very types of samples on which pyrosulfate fusion is impossible or undesirable because of their high calcium content. For example, samples of bone ash are preferably dissolved in boiling 72 percent perchloric acid to oxidize the last traces of unburned carbon and then evaporated until most of the perchloric acid has been volatilized and the solution has a thick syrupy consistency. Evaporation should not be carried to the point at which the solution begins to solidify on the hot plate. Even beryllium, thorium and protactinium present in bone during ignition are easily solubilized under these conditions. The same effect can be observed visibly by evaporating several milligrams of the high-fired oxides with 85 percent phosphoric acid to a syrupy consistency and observing how easily and completely the turbid suspension clears. However, the cake must be dissolved in dilute acid and boiled for 15 minutes or so to hydrolyze the condensed phosphates before any chemical reactions are attempted. Protactinium has been removed to the extent of 98 percent in a single diisobutylcarbinol extraction from a solution of 100 grams of bone ash in 8*M* hydrochloric acid after such treatment.

The extraction of beryllium into acetylacetone from a solution of bone

ash illustrates the principles involved and the great potential for perturbations in the analytical scheme if not provided for. Under the proper conditions [9], beryllium can be extracted into acetylacetone to better than 99 percent in a single extraction. The presence of 0.5 ml of 85 percent phosphoric acid produces no significant effect on the extraction, but addition of 0.5 gram of sodium pyrophosphate, which contains less than one-third as much phosphorus, reduces the extractability to about 0.9 percent under the same conditions. Boiling the solution for 15 minutes in 1*M* hydrochloric or perchloric acids restores the original complete extractability. Sulfuric acid is not as efficient as the strong acids.

Similarly, if bone ash is dissolved in a slight excess of 72 percent perchloric acid and the heating is stopped as soon as the ash dissolves, ionic beryllium can be extracted quantitatively. The condensed phosphates produced during ignition of the bone are rapidly hydrolyzed by the boiling with strong acid used to dissolve the ash, and the subsequent extraction proceeds normally. On the other hand, if the solution is evaporated to a syrupy consistency to ensure complete dissolution of refractory beryllium oxide or phosphate, the condensed phosphates are reformed and subsequent extraction of beryllium is reduced to less than 30 percent, even though the cake was dissolved by boiling with water for 15 minutes without added acid. If the solution is boiled for 15 minutes after having added enough perchloric or hydrochloric acids to make the solution about 1*M*, the subsequent extractability of beryllium goes back up to better than 99 percent.

Similar competing reactions of condensed phosphates in many other systems have been observed repeatedly. Samples containing both calcium and phosphate, such as vegetation or soft tissue ash, and dissolved by pyrosulfate fusion will frequently dissolve in dilute hydrochloric acid completely at first and then suddenly precipitate profusely after a few minutes boiling which destroys the sequestering agent. The efficiency of carrying large polyvalent ions on barium sulfate is decreased drastically if the solution from pyrosulfate fusions of phosphatic samples is not boiled extensively with strong acid before the precipitation is carried out. It may confidently be predicted that condensed phosphates produced *in situ* from orthophosphates present in the sample by strong heating will cause severe deviations from the expected chemistry in most analytical procedures.

One other perturbing characteristic of phosphoric acid needs to be emphasized. If carrier-free thorium-234 tracer is evaporated to complete dryness on a hot plate with a few drops of 85 percent phosphoric acid, a bone-white amorphous powder is obtained which is almost totally insoluble in even boiling concentrated hydrochloric acid and which will contain

over 95 percent of the total thorium tracer present. The material can be dissolved and the thorium released by fusing with pyrosulfate. The white, insoluble material is obviously a phosphoric acid polymer of some unknown composition because only phosphoric acid was present. It is to be expected that heating phosphatic samples such as urine, feces, *etc.* to dryness will certainly produce these acid-insoluble residues, and severe losses of almost any element being sought will occur. For example, more plutonium was found in the acid-insoluble residue from a fecal sample that had been dry-ashed in a muffle furnace than was found in the acid-soluble portion. These experiences again emphasize a cardinal rule for analytical chemists: Be extremely careful about discarding any insoluble residues!

IV. Evaporation to Dryness

One of the most common operations performed at least once in many analytical procedures is that of evaporating aqueous solutions to dryness. Whether for purposes of eliminating acids or other unwanted volatile materials, the evaporation is all too frequently carried out with almost total disregard for whether or not the residue will redissolve on subsequent treatment with water or even strong acids. Obviously, if the proper ionic condition is not restored, severe deviations from the expected chemistry will result. If part of the residue remains firmly attached to the walls of the container, additional problems will be introduced in subsequent samples due to contamination if proper cleaning methods are not employed.

One of the outstanding characteristics of tervalent and particularly quadrivalent ions is their ability to form extremely insoluble hydroxides at very low hydroxide concentrations. When an acidic solution containing a metallic salt of a volatile acid is evaporated to dryness, the excess acid is volatilized, and the remaining neutral salt undergoes progressive hydrolysis in its own water of crystallization. The number of hydroxyl ions taken up depends greatly on the charge of the metallic ion. For each hydroxyl ion taken up by the metal ion, an additional molecule of the volatile acid is formed, which is immediately driven off. On prolonged heating, complete conversion to a refractory oxide occurs in many cases that is redissolved very incompletely or not at all on subsequent treatment even with strong acids.

To permit this effect to be observed visibly, four solutions each containing a few milliliters of water and about 100 mg of thorium nitrate were evaporated just to dryness on a bare electric hot plate. The four residues

dissolved completely within about 1 minute by heating with a few milliliters of concentrated hydrochloric, nitric, perchloric, and sulfuric acids, respectively. However, after reevaporating the four different acid solutions just to dryness so that no residual nitrate remained and each residue was then treated with its respective acid, only the nitrate dissolved in concentrated nitric acid. The chloride, perchlorate and sulfate residues did not dissolve completely in hydrochloric, perchloric and sulfuric acids, respectively. In contrast, addition of about 1 gram of sodium hydrogen sulfate before evaporation to dryness with any of the acids gave a residue that dissolved completely and almost instantaneously in cold water. Another run with 1.5 grams of sodium hydrogen sulfate was evaporated, and the residue was heated over a blast burner until the bottom of the Pyrex flask sagged and evolution of sulfuric acid fumes had completely stopped. The residue dissolved almost but not quite completely either in cold water or on subsequent boiling, probably due to partial hydrolysis at the low acidity resulting from such severe heating. The turbidity does clear up on addition of a few drops of nitric acid.

These tests show that sodium hydrogen sulfate is remarkably effective in preventing hydrolytic deposition of quadrivalent thorium. The reasons are obvious. Sulfate forms a relatively stable complex with thorium, requiring significant hydroxide concentrations before even a monohydroxo thorium ion forms to any significant extent. Furthermore, unlike hydrochloric, nitric and even sulfuric acids, which are volatile, sodium hydrogen sulfate is virtually nonvolatile, acting as a buffer in preventing the acidity from going any lower on strong heating than that of the sodium hydrogen sulfate itself. In fact, strong heating converts sodium hydrogen sulfate to molten pyrosulfate, which is a powerful solubilizing flux for the very oxides that are being produced on heating the other systems containing volatile acids. When only small quantities of elements are present, the quantity of sodium hydrogen sulfate required is so small as to be compatible with almost any system except in the presence of those elements forming very insoluble sulfates.

Four solutions of thorium in hydrochloric, nitric, perchloric and sulfuric acids were again evaporated to dryness, but the residues were allowed to remain on a bare electric hot plate for several hours. None of the residues—not even the nitrate—dissolved significantly on boiling with concentrated nitric acid, which was the most effective solvent in the earlier tests. Addition of 1 drop of 48 percent hydrofluoric acid produced no change, but on dilution of the concentrated acid with water, all four solutions dissolved rapidly to give completely clear solutions. To determine whether this effect was due to the fluoride ion or the water, the test was repeated, heating the residues on the bare hot plate for 1 hour. On boiling

with 30 ml of concentrated nitric acid, very milky suspensions were obtained with the chloride and sulfate residues, less turbid with the nitrate and least turbid with the perchlorate. On addition of an equal volume of water to each flask, the sulfate residue cleared completely and almost immediately while the other residues showed little change. Addition of 1 drop of 48 percent hydrofluoric acid to the three turbid suspensions in dilute nitric acid caused immediate and complete dissolution of the turbidity. This demonstrates the powerful complexing action of fluoride in dissolving refractory oxides of thorium (and many other metals), but water is still necessary to dilute the concentrated acid. The sulfate system provided its own complexing agent to dissolve the hydroxo compounds after addition of water.

Although the above tests were performed with macro quantities of thorium for convenience of visual observation, the principles demonstrated are clearly applicable to much smaller, invisible quantities. Similar experiments were performed with carrier-free plutonium-239 nitrate in 10 percent nitric acid to demonstrate that extremely small quantities of quadrivalent ions would act the same way. Identical 1-ml aliquots of the plutonium-239 tracer were evaporated to dryness in 250-ml Erlenmeyer flasks with 10 ml of concentrated nitric, hydrochloric, and perchloric acids, and 100 mg of sodium hydrogen sulfate, respectively. After baking for 1 hour on a bare electric hot plate, all four residues were boiled vigorously for 2 minutes with 3 ml of concentrated nitric acid, fused with aluminum nitrate and extracted with 30 percent Aliquat-336 in xylene under conditions known to extract plutonium quantitatively [8]. After extraction, the residual aluminum nitrate aqueous phase was analyzed for plutonium. Only 0.2 to 0.4 percent of the plutonium taken initially remained unextracted in the aqueous phase, all of which can be attributed to phase separation. However, even after rinsing three times with water to remove any traces of soluble plutonium remaining from incomplete transfer of the original solution, the empty flasks in which the evaporation to dryness had been carried out contained 1.3, 0.4, 2.3, and 0.006 percent of the initial plutonium activity from the nitric, hydrochloric, and perchloric acids, and the sodium hydrogen sulfate, respectively.

The experiment was repeated except that the baked residues were boiled for 2 minutes with 4 ml of 72 percent perchloric acid before the extraction, and sulfuric acid was used in place of the sodium hydrogen sulfate. The results were virtually identical. Only 0.5 percent of the original plutonium remained unextracted in the aqueous aluminum nitrate phase, and 1.7, 1.5, 0.3 and 0.005 percent of the plutonium remained stuck to the bottom of the respective flasks. The efficiency of even the residual sulfate from the evaporation of sulfuric acid in keeping trace

quantities of plutonium off the container is remarkable. Although the quantities of carrier-free tracer lost on the bottom of the flask under the present conditions is not very large relative to the total recovery of the plutonium present, they will be extremely significant with respect to contamination of the next sample to be processed in the same container if not cleaned up. The flasks can be cleaned completely and reliably by making a pyrosulfate fusion such that no detectable plutonium can be removed in a subsequent fusion and analysis.

Most of the plutonium that was dissolved out of the flask with nitric and perchloric acids was in a form capable of being extracted, but this might not always be the case, particularly with less energetic treatment. For example, to convert a plutonium-239 nitrate solution in 10 percent nitric acid to the chloride form for extraction into triisooctylamine from hydrochloric acid solution, a 1-ml aliquot was evaporated to dryness and inadvertently heated on an electric hot plate for about 5 minutes. The residue was boiled for 2 or 3 minutes with concentrated hydrochloric acid in an attempt to redissolve the plutonium before extraction with 50 ml of 10 percent triisooctylamine from 60 ml of 10*M* hydrochloric acid. The quantity of plutonium remaining unextracted in the aqueous phase was 93.3 percent of that taken initially.

The experiment was repeated except that the plutonium nitrate was converted to the chloride form by evaporation with 5 ml of concentrated hydrochloric acid to about 1 ml in an uncovered 30-ml beaker without letting the solution go dry even in local spots on the bottom. After extraction, 9.3 percent of the plutonium still remained in the aqueous phase, probably due to slight drying and heating on the sides of the uncovered beaker.

The experiment was repeated again, but the plutonium nitrate was converted to the chloride form by boiling with concentrated hydrochloric acid in a covered 30-ml beaker without allowing any evaporation to occur. After extraction under identically the same conditions used above, only 0.52 percent of the original plutonium remained unextracted in the aqueous phase. A repeat experiment under the same conditions gave 0.77 percent in the aqueous phase, both losses being about what would be expected from incomplete phase separation in the time allotted.

It is clear that evaporation of solutions of tervalent and particularly quadrivalent ions in volatile non-complexing acids to dryness is very risky, particularly if the residues are heated very hot or very long. Hydrochloric acid is not as effective as the higher boiling nitric or perchloric acids in redissolving the residues except for those cases in which strong chloride complexes are formed as in the case of ferric iron.

For example, evaporation of solutions of the large tervalent and quadrivalent alpha-emitting elements to dryness prior to electrodeposition for alpha spectrometry on the mistaken assumption that the residue will redissolve completely in hydrochloric acid gives decreased yields and contaminated beakers that require pyrosulfate fusions to clean completely. Addition of even 100 mg of sodium hydrogen sulfate before evaporation to dryness eliminates the problem.

V. Neutralization with Alkali

Another very common operation in analytical chemistry that is performed with surprising casualness and indifference to the consequences is that of neutralizing a solution containing heavy metals to some preselected pH by the addition of an alkali. Generally, the analyst feels that because the final equilibrium pH is below that necessary to produce the beginning of permanent precipitation that no perturbation has in fact been incurred. Obviously, with the small quantities of elements present in trace analysis, there will be no turbidity or other visual evidence to suggest otherwise. Yet, this is undoubtedly one of the most common sources of erraticism, poor separations, and incomplete recovery to be encountered in routine analysis where such operations are performed. The pH required for incipient precipitation of elements forming insoluble hydroxides is dependent on the $K_{s.p.}$, the charge and concentration of the metal ion, the ionic strength and many other factors. However, in many cases, reaction rates are a more important and limiting consideration than the final equilibrium conditions. As alkali is added, the pH in the local vicinity of the individual drops generally is sufficiently high to precipitate part or all of the element present in that region, depending on the strength of the alkali added. Whether or not the precipitated hydroxide redissolves after the solution is stirred and the excess alkali is neutralized to the pH of the main body of the solution depends on the time allowed and how closely the final pH is allowed to approach that at which permanent precipitation occurs under the particular conditions used.

For example, in the determination of the large tervalent and quadrivalent alpha-emitting radionuclides of thorium through californium by alpha spectrometry, the pH of the solution is generally adjusted with ammonium hydroxide to the acid side of a methyl red endpoint (*ca.* pH 4) prior to electrodeposition. When electrodepositing standards of carrier-free tracers, excellent yields, greater than 98 percent, can be obtained easily and routinely using many current procedures. However, when the

same procedure is applied to the same radionuclides after their chemical separation from soil, the yield on electrodeposition usually drops by 10 to 25 percent or more.

Recent information obtained in this Laboratory demonstrates conclusively that both the yield and tolerance to other elements can be increased markedly by adjusting the pH to the acid side of thymol blue indicator (pH 1.2 to 2.8) rather than methyl red (pH 4.8 to 6.0). Apparently, when the radionuclide being electrodeposited is present in only carrier-free quantities, its precipitation is so slow even at pH's above 6 that subsequent redissolution is virtually complete in a reasonable length of time even at pH's only slightly below 4. However, if even microgram quantities of cerium, lanthanum, iron, *etc.* from the sample survive the chemical separations, the added carrier causes precipitation of the radionuclide to be more complete and the subsequent dissolution at pH 4 considerably less, as should be expected of elements forming such insoluble hydroxides. When thymol blue is used, much less alkali is added, less precipitation of either radionuclide or inert carrier occurs, and more acid is present after neutralization to the acid side of the indicator range to redissolve the small quantities that might have precipitated. Obviously, if the radionuclide is already precipitated in the solution at the beginning of electrodeposition, it cannot be electrodeposited at the cathode. One should always be very cautious in adjusting the pH of a solution containing heavy metals with strong alkali closer than one or two pH units to the pH at which precipitation occurs unless suitable complexing agents are present to prevent significant local precipitation around the drops. A much safer way, particularly if tervalent and/or quadrivalent elements are present in the absence of complexing agents, is to make the final pH adjustment with a buffer solution of pH only slightly higher than the final one desired.

A very different and unexpected type of problem was encountered in a fluorometric determination of thorium [10]. In the original procedure no longer used, a constant quantity of sodium hydroxide was neutralized with sulfuric acid to produce a high and constant salt concentration from variable quantities of sulfuric acid present. To keep the procedure as simple as possible, the diethylentriaminepentaacetic acid (DTPA) and triethanolamine (TEA) being used to prevent precipitation of thorium throughout the entire alkaline range were incorporated in the sodium hydroxide. After adjustment of the pH to about 5 with dilute sulfuric acid, a pH 11 buffer and the fluorometric reagent morin were added, and the fluorescence was measured. The fluorescence produced from 5-μg thorium standards was very erratic and in most trials was reduced completely

to the level of the blank. The trouble was traced to precipitation of part or all of the thorium at the high alkalinity of the strong sodium hydroxide solution despite the presence of TEA, which did not then redissolve on subsequent acidification because of the presence of DTPA. This was a very surprising discovery because of the stability and water solubility of the Th-DTPA complex. If the complexing agents are omitted while the solution is strongly alkaline, thorium will be precipitated completely but will also redissolve rapidly and completely on reacidification. The complexing agents can then be added to the slightly acidic solution and the pH raised to the mildly alkaline conditions required for the fluorometric determination by adding a buffer solution, and the full fluorescence expected from 5-μg standards can be obtained and reproduced with high precision. However, the problem was solved more simply and conveniently by adding a small quantity of sodium sulfate and evaporating the excess sulfuric acid to dryness. The sodium hydrogen sulfate cake dissolves immediately and completely in water, after which the full fluorescence is obtained with equally high reproducibility.

This explanation was developed from a simple experiment. If 2 or 3 mg of thorium in 25 ml of water is treated with sodium hydroxide, the precipitate of thorium hydroxide will redissolve rapidly and completely on addition of a single drop of 72 percent perchloric acid beyond a methyl red endpoint. If a small quantity of ethylenediaminetetraacetic acid (EDTA) is present, no precipitate is formed on addition of a slight excess of alkali. However, if 1 ml of 10 percent disodium EDTA is added after the formation of thorium hydroxide, the precipitate does not dissolve easily or completely even on addition of many more drops of 72 percent perchloric acid than are required to neutralize the alkali and to exceed the total buffer capacity of the EDTA. The cause of this anomaly is not known, but the experiment demonstrates that hydrolytic species will not always redissolve in the presence of foreign materials as easily as they do in pure solution, even if freshly prepared.

VI. Contamination

Contamination is perhaps the most persistent and severe problem with which the analyst has to contend in trace analysis, and requires continuous and unrelenting care for its elimination and/or control. The many difficult problems involved in purification of water, acids and reagents, control of laboratory environment, and availability and choice of containers for storage of solutions and chemical reactions for the parts-per-million

and lower range have been discussed repeatedly elsewhere. Although these problems are admittedly severe and many of the current solutions are not entirely satisfactory, there are many other chemical problems that are potentially more severe and less reproducible for which the analyst himself is responsible, and which can and must be controlled through proper information and attention to detail.

It has always seemed somewhat incongruous to the present author to watch a professional chemist busily scrubbing with soap suds and brush until the beaker is spotlessly clean. Such treatment is fine for getting the greasy fingerprints off the outside of the beaker, but it doesn't do very much to eliminate the inorganic impurities on the inside where it matters. If the beaker was used to hold a solution of an ore on which an ammonium hydroxide or sodium carbonate separation was made, hydrochloric acid containing hydrogen peroxide or other reducing agent will inevitably be required to remove the manganese dioxide from the sides of the container. If zirconium, niobium, tantalum, *etc.* might also have been present, the further addition of hydrofluoric acid would also be desirable and prudent. In other words, unless the analyst plans to use a new container for each analysis, the time to begin controlling contamination is before or immediately after it has been incurred, before its recent use history has been lost by placing it back in the drawer, namely during the cleanup of glassware and other pertinent equipment. Moreover, the cleaning process must not be carried out blindly and by a constant, standard procedure, but must reflect the best chemical remedy for the problem at hand.

During the recent development of a procedure for the simultaneous determination of virtually all alpha-emitting elements in a single sample of soil [8], severe contamination was encountered repeatedly that clearly related to the quantities of the same radionuclide in the previous sample put through the same equipment. Consequently, every empty container was checked after use to determine the source and extent of the contamination from each piece of apparatus, and therefore from the particular chemical operation responsible, and the most efficient way to remove the contaminant. It was found that beakers in which barium sulfate had been precipitated and filtered from a boiling solution to precipitate the large polyvalent ions invariably contained a thin milky deposit of barium sulfate on the sides of the beaker, due to postprecipitation after the filtrate had cooled. This deposit was not removed efficiently or completely on washing with a solution of hydrochloric and hydrofluoric acids, but was dissolved in the subsequent sample releasing the activity it contained. Treating the beaker with any solvent for barium sulfate such as fuming sulfuric or perchloric acids or hot alkaline DTPA eliminated the problem completely. Erlenmeyer flasks in which nitric acid solutions of plutonium,

thorium, americium, *etc.* had been evaporated to dryness prior to electrodeposition required a pyrosulfate fusion to dissolve the refractory oxides produced on strong heating. Addition of sodium hydrogen sulfate before evaporation to dryness decreases the loss materially as described above, but pyrosulfate fusion is still recommended to guarantee complete decontamination.

Separatory funnels used in liquid-liquid extractions should be rinsed with alcohol to remove the organic solvent and then with hydrochloric acid containing a small quantity of hydrofluoric acid to remove any hydrolyzed compounds, particularly of zirconium, protactinium, *etc.* The most severe source of contamination by far was in the cell used for electrodeposition. Because electrodeposition of electropositive elements depends on deposition of the hydroxides at the cathode, during which time the pH of the entire solution increases to about pH 7.5, small quantities of insoluble hydroxides inevitably find their way to the walls of the cell. Casual treatment with mixtures of various acids, including hydrofluoric, and hydrogen peroxide at room temperature is totally inadequate to achieve complete decontamination. The most effective treatment involves boiling the cell, anode and "O" ring with concentrated nitric acid, repeating the process in fresh acid when more than a few hundred disintegrations per minute had been electrodeposited. However, hot concentrated nitric acid precipitates polonium extensively so other means must be employed for its removal.

Another source of high and variable contamination is the dropping bottles or reagent solutions that are dispensed by dip-type pipets and used repeatedly during a series of analyses. The safest way to avoid a continuing buildup of contaminant is either to rinse the outside of the pipet every time it is used before it is allowed to reenter the reagent bottle, or to pour a small quantity of the original reagent into a small beaker or graduate cyclinder and discard what is not used. Wasting small judicious quantities of reagents is infinitely less expensive than redoing the analysis to say nothing of the effort expended in identifying the problem initially. Since identifying all the sources of contamination in the plutonium-soil procedure over 4 years ago, and implementing the present program of appropriate chemical cleaning of all glassware and prevention of contamination of reagent solutions, not a single case of contamination has been observed.

Another problem having an effect similar to contamination in that it is variable, severe and cannot be corrected for is failure to obtain absolutely complete decomposition of organic matter, including organic compounds deliberately used in the analysis. In a fluorometric procedure for berylli-

um using morin [9], acetylacetone is used to extract the beryllium, and the extract is then wet-ashed with nitric and perchloric acids before the fluorometric measurement. When complaints were received about high and variable blanks being obtained, investigation showed that the entire problem was due to the analysts' removing the cover glass so that the perchloric acid could escape rather than leaving the cover in place to cause significant refluxing of the perchloric acid and extend the digestion time as had been intended but not specifically mentioned in the published article. Consequently, the acetylacetone was incompletely decomposed, and the decomposition products remaining included a compound exhibiting a blue fluorescence which contributed substantially to that being measured. Leaving the cover glass in place until most of the perchloric acid had been volatilized rectified the entire problem.

One of the strangest and most unimaginable problems was encountered in the development of a procedure for the determination of lead-210 [11]. Lead and bismuth were extracted into a chloroform solution of diethylammonium diethyldithiocarbamate (DDTC) from a strong acid solution. After wet-ashing the extract, both elements were extracted into dithizone from an alkaline citrate-cyanide solution, and the lead was then stripped out of the organic phase with a pH 2.7 buffer, separating it from bismuth. Although the dithizone separation of lead from bismuth worked extremely well when tested alone, the separation failed completely when applied to the wet-ashed DDTC extract. The lead could not be stripped completely from the dithizone extract with the acid buffer, certainly not the way the lead dithizone complex is known to act. The trouble was traced to incomplete destruction of the dithiocarbamate entity, despite repeated severe treatment with nitric, sulfuric and perchloric acids, and the lead was being retained in the chloroform extract as the dithiocarbamate complex, which is extractable from strong acid.

How a simple molecule like diethyldithiocarbamic acid, containing both a thiono and a powerful reducing thiol group and forming only a 4-membered carbon-sulfur hydrogen-bonded heterocyclic ring, can survive the vigorous wet-ashing conditions used is very difficult to imagine. However, if DDTC is evaporated to fumes with concentrated nitric and perchloric acids and water is added, a clear, colorless and odorless solution is obtained that becomes turbid and develops a strong odor of hydrogen sulfide on standing overnight. The solution still has definite ability to form strong chloroform-soluble complexes with both lead and bismuth from strongly acid solutions that prevents complete stripping of lead in the subsequent dithizone extraction. The ability to extract lead from strongly acid solutions is not shared by many organic compounds

and suggests that some of the diethyldithiocarbamate itself does in fact survive the oxidative attack.

After oxidation with nitric and perchloric acids, the residual organic matter is not charred significantly even by boiling concentrated sulfuric acid but is easily charred on evaporation to dryness. The resulting char can then be oxidized smoothly and completely with nitric and perchloric acids, leaving nothing capable of extracting lead under any conditions. Sodium hydrogen sulfate is added as an acid buffer to prevent hydrolysis and thermal decomposition of lead and bismuth sulfates during heating to dryness and to prevent precipitation of very insoluble anhydrous sulfates of iron and other metals that might be present. When these conditions of wet-ashing the DDTC extract were used in the procedure, all difficulties with stripping lead from the dithizone extract disappeared.

These few examples are intended to show the many kinds of complications that can arise inadvertently that completely change the course of the intended chemical reactions with a consequent decrease in accuracy, precision and reliability of the determination. The analyst must know the characteristics and limitations of his procedure if good results are to be obtained consistently. Although emphasis has been placed on the tervalent and quadrivalent elements, the same principles apply to the lower valent elements in somewhat lower degree.

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DISSOLUTION OF ORGANIC MATERIALS

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The initial stage in the determination of trace elements, the destruction of the organic matter, is likely to introduce more errors than all the other stages put together.

There are two main families of decomposition methods, those involving air or oxygen and carried out at relatively high temperatures and those involving relatively large quantities of liquid reagents and carried out at temperatures limited by the boiling points of the liquids. Each of them has its advantages and disadvantages. The dry methods generally being characterized by high and relatively less controlled temperatures and with little or no separation of the required elements and the other solid components of the system, while with regard to the wet methods, the temperature is generally lower and there is separation at all times between the required elements and the solid components of the system. The technique using activated oxygen falls into a separate class of its own.

The problems during the decomposition stage are of two kinds, losses of the required elements, or contamination of the sample with extraneous material. The losses can be due to volatilization or to fixation on the solid components of the system and such losses are generally less serious with wet methods than with dry. Contamination on the other hand is often related to the quantities of reagents added and in this instance the dry methods are probably superior to the wet.

The problems encountered, particularly with regard to losses, will vary greatly with the element to be determined and the nature of the sample in which it is to be determined. Elements such as mercury and selenium are always difficult and samples containing chlorine in any form generally pose problems.

Keywords: Ashing techniques; dissolution; dry ashing; organic materials analysis; trace element analysis; wet ashing.

I. The Ashing of Organic Samples

The determination of trace elements in organic materials always involves three specific stages. The first of these is the sampling of the organic material in a representative manner; the second is the isolation of the desired trace element or some property of it; and the third is the measurement of that trace element or property. This paper is concerned with the second of these stages, the isolation of a trace element from other material that might be likely to interfere in the final stage, the determination. Quite clearly it is not always necessary to carry out any kind of physical removal of interfering materials as it is sometimes possible to separate some property of the desired element by electronic or optical means. Examples of this kind of separation would be the use of activation analysis and gamma spectrometry for some elements, or flame photometry or atomic absorption spectrometry for elements occurring in some kinds of dilute solutions. However, this paper is concerned with those instances where it *is* necessary to bring about the physical removal of material which, in the vast majority of instances, is the organic material that makes up the bulk of the samples with which we are concerned.

Each of these three stages, the sampling, the separation, and the measurement, can give rise to errors but it is probably true to say that in many instances the separation stage is likely to introduce more errors than the rest of the analysis put together. The errors that occur can be either positive or negative in that material may be added to the sample by contamination, or taken away from it by various kinds of loss, but we will be concerned mainly with the second of these, losses of the significant element during the separation stage.

Having discussed using a separation, it is important to recognize that this separation should only be carried as far as is necessary. It is, in many ways, very satisfying to prepare a sample from which all traces of organic material have been removed, but if a perfectly adequate determination can be carried out on a sample which has suffered a lesser degree of disruption, then it might be considered to be tempting fate to carry this disruption process further than is necessary. As was said above, some samples can be handled by techniques such as atomic absorption without any further treatment at all, while others can be handled after various degrees of dilution, hydrolysis, extraction or partial ashing, or indeed by any technique up to the complete and total removal of the organic material. Generally speaking, these limited disruption techniques are acceptable when they are used on repetitive samples where their suitability has been demonstrated. It is perhaps unreasonable to try to use them on occasional samples of a new kind.

However, having decided that complete destruction of the organic material *is* necessary, there are a very large number of factors that need to be considered in selecting the ashing technique to be used. The choice will be influenced by the identity of the element to be determined, the level at which it occurs within the sample, and by the nature of the sample itself. It is not much of an exaggeration to say that each combination of a specific element, type of sample, and the level of occurrence, introduces a unique set of circumstances which will affect the choice of method. However, to offset this there are very nearly as many possible methods of destruction as there are situations for them to meet. The basic division is into the wet methods where the oxidizing materials are present as liquids, and the dry methods where the oxidizing agent is gaseous, usually being air but sometimes being oxygen, or even, very rarely, other oxidizing gases. To further complicate the picture, there are a number of possible liquid oxidizing reagents which may be used, singly or in combination, with sulfuric acid, nitric acid, perchloric acid and hydrogen peroxide being the most common. There are also a variety of ashing aids that are used in conjunction with the dry ashing methods. In addition to this, the nature of the vessels in which the ashing is carried out can have a significant effect on the results and the whole picture can be affected by organizational considerations, such as the time available, the number of samples to be handled, the equipment available, and the number and skill of the operators to do the work. Having stirred up this highly complicated mixture of factors, we shall now try to go through a few of them systematically so that we can attempt to draw out some threads which might help us find our way through this maze.

However, before we go on to talk about the problems that can arise, it is necessary to be reminded briefly of the characteristics of the main methods used for the destruction of organic materials. The two primary families, wet oxidation and dry ashing were mentioned above; they must to a very large extent be regarded as complementary rather than competitive with one another. This means that none of them is absolutely good or absolutely bad in any particular instance, merely that one is better (or worse) than the other according to the conditions. As is shown in table 1, wet oxidation generally involves less elapsed time but more operator time. The temperatures used are lower so that problems of volatilization and retention should be less important, while the reagent blank is likely to be larger so that contamination problems are increased. Wet oxidation methods generally are not particularly suitable for handling large samples, but with a sample of a suitable size the use of relatively large quantities of reagents that are frequently strong acids does tend to overwhelm any chemical variation in the sample, so that the actual nature of the sample

becomes rather less important. By contrast, dry ashing tends to be at the other end of the spectrum, the elapsed time is frequently rather long, but as little supervision is required this can very conveniently be the overnight period. The temperatures are higher but by comparison the reagent blank problems are smaller. Large samples can be dealt with quite readily although, in the absence of ashing aids, variations in the sample can influence recovery.

From the point of view of the author, I believe that for single samples it is probably better to use a wet oxidation method. If a large number of similar samples are to be handled routinely then it is clearly important to determine the best method for that specific application. One technique that has been used quite extensively over the last 10 or so years is the low temperature ashing method first described by Gleit and Holland. In this method the sample is oxidized by oxygen at low pressure—about 1 torr—after excitation by a radio frequency field. Under these circumstances, the temperature does not rise above 130 °C to 150 °C and oxidation of the quite small samples used is complete in a few hours. Using this method it is clear that one of the disadvantages of dry ashing—the high temperatures needed—is removed and it would be expected that better recoveries would be obtained. Many very good results have been reported with this technique but there have also been a number of others where losses have been found despite the very low temperatures. However, the good results far outweigh the bad, although the size of the sample that can be analyzed and the rate of handling must be considered disadvantages.

However, returning to the problems to be encountered in the more traditional ashing techniques, it seems that as the whole object of the exer-

TABLE 1. *Comparison of wet and dry oxidation*

Wet oxidation	Dry ashing
·More rapid	·Rather slow
·Temperature lower—less volatilization and retention	·Temperature higher—more volatilization and retention
·Generally less sensitive to nature of sample	·Generally more sensitive to nature of sample
·Relatively more supervision	·Relatively less supervision.
·Reagent blank larger	·Reagent blank smaller.
·Large samples inconvenient	·Large samples easily handled.

cise is to determine certain specific elements in organic materials, then consideration of the elements to be determined will perhaps form a very suitable place to start the discussion.

If an element is to be lost during the disruption of an organic sample, there are three possible routes. The most obvious is that the element has been removed completely from the system by volatilization, so that it is no longer present and can therefore not be determined. Secondly, it might be bound securely to solid material present in the system, so that when the sample is transferred for measurement it is left behind. Thirdly, there is a possibility that although the element is present and is transferred, it is present in such a form that it does not react in the expected manner, so that the result obtained by the measurement will be low. All three of these possibilities have been demonstrated or postulated to explain losses that occur during sample oxidation.

Starting first with the problems of volatilization, it is clear that some elements such as mercury are volatile in virtually all of their chemical forms and any method which involves high temperatures in open systems must be potentially susceptible to the losses. Other elements are only volatile in *some* of their chemical forms so that the initial form in which they occur and the nature of the chemical reactions that occur during the disruption will all have an effect on the volatilization losses. During the average decomposition process, both oxidizing and reducing conditions can occur and there are also circumstances in which some fairly reactive intermediate materials are produced by the interaction of the sample of the oxidizing mixture. The most significant of these reactive materials produced during decomposition is probably hydrochloric acid which can be produced from inorganic chlorides, organic materials containing covalent chlorine, or even from perchloric acid used in the oxidizing mixture.

Some of the elements that have caused difficulty because of volatilization losses, divided up into the four categories: intrinsically volatile, volatile under oxidizing conditions, volatile under reducing conditions, and volatile in the presence of HCl, are shown in table 2. The melting and boiling points of the relevant chlorides of the elements in the fourth column are shown in table 3 from which it can be seen that arsenic and germanium are likely to be difficult under any conditions in the presence of HCl while lead and zinc are unlikely to be troublesome at low temperatures but might well be expected to cause difficulty at the higher temperatures used in dry ashing.

The second major cause of loss of material is the retention of the element concerned by solid material present in the system. This solid materi-

al can sometimes be the material of construction of the ashing vessel itself or sometimes solid material existing in the sample or produced within the reaction mixture. If we consider first the loss of elements by reaction with the ashing vessel it is clear once again that this is a problem that will be found more commonly in dry ashing than in wet, as the degree of contact is very much greater and the temperatures that are used are generally much higher. When we turn to materials present in the reacting system, losses by retention are found in both wet and dry ashing, although the reasons for the losses may be different.

With oxidation systems containing sulfuric acid and with samples high in calcium, there is the risk of loss of elements such as lead by co-precipitation of the relatively insoluble sulfates. In dry ashing techniques the presence of high levels of silica in the sample may well cause difficulties with elements such as lead, zinc and copper which may be very strongly retained on silica materials. The third type of loss, due to the nonavailability of the element to the method of detection is rather less common but it has been put forward to explain problems encountered

TABLE 2. *Elements that are volatile under certain conditions*

Intrinsically volatile	Volatile under oxidizing conditions	Volatile under reducing conditions	Volatile with hydrogen chloride
Mercury	Technetium	Selenium	Arsenic
	Rhenium	Tellurium	Germanium
	Ruthenium	Polonium	Lead
	Osmium		Zinc

TABLE 3. *Melting and boiling points of some inorganic chlorides*

Element	Volatile form	Melting point, °C	Boiling point, °C
Arsenic	$AsCl_3$		130
Germanium	$GeCl_4$		83
Lead	$PbCl_2$	501	
Zinc	$ZnCl_2$	262	

with tin where conversion of the element to a very insoluble stannic oxide might be responsible; with antimony where the presence of the element in the wrong valency state was said to be the reason for its failure to react with rhodamine B; with iron, where dehydration during heating in residual concentrated sulfuric acid was said to give an insoluble anhydrous ferric sulfate; and with iron again where dehydration of orthophosphates to pyrophosphates and other condensed phosphates led to complex formation. This type of loss is not very common and is probably not worth considering further in the short time available, but it might be worth suggesting that similar problems could arise with the platinum metals during dry oxidation when reduction to the metal would probably occur and difficulties might be experienced unless the appropriate measures were taken to get the metals back into solution.

A rough breakdown of the bulk of the elements in terms of the problems that they cause during ashing is shown in the next group of tables. It is quite possible to find reports of losses for virtually every element, but there is always a risk that the losses being reported are not due to the ashing step as it has often not been possible to separate the ashing losses from the losses due to sampling or the determination itself. Additionally, many elements can occur as volatile organic compounds and these are naturally likely to cause substantially more trouble than when they occur in inorganic form but as far as table 4 is concerned, the elements which in the main do not cause a great deal of difficulty have been listed. As you look at them you will agree that it is understandable that they should be fairly straightforward and we can only regret that there are not more of them. The elements which cause trouble on wet ashing only are shown in table 5; as you can see, this is a fairly rare circumstance. All four of these elements, technetium, rhenium, ruthenium and osmium, are characterized by

TABLE 4. *Elements generally causing little trouble*

Magnesium	Scandium	Actinium
Calcium	Yttrium	Thorium
Stroutium	Rare earths	Protactinium
Barium		Uranium
Chromium	Manganese	Vanadium
Molybdenum		Niobium
Tungsten	Bismuth	Tantalum

TABLE 5. *Elements causing problems on wet ashing only*

Technetium	Ruthenium
Rhenium	Osmium

forming volatile oxides in their highest valency states and they are the elements quoted earlier as showing losses under oxidizing conditions. This will mean that the use of highly oxidizing acid mixtures will be likely to convert some of the element to this volatile form, so that it would be lost. Elements which, although fairly satisfactory during wet ashing, cause problems when dry ashing techniques are used are shown in table 6, and elements which just cause problems are shown in table 7. These are the ones which are prone to difficulties however you handle them, and pre-eminent in this group is mercury which is highly volatile itself and is also volatile in the form of most of its compounds.

Having looked at these groups of elements it is clear that more than one mechanism must operate in causing the losses. I have referred already to the three main groups of losses, by volatilization, by retention, or by nonavailability; within each of these there are also a number of further

TABLE 6. *Elements causing problems on dry ashing only*

Lithium	Copper	Zinc	Antimony	Rhodium
Sodium	Silver	Cadmium		Palladium
Potassium			Iron	Iridium
Rubidium	Beryllium	Thallium	Cobalt	Platinum
Cesium			Nickel	

TABLE 7. *Elements causing problems under most conditions*

Gold	Selenium
Mercury	Lead
Germanium	Arsenic
Tin	

subdivisions which all contribute to the complexity of the whole. If we start with the first type of loss, loss by volatilization, this can be brought about by at least five different mechanisms. The first of these relates simply to the volatility that is inherent in the element and its compounds, and must in this instance relate to mercury. Most oxidizing mixtures used in wet digestion involve temperatures close to the boiling point of sulfuric acid and at this temperature substantial amounts of mercury will escape from an open system. Accordingly, the technique which has generally evolved is to trap the distillate from the oxidation system and recombine it with the residue prior to the determination; this works reasonably well.

However, as this loss is related to the relatively high temperature of boiling sulfuric acid, it might be considered that an advantage would be gained by the use of perchloric acid mixtures which can be handled in

such a way as to give a readily maintainable temperature ceiling of about 200 °C (fig. 1). If we look at the bottom line on this figure, we can see that distilling a mixture of nitric, perchloric and sulfuric acid at temperatures which do not exceed 200 °C does lead only to a fairly small transfer of mercury amounting to some 10 percent. However, there is another problem in that perchloric acid is reduced at high temperature to give hydrochloric acid and, if rather hard to oxidize materials are being handled, hydrochloric acid is generated at a temperature at which mercuric chloride is substantially volatile and the rate of loss shoots up rapidly.

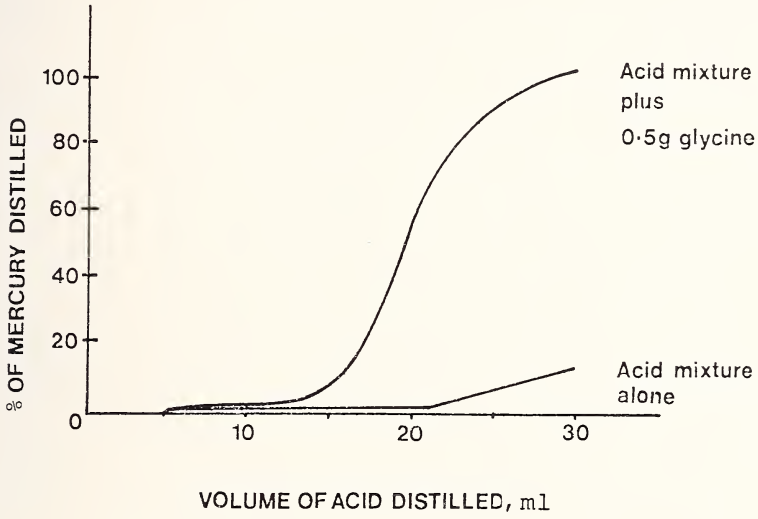

Figure 1. Effect of organic matter on distillation of mercury.

So far, we have shown two loss mechanisms, inherent volatility and interaction with a reagent, or at least a breakdown product of one. The next one is a similar case in which the volatile form of the element of interest is produced *in situ* by interaction with a breakdown product of the sample being digested. This type of loss occurs with a number of elements, but the example I would like to quote is arsenic and some relevant figures are shown in table 8. From these figures it can be seen that when an organic material containing no chlorine was oxidized with sulfuric acid and hydrogen peroxide the recoveries were very good, but when either inorganic chlorine as sodium chloride or covalently linked chlorine as in the PVC were present, the recoveries fell off sharply. It could be suggested that with the sodium chloride preliminary dilution and evaporation at a low temperature would have removed the chloride iron and prevented the loss, but in the case of the PVC the chloride was produced only at a high

temperature when the plastic was decomposed. It is hard to see how this sort of problem can be eliminated.

The fourth of the volatilization mechanisms has already been mentioned. This is the oxidation of the material to a volatile form and occurs with technetium, rhenium, ruthenium and osmium, all of which have a volatile oxide corresponding to the highest valency state. These will be lost when the strong oxidizing mixtures used for the destruction of organic matter are applied to samples containing the elements.

The last of the volatilization mechanisms is the reduction of an element to a volatile form. This mechanism certainly operates in the case of selenium and probably also with tellurium and polonium, the related elements. Some recoveries of selenium under various oxidizing conditions are shown in table 9. As can be seen, dry ashing led to almost complete loss of selenium due to the reducing conditions that were rapidly established. Nitric and perchloric acids on the other hand gave excellent recoveries as an oxidizing condition was maintained throughout. The last two pairs of results both refer to the use of nitric and sulfuric acids but in one case the oxidation was carried out very slowly with excess nitric acid present at all times so that the mixture never darkened, while the last set of results were carried out with rapid heating so that the mixture charred heavily. As can be seen, the experiment in which oxidizing conditions were maintained gave relatively good recoveries, while the heavily charred system gave almost total loss.

TABLE 8. *Arsenic recoveries after digestion in sulfuric acid and hydrogen peroxide*[a]

Sample material	Recovery %	
Cocoa & sodium chloride	64	56
Polythene	97	99
PVC	3	3

[a] As-74 tracer.

TABLE 9. *Selenium recoveries under various oxidizing conditions*

Oxidation method	Recovery %	
Dry ashing	3	4
Nitric and perchloric acids	100	101
Nitric and sulfuric (no charring)	95	98
Nitric and sulfuric (heavy charring)	4	4

For comparison with the position described with arsenic, some further results for selenium, obtained using sulfuric acid and hydrogen peroxide, with the two plastic materials PVC and polyethylene are shown in table 10. You will remember that loss of arsenic was high with PVC but low with polyethylene. In this case it can be seen that the selenium was recovered quantitatively when no organic material was present in the system, but the addition of either PVC or polyethylene led to substantial losses, again suggesting that the mechanisms are quite different. In this instance the loss in the presence of polyethylene was greater than with PVC, possibly due to the larger quantity of carbon in the same weight of sample.

TABLE 10. *Selenium recoveries after digestion in sulfuric acid and hydrogen peroxide*

Organic materials	Recovery %	
None	98	99
PVC	66	72
Polythene	11	16

Most of the elements mentioned so far exhibit volatilization losses even under wet oxidation conditions so that the problems will naturally tend to be worse at dry ashing temperatures. Furthermore some other elements will show losses at these higher temperatures; the main addition to the list of volatile elements will be the alkali metals where quite serious losses have been reported at temperatures below 600 °C. The losses are likely to be more serious with chlorides, particularly of the higher alkali metals, but in most instances the use of sulfuric acid as an ashing aid is adequate to prevent volatilization.

The type of loss that is particularly characteristic of dry ashing is the retention of the element of interest on either the ashing vessel or on some solid material present in the sample, *i.e.*, the second of the three major types of loss mentioned earlier.

If we take the ashing vessel retention first, then it is clear that the behavior of the individual elements will be affected greatly by the type of ashing vessel used. In most cases the choice lies between platinum, silica and porcelain, and in the terms of this discussion the main distinction is between platinum on the one hand and the two silica based materials on the other. As these two types of materials will have very different reactivities, the nature of the problems encountered will also be different. With metallic platinum, the likely form of interaction will be diffusion of metallic element into the platinum surface. This is only likely to occur when the

element is already present in a metallic form or can be readily reduced to one and it would therefore seem that problems are likely to be encountered with silver, gold, and the platinum metals and, as will be mentioned later when discussing silica ashing vessels, also copper.

If we now turn to the silica based vessels, which do have some economic advantages, it can be recognized that the most probable mechanism for retention would be reaction between the oxide of the element being determined and the surface of the silica based material. What would be happening would be the formation of complex, glass-like materials, by solid-state interactions between the oxide of the element to be determined and the surface of the vessel, and when looked at in this light, the amount of the loss might well be expected to show some relationship to the readiness with which these glass-like compounds can be formed. There is some evidence for this type of relationship and lead, which is one of the elements most easily incorporated into a silicate glass network, is also one of the elements most readily lost by retention on silica vessels during dry ashing. However, having formed a glass, there will be no loss of the element unless this glass is stable to the acid reagents generally used for dissolving the ash, and it is therefore interesting to note that copper does show serious retention losses although its silicates are very vulnerable to acid attack. One possible explanation of this is that copper does not combine with silica vessels in this way but by reduction of the oxide to copper during the ashing stage and diffusion of the metal so formed into the surface of the silica vessels.

This discussion has been concerned mainly with ashing vessels made of silica but it is clear that as silica is also a very common component of the ash of organic materials then many of the arguments should apply to the retention of trace elements on this type of ash also. Results have been quoted over many years to show that the losses of some trace elements can be related directly to the amount of silica present in the ash.

Talking about the silica present in the sample leads very readily to a discussion of other inorganic materials which may be present in the sample or which may be added purposely before dry ashing is carried out.

With regard to the inorganic materials occurring naturally in the biological samples being analyzed, we might reasonably expect the common ones to include sodium chloride and various kinds of phosphate. There is not very much information in the literature on the effects of these materials or recoveries but a few figures obtained for the retention of lead after heating in silica crucibles at 650 °C in the presence of various inorganic compounds, where the high temperature was used to emphasize trends not to represent actual conditions are shown in table 11. From this table

TABLE 11. *Effect of inorganic materials on lead recovery during dry ashing*[a]

Material	% lead retained	
None	22	23
H_3PO_4	46	38
NaH_2PO_4	2	2
Na_2HPO_4	2	2
Na_3PO_4	14	16
NaCl	62	56

[a] Conditions: 2 μg $PbNO_3$ with 10 mg of inorganic material; heated in silica crucible at 650 °C.

it can be seen that although phosphoric acid does cause serious retention losses none of the sodium phosphates give rise to losses as serious as those found in the absence of any inorganic material. It would therefore seem that the types of phosphate to be found in biological materials are not likely to cause serious retention problems. When we look at the figures for sodium chloride, however, a very different picture emerges. The quoted retention losses of something like 60 percent are probably underestimates of the true position because of the nature of the determination, but even so they indicate that the majority of the lead is rendered nonrecoverable when heated in the presence of sodium chloride, albeit at a fairly elevated temperature. That this is not an isolated phenomenon is demonstrated by the data in table 12 which shows the results of similar experiments with iron, cobalt, zinc, chromium and antimony, at a slightly lower temperature. As you can see, there are very serious retention losses in each case and, indeed, I have reason to believe that all of these losses are underestimated because of certain limitations of the technique used.

A possible explanation for these losses, all of which occurred when the elements were heated in silica crucibles, is that the presence of sodium chloride weakens the surface of the silica allowing the interaction with the

TABLE 12. *Effect of sodium chloride on the recovery of trace elements during dry ashing*[a]

Element	% retained	
Iron as nitrate	29	33
Cobalt as nitrate	7	15
Zinc as nitrate	59	47
Chromium as chromate	46	34
Antimony as antimonite	36	31

[a] Conditions: 2μg of the trace element with 10 mg NaCl; heated in silica crucible at 600 °C.

trace element to take place more readily. Salt is well known for its action on silicate materials and its use as a simple glazing material is long standing.

Reports of low recoveries of trace elements in the presence of sodium chloride have long been known and it has been suggested that they are caused by volatilization through the formation of volatile chlorides by reaction of the trace element with the sodium chloride. It was for that reason that the five elements shown in the last table were originally examined, as they all do form volatile chlorides. However, the technique used allowed the total amount of the trace element, both recoverable and retained, to be measured and it was clearly shown that there were no volatilization losses at all. In addition, the free energy changes that would be involved in the reaction between the compound used and sodium chloride are all strongly positive so that the production of volatile chlorides by this mechanism is not feasible. See table 13.

However, the fact that volatile species are not produced by reaction with sodium chloride does not mean that they cannot be produced by reaction with other chlorides. The recoveries obtained when a number of elements were heated with other inorganic chlorides, mainly ammonium chloride but including a few others as well are shown in table 14. In this case the recovery figure would include any material that was retained by the crucible so that the losses shown are due solely to volatilization.

Just to complete this gloomy picture of the problems that arise when dry ashing chloride containing materials, I would like to put in the next table which isn't quite in the right place. It is a very simple table which shows the losses of lead that occurred when dry ashing polyvinyl chloride (table 15). As you can see, virtually no lead was recovered and none was retained, presumably due to the production of a reactive chloride at a fairly high temperature, which converted the lead to lead chloride and allowed it to volatilize. Although a similar problem has not been demonstrated for other elements, there is every reason to believe that similar difficulties would be encountered for elements such as zinc or antimony.

I would now like to pass on briefly to the inorganic materials that are added on purpose before dry ashing, *i.e.*, the so-called "ashing aids." They are added for one or both of two purposes, either to hasten the oxidation of the organic material or to improve the recovery of the trace element of interest. The simplest of the auxiliary oxidizing materials is nitric acid which is very frequently added for the sole purpose of speeding up the oxidation of residual carbonaceous material when the bulk of the sample has been ashed. Similarly, it is one function of the inorganic nitrates such as magnesium nitrate or aluminum nitrate, to hasten the decomposi-

TABLE 13. *Free energy changes for reactions with sodium chloride*

Reaction	△ Fo
Sb_2O_3 + 6NaCl	+ 262
CoO + 2NaCl	+ 91
Fe_2O_3 + 6NaCl	+ 314
PbO + 2NaCl	+ 73
ZnO + 2NaCl	+ 79
FeO + 2NaCl	+ 88
As_2O_3 + 6NaCl	+ 272

TABLE 14. *Recoveries of trace elements on heating with inorganic chlorides.*

Element	Added chloride	Recovery %
Antimony	Ammonium	11, 6, 19, 8.
Lead	Ammonium	69, 75, 54, 76.
Zinc	Ammonium	7, 7, 9, 6.
Zinc	Magnesium	53, 53, 52.
Zinc	Calcium	4, 4, 4, 4,.
Zinc	Barium	99, 101, 100, 100.

TABLE 15. *Recovery of lead from polyvinyl chloride during dry ashing*[a]

Organic material	Lead recovered %	Lead retained %
PVC	1	1

[a] Conditions: 2μg lead as the nitrate with 2 g polyvinyl chloride; heated at 600 °C.

tion of the organic material, but in addition they also serve the second purpose of improving recovery by separating the reactive trace element from the reaction vessel or other inorganic material present in the sample and so reducing the likelihood of retention losses.

Thirdly, we come to the materials such as sulfuric acid whose only purpose is to improve recovery. The great advantage of sulfuric acid is that, almost regardless of the original chemical form of the trace element, it will convert the element to its sulfate which, generally speaking, is the form least likely to volatilize. A further extremely important function is that it will very often remove potentially interfering materials, such as chloride, and so prevent volatilization and retention losses. When this is the intention, the sulfuric acid should be added in dilute solution so that the

hydrochloric acid produced will be removed at a temperature of about 100 °C, when there is little risk of loss of the trace elements by volatilization as the chloride. Nonetheless, in applying these techniques, it is helpful to be aware of the potential problems.

That really covers all that there is time for within the short period of this lecture. I am sure it must all seem very confusing, so just to finish off I want to add a couple of summary tables to try to remind you of the problems that can be encountered during the oxidation of organic samples and as an indication of the mechanisms that are responsible. At the beginning three major problem areas, volatilization, retention, and nonavailability were mentioned and the major aspects of the first two of them are summarized in tables 16 and 17. It should be pointed out that not all of these mechanisms can be taken as certain, and I am sure that some, or even all, of them can be open to attack. However, they do go some way to explain the observed facts and they might be helpful in deciding the best methods to use.

TABLE 16. *Mechanisms of volatilization losses*

1. Inherent volatility.
2. Oxidation to a volatile form e.g., RuO_4.
3. Reduction to a volatile form e.g., selenium.
4. Interaction with sample e.g., production of HCl from PVC.
5. Interaction with reagent e.g., reduction of $HClO_4$ to HCl.

TABLE 17. *Mechanisms of retention losses*

1. Coprecipitation, e.g., lead on calcium sulphate.
2. Reduction and diffusion, e.g., gold in platinum, copper in silica.
3. 'Glass' formation, e.g., lead in silica.
4. Attack by sample, e.g., NaCl on silica.

II. Conclusion

Having said all that, what conclusions can we draw? First of all, it is clear that the decomposition of an organic material prior to its analysis is not the simple matter it was believed to be in the days when a paper or trace element analysis could dismiss the whole subject in the words "the sample was ashed." It is clear also that some elements such as mercury and selenium, and perhaps also gold and lead, must always be treated with care as they are quite capable of causing trouble in both wet and dry ox-

idation techniques. For most of the other elements and, indeed, even for these four, it is possible to define oxidation techniques which will give good recoveries provided the sample itself does not introduce extra problems. The commonest problem that the sample can introduce is the presence of large amounts of chlorine in it, either as chloride ion, or as covalent chlorine. As has been shown, both of these are capable of causing extreme problems although, if its presence is known, chloride ion can generally be dealt with by acidification with dilute acid and evaporation to remove the chloride as hydrochloric acid at a low temperature. If the chlorine is present as covalent chlorine then it is likely to cause problems with quite a wide range of elements. When, in addition, it is bound into a material such as polyvinyl chloride, which is very resistant to attack, the problems can be very great indeed. However, by their nature, these extreme cases are exceptional and for the majority of elements in the majority of samples perfectly adequate techniques are available today.

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THE ROLE OF THE ANALYTICAL BLANK IN ACCURATE TRACE ANALYSIS

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The inability to control the analytical blank, *i.e.*, contamination from all sources external to the sample, has seriously affected the accuracy of low level trace determinations. Most of the sources of the blank are variable and it is this variability that determines the uncertainty of the blank correction and, therefore, the lower limit of trace element concentration that can be determined with reliability. To improve both the accuracy and lower limit of trace determinations, it is imperative to control the variability of the analytical blank. The only practical way to accomplish this is to reduce the size of the blank itself by controlling the sources of the blank.

The analytical blank is composed of contamination from four principal sources, namely: the environment the analysis is performed in, the reagents used in the analysis, the apparatus used, and the analyst performing the analysis.

Environmental contamination is caused by particulates and gaseous compounds in the ambient air. Methods for reducing the blank from this source by the use of isolation chambers or "absolute" filters are described.

While commercially available high-purity reagents have helped the analyst to control the blank from reagents, they are frequently not low enough in trace element concentration and must be purified before use. Methods for the preparation of high-purity reagents, especially water and the mineral acids, are reviewed.

Contamination from beakers, containers, mortars and other apparatus can seriously affect the blank. Techniques for controlling this type of contamination are described with emphasis on the purity of materials.

The last source of contamination is that caused by the analyst during the analysis due to carelessness or poor technique. Suggestions are given to aid the analyst in this regard.

Keywords: Accuracy; analysts; apparatus; blank; contamination; environment; purity; reagents.

I. Introduction

The analytical blank may be considered the "Achilles' heel" of trace analysis. As the Greek warrior of Homer's Iliad had his vulnerable point, so also does trace analysis in that the size and variability of the analytical blank may render useless the information from the analysis. First, let me define what is meant by the analytical blank. The analytical blank is simply the contamination by the element or compound being determined from all sources external to the sample. I am not including in this definition the so-called instrumental blank or noise level which really refers to the sensitivity or level of detection of the analytical method. Also not included in this definition is the so-called negative blank, that is loss of an element during analysis by physical processes such as adsorption. This is really a separate subject unto itself. Obviously, this definition includes both inorganic and organic contamination, but my remarks will deal mainly with trace inorganic cation contamination.

Modern methods of analysis have lowered the threshold of determining trace elements to the low parts per billion (ng/g) for many elements but the inability to control the analytical blank has seriously affected the accuracy of these methods. The blank is a problem common to all trace element techniques with the exception of certain nuclear techniques such as neutron activation analysis and fission track analysis.

Let's look at the effect of the blank on the accuracy of a trace analysis. During the analysis, the analyst normally carries "blanks" thru all the steps of the analysis and makes a "blank-correction" of the analysis based on the estimation of the contamination from these blank determinations.

Figure 1 shows the effect on accuracy of a 10 ng blank that exhibits a variability of ± 5 ng. This represents a very low blank with a reasonable uncertainty. It is not uncommon, even in very careful work to have blanks an order of magnitude higher. Notice that at the microgram level, the effect on accuracy of a blank of this size is small, but that it increases rapidly until at the 0.01 μg or 10 ng level, a 50 percent uncertainty would be introduced by the blank. If the blank were an order of magnitude higher with the same percentage variability, a determination at the 0.1 μg level would have a 50 percent uncertainty due to the blank alone.

It is the variability or the uncertainty in the blank correction [1] and not the absolute value of the blank that affects the accuracy of the analysis. As the variability becomes a significant percentage of the sample amount, more and more determinations of the blank are necessary so that the variability can be estimated with some degree of reliability. It is a common mistake of analysts to base the blank correction on a single

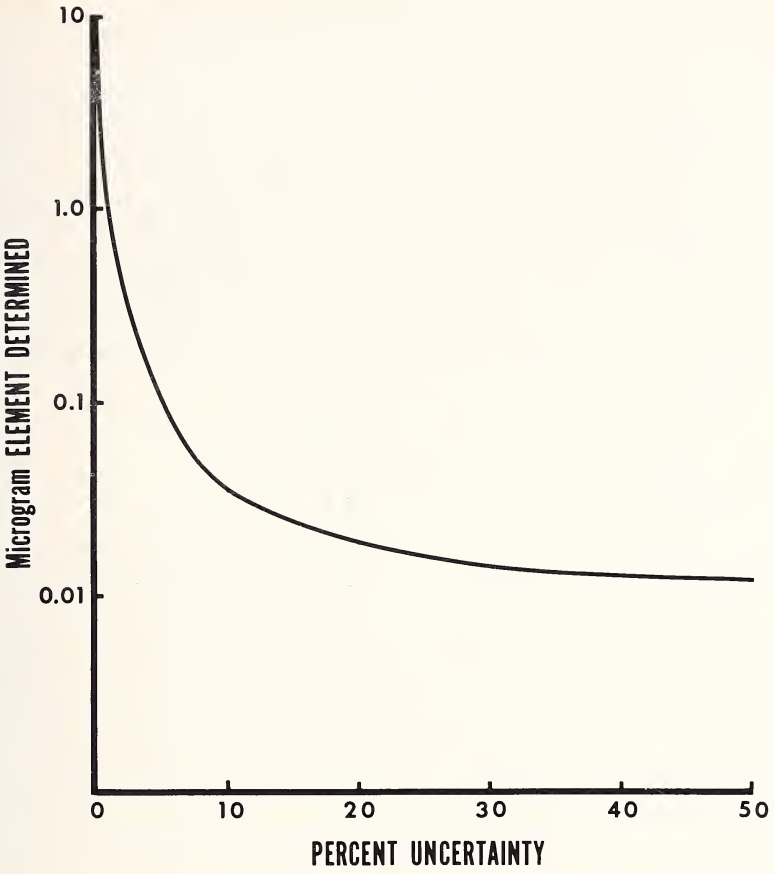

Figure 1. Effect of a 10 ± 5 ng blank on accuracy of analysis.

determination and have no information on the approximate variability or effect on accuracy.

One might argue that, since it is the variability and not the absolute value of the blank that affects accuracy, the way to increase accuracy is to have large blanks but with reduced variability. This is certainly not true in the real world of trace analysis. Firstly, it is inherently inaccurate to base a result on the difference between two signals or numbers of relatively the same size. But secondly, and more importantly, real blanks, large as well as small, do exhibit marked variability since most of the sources of the blank are not constant. The only practical way to lower the variability and increase the accuracy of the blank correction is to reduce the size of the blank. It is obvious from figure 1 that as one goes to lower levels of trace concentrations the blank becomes more and more of a factor. Figure 2

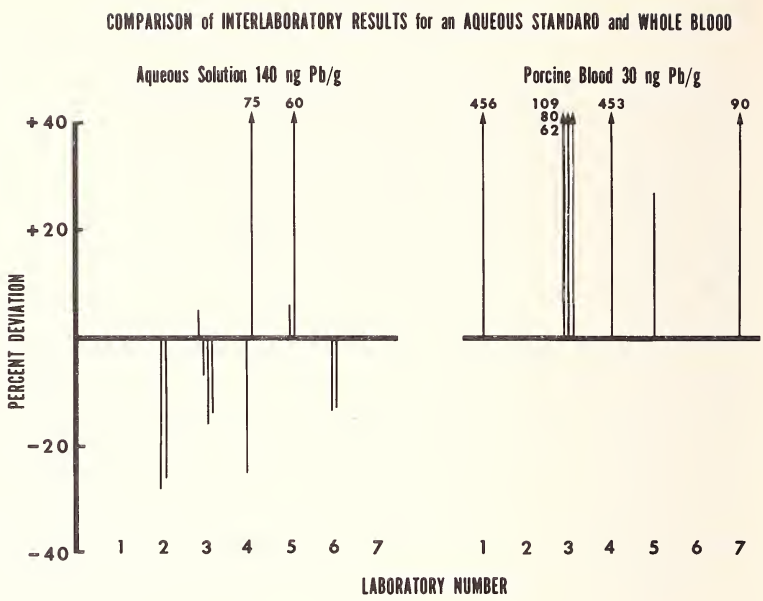

Figure 2. Comparison of interlaboratory results for an aqueous standard and whole blood.

represents two parts of a study to develop a referee method for lead in blood by atomic absorption spectrophotometry. The left portion represents the results from different laboratories for the analysis of an acidified aqueous standard. No chemistry was necessary and the results fluctuate, not very accurately, around the "base-line" value. But notice what happens when the same laboratories analyze a real blood sample as shown on the right portion.[1] The level of lead was a factor of five lower than the standard and some chemistry was necessary. The results varied from 30 to 450 percent high, in all probability due to lack of control of the analytical blank.

This is but one example of the fact that as one goes to lower trace concentrations, the blank becomes the dominant factor on accuracy and determines the lower limit of trace element concentration that can be determined with reliability. It is imperative to control and reduce the analytical blank if improvements in precision and accuracy of trace determinations are to be made.

[1]This is for porcine blood which contains much lower lead than human blood.

II. Sources of Contamination

How does one go about lowering a blank? The sources of the blank must be considered and steps taken to reduce the contamination from each source.

The analytical blank is composed of contamination from four principal sources, namely, the environment the analysis is performed in, the reagents used in the analysis, the apparatus used, and the analyst performing the analysis. Of these factors only the contamination from reagents is relatively constant while the contamination from the other factors is variable and determines the variability of the analytical blank.

A. Environment

Let us consider environmental contamination. Fall-out or absorption of particulates or gases from the laboratory air can cause significant and variable amounts of contamination of the sample or a solution of the sample. As much as 200 μg of particulates per m^3 of air have been reported in the air of an analytical laboratory [2] and it was shown to contain large amounts of calcium, silicon, iron, aluminum, sodium, magnesium potassium, thallium, copper, manganese and lesser amounts of several other elements. Analysts in Dortmund, Germany, were not able to lower iron blanks lower than 0.04 μg/ml because 20 tons of ferric oxide were dispersed into the air from nearby plants [3]. Table 1 shows the lead particulate concentrations from three different locations. The lead concentration from downtown St. Louis, 18.84 $\mu g/m^3$, was much higher than rural Missouri, 0.77 $\mu g/m^3$, [4] due to automotive exhaust pollution. Our

TABLE 1. *Examples of lead concentration in air*

Site	Lead concentration $\mu g/m^3$
Downtown, St. Louis, Missouri[a]	18.84
Rural Park, Southeastern, Missouri[a]	0.77
Laboratory air, NBS, Gaithersburg, Maryland[b]	0.4

[a] See reference [4].
[b] See reference [5].

laboratory air at NBS, Gaithersburg, Maryland [5] compares favorably to rural air but still contains too much lead for low level work. Wide variations in the particulate content of air is to be expected. Useller [6] has stated that in a typical rural area airborne particulate counts larger than 0.5 μm of 1,400,000/m^3 are not unusual and that in metropolitan areas counts of 53,300,000/m^3 are normal.

The use of evaporation chambers has been one approach to reducing airborne contamination. Evaporations are performed in a closed system under filtered air or nitrogen. Figure 3 shows some different evaporation chambers from the literature. Chamber *(a)* was designed by Thiers [7,8]. Filtered air is passed over the solution and out the bottom while the sample is heated from below with a hot-plate and above with a heat lamp. Chamber *(b)* is a German design by Koch [9] in which filtered air or nitrogen is passed over the sample solution. A heated quartz dome

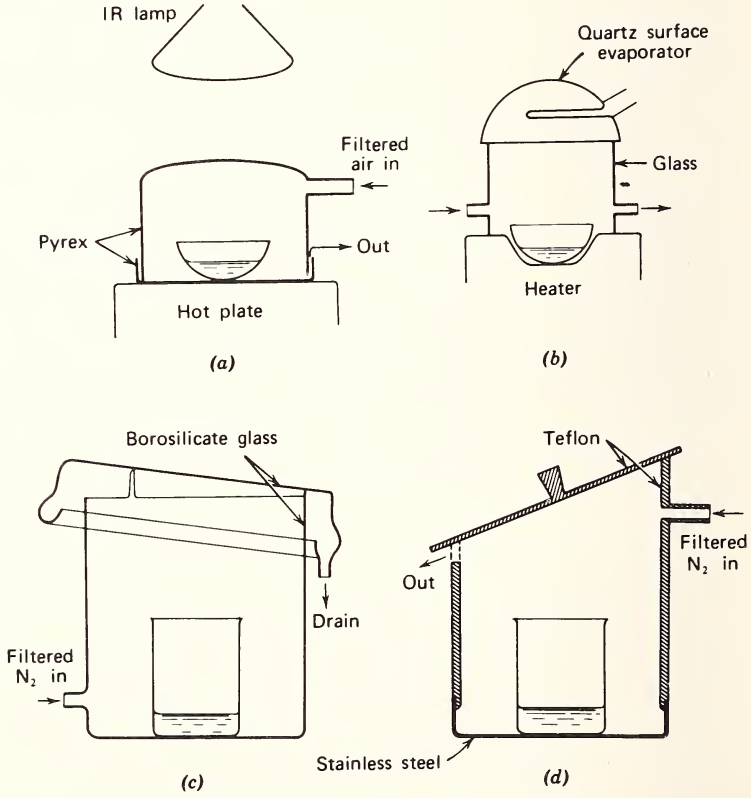

Figure 3. Evaporation chambers from the literature, reference 35. Reprinted with permission of John Wiley & Sons, Inc.

prevents condensation of liquids. The last two chambers, *(c)* and *(d)*, were used by Chow and McKinney [10] for the analysis of high-purity HCl (Glass) and HF (Teflon).

Figure 4 is a drawing of an evaporation chamber that has been used by Alvarez at NBS [11]. The solution is evaporated by heating from above and below the chamber while filtered nitrogen passes over the solution until only a drop remains. The drop is then drawn up into the capillary and loaded onto gold wire for analysis by spark source mass spectrometry.

Table 2 shows the results of Chow and McKinney [10] for the analysis of 500 ml samples of high-purity hydrochloric acid which was evaporated

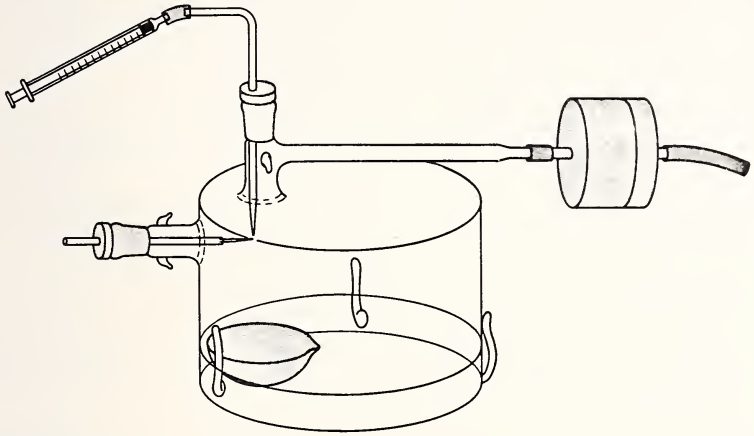

Figure 4. Clean environment evaporator and transfer system.

TABLE 2. *Lead contamination of labware after exposure to air*[a]

Beaker	Condition	Laboratory	Time (days)	Lead μ(g)
Teflon	Open	Ordinary	8	4.07
				2.32
	Nitrogen-flushed container	Ordinary	8	1.13
	Open	Pure air	8	0.44
	Nitrogen-flushed container	Pure air	8	0.18
				.13
	Nitrogen-flushed container	Pure air	1	0.02
Borosilicate glass	Nitrogen-flushed container	Pure air	1	.03

[a] See reference [10].

slowly under different conditions. When the HCl was evaporated over a period of 8 days from an open Teflon beaker, the lead contamination was high and variable. Using a clean air chamber under nitrogen reduced the lead contamination only by a factor of about three. Both of these evaporations were performed in a normal fume hood. (Since the volume of air moving across an area in a fume hood is many times greater than the air moving across the same area in the laboratory, the contamination is greater in the hood than elsewhere in the laboratory.) When the same evaporations were performed in a clean air laboratory in which dusts and aerosols were removed by electrostatic precipitation and filtration, the lead contamination was reduced by a factor of 10 when the HCl was evaporated open and by a factor of 20 when it was evaporated in an evaporation chamber flushed with nitrogen.

The major development to date for providing particulate free air is the high efficiency particulate air filter, better known as the HEPA filter [12]. This filter was developed during World War II for the Manhattan Project to remove fissionable particulates from air and to this date remains the most efficient means of air filtration. The HEPA filter has an efficiency of 99.97 percent for 0.3 μm particles and are routinely employed for supplying air that meets the Class 100 specification [13], that is less than 100 particles per cubic foot of air larger than 0.5 μm. These filters, coupled with the laminar airflow principle [14] in which the "clean" air is moved unidirectionally, have been used for the last decade to provide clean particle-free work areas.

Figure 5 is a view of the clean room located in the Analytical Chemistry Division at the National Bureau of Standards. The HEPA filters are located on the far wall. In fact the entire wall is composed of HEPA filters causing a wall of clean air to pass unidirectionally down the room. This room is really a particulate-free room and cannot be used as a clean laboratory since it was not designed for acid vapor.

Figure 6 is a cut-away view of the clean laboratory also located in the Analytical Chemistry Division at NBS. This room was designed for trace inorganic analysis and can be used for chemical operations such as acid dissolutions and evaporations. All of the air entering the room is passed thru prefilters and then thru the HEPA filters located over the center work-bench of the room. The Class 100 clean air is directed unidirectionally down and out across the bench to prevent particles from entering the area. Plexiglas dividers prevent cross-contamination. The air is recirculated thru the filters by drawing air at the base of the outside walls. In addition to the "clean" area under the center bench, there are several Class 100 hoods that provide clean work areas on the outside bench. Acid dissolutions and evaporations are performed in the two

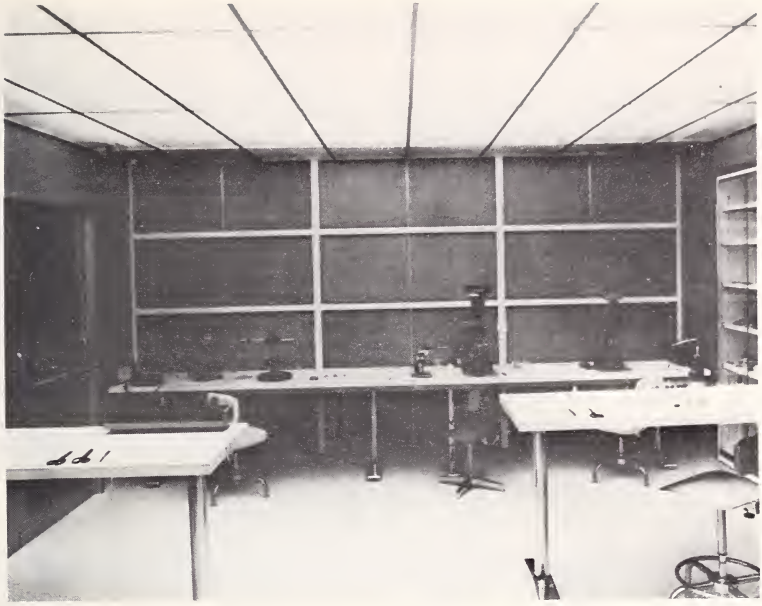

Figure 5. Particulate free clean room – National Bureau of Standards.

hoods at the rear of the room. These are Class 100 clean air hoods lined with sheet Teflon and are so designed that all air 12 cm from the front is exhausted while a curtain of air moves out the front to prevent particulates from entering the hood.

Figure 7 is a schematic of a laboratory used at Bell Telephone Laboratories for trace analysis [15]. The inlet air to the room is non-laminar flow clean air which has been filtered thru HEPA filters. The air to the clean hood is further purified by recirculation thru HEPA filters and the room is kept under positive pressure. Using this room, no significant contamination of water samples could be detected by spark source mass spectrometry or activation anaysis.

Table 3 shows a comparison of particulates in the air of an ordinary laboratory, a clean room, and a Class 100 hood, all at NBS [5]. Both the clean room and Class 100 hood air showed a dramatic reduction in contamination. Lead concentrations were reduced by a factor of over 1000, iron by a factor of 200 and the other elements, which were low to start with, by a factor of 10.

While HEPA filters are a powerful tool in reducing particulate contamination, they are not the complete answer to environmental con-

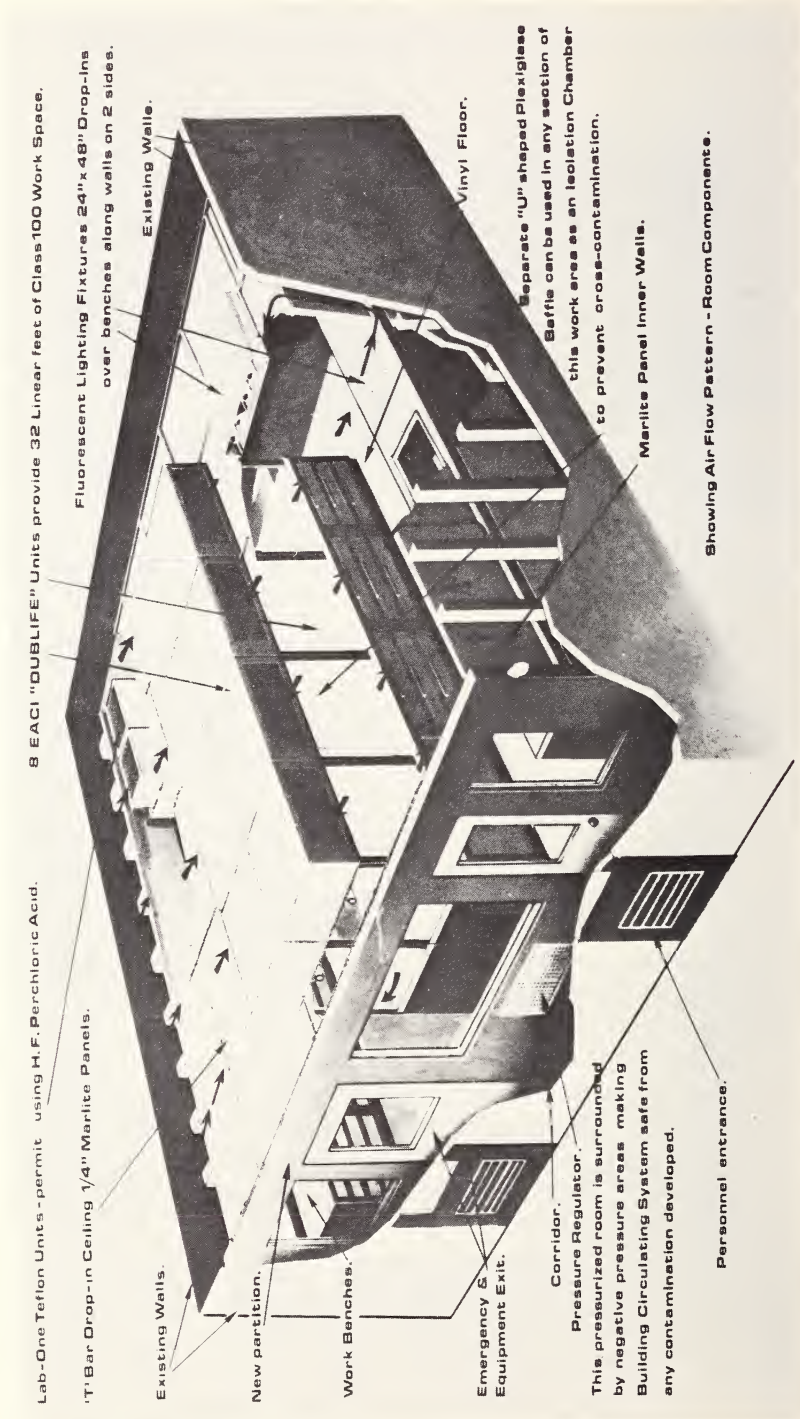

Figure 6. Cut-away of clean laboratory facility for trace level sample preparation.

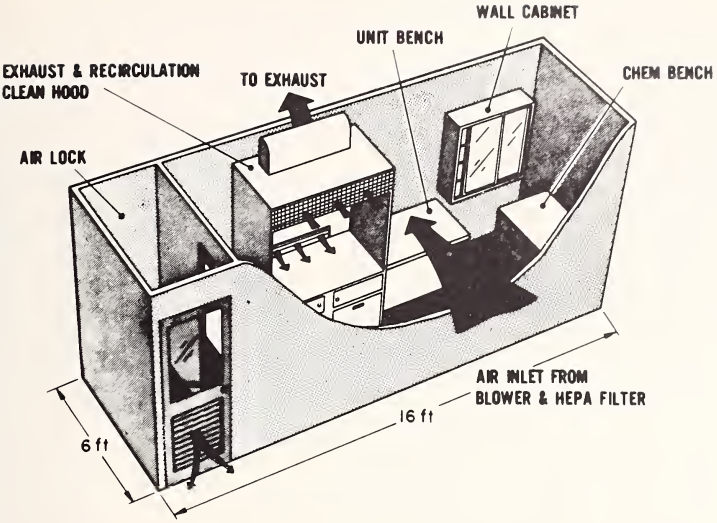

Figure 7. Schematic of clean laboratory—Bell Telephone Laboratories, reference 22. Reprinted with permission of Analytical Chemistry.

TABLE 3. *Particulate concentration in laboratory air*[a]

	Concentration, $\mu g/m^3$			
	Iron	Copper	Lead	Cadmium
Ordinary Laboratory	0.2	0.02	0.4	0.002
Clean Room	.001	.002	.0002	n.d.
Clean Hood	.0009	.007	.0003	0.0002

[a] See reference [5].

tamination. Figure 8 shows the particle size of some common contaminants. Since HEPA filters are efficient for the removal of particles 0.3 μm or larger, particles as small as bacteria are removed, but some common particulates such as tobacco smoke are not efficiently removed since they are smaller than 0.3 μm. Obviously gases, both organic and inorganic, are not removed. To remove these contaminants, the air must be passed thru absorbants and scrubbed, which is an expensive operation. Most laboratories either live with the small amount of contamination that results from clean room operation or resort to the evaporation chamber technique in the clean room to further reduce environmental blanks.

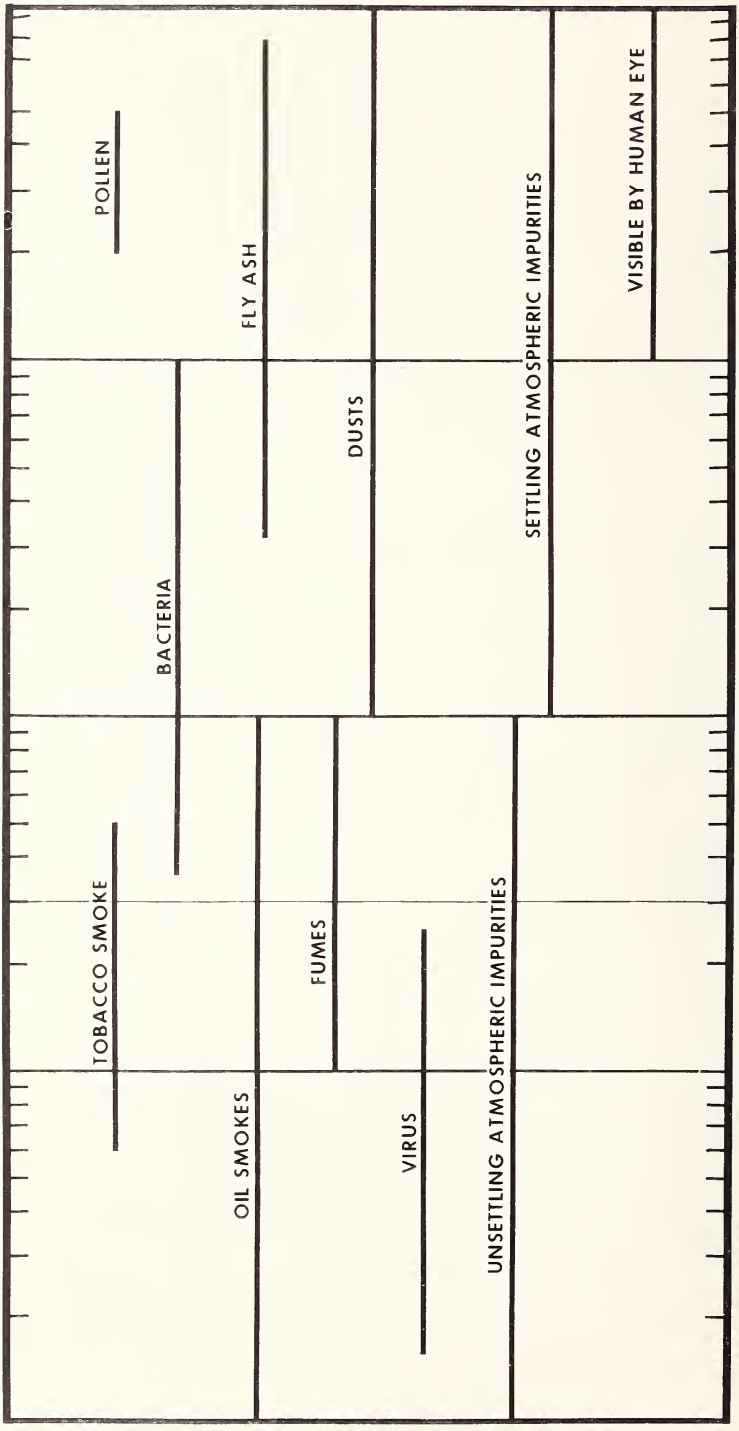

Figure 8. Relative size of common air contaminants.

B. Reagents

The chemical reagents used during the course of an analysis are an important source of the analytical blank. The first order of priority for a trace laboratory in regard to reagents is a readily available supply of extremely high-purity water. There are several commercial systems on the market for producing "conductivity water"; that is, water whose conductivity approaches the theoretical conductivity of 0.055 micromho/cm at 25 °C [15,16]. These systems are based on the use of mixed-bed or monobed ion exchangers, in which cation and anion exchange resins are mixed in a single unit, and sub-micron filtration. Measured conductivity of deionized water has been reported as low as 0.05 micromho/cm (temperature not specified) [16]. However, only ionized compounds are detected by conductivity measurements and soluble unionized compounds or particulates are not detected. Both soluble organic materials and particulates are generated by ion-exchange columns. Studies at the Philips' Laboratories [17] have shown that deionized water with a conductivity of 0.06 micromho/cm contained up to 100 ppb of cation contamination. They postulated that the detected impurities were present in particulate or colloidal form and were not appreciably ionized. Another possibility is that the cations form unionized complexes with dissolved resin material. In fact there is some literature evidence [18-20] to show that small amounts of heavy metals can be masked in deionized water and lost to polarographic analysis due to chelation by soluble resin. Whatever the reason, there is ample evidence in the literature [17] to prove that conductivity is not a reliable measure of trace element concentration in water.

Dr. Ralph Thiers [8] of Harvard wrote in 1957, "Since non-ionic materials are not held by ion-exchange resins, and since traces of nitrogeneous compounds are leached off the resin by water, probably the best treatment for obtaining purest water in all ways is ion-exchange followed by distillation from fused quartz." Nothing has happened in the last two decades to change the validity of that statement and this position has been supported by other investigators [21-23].

The system used by our group at NBS consists of taking the "building distilled" water as it is supplied to the laboratory and redistilling it in a conventional still equipped with a quartz condensing system. The distillate from this still is fed to a quartz "sub-boiling" still (Quartz Products Corp., Plainfield, N.J.) where the water is again distilled, but by a non-boiling technique which will be described later. The "building distilled" water consists of ordinary city water that has been passed thru a mixed-

bed deionizer, distilled in a tin-lined still, stored in a tin-lined tank and distributed thru tin pipes to the laboratory.

Table 4 shows the results of the analysis of the "sub-boiling" distilled water and the "building distilled" water by spark source isotope dilution mass spectrometry (SS-IDMS). There was better than a tenfold reduction in impurities from "building distilled" water which is really good quality water for a high-volume operation. Copper at 3 ppb is the principal impurity in this water. The analysis of the "sub-boiling" distilled water, which totaled 0.5 ppb for the elements determined, really represents an upper limit for most impurities since no attempt was made to correct for a blank during the evaporation of the water. This water compares favorably with the best water reported in the literature. While it is one problem to produce high quality distilled water, it is quite another to keep it pure before use. We have been using quartz for storage and this analysis is for water stored in quartz for 2 weeks. We are currently studying the storage of water in Teflon.

Studies in the literature have shown that the trace element content of water stored in Teflon or polyethylene increased by a factor of 5 to 10 for some elements after 30 days storage [22]. As a general rule, distilled water should be used as soon as possible after preparation.

TABLE 4. *Impurity concentration in distilled water*

	Sub-boiling distilled (ng/g)	Building distilled (ng/g)
Pb	0.008	0.1
Tl	.01	<.01
Ba	.01	.004
Te	.004	.02
Sn	.02	.2
Cd	.005	.03
Ag	.002	.005
Sr	.002	.002
Zn	.04	1
Cu	.01	3
Ni	.02	0.1
Fe	.05	.4
Cr	.02	—
Ca	.08	1
K	.09	0.06
Mg	.09	2
Na	.06	0.1
Total Impurity	0.5 ppb	8.0 ppb

The next most important group of reagents for the trace analyst are the mineral acids. In recent years high-purity acids have been available from commercial suppliers. While these acids may prove quite satisfactory for a number of trace applications, the concentrations of some trace elements are too high for low level trace analyses so the analyst will have to purify his own acids in these cases. High-purity hydrochloric acid, hydrobromic acid, and hydrofluoric acid have been produced by saturating pure water with the pure gaseous compound [7,8,24-30] or by isopiestic or isothermal distillation [31,32]. The two-bottle sub-boiling method of Mattinson [33] has been used to produce high-purity HCl and HF [34]. Nitric acid, perchloric acid and sulfuric acid are usually purified by repeated distillations [35].

We have been purifying these acids by "sub-boiling" distillation from quartz or Teflon stills [11,36]. Figure 9 is a schematic of a quartz "sub-boiling still which is used for the production of high-purity water and the mineral acids with the obvious exception of hydrofluoric acid. The still is fed by a 6- to 8-lb bottle of ACS Reagent Grade acid through a liquid level control. Heating of the liquid being distilled is accomplished by a pair of infrared radiators positioned on both sides of the condenser.

The surface of the liquid is heated and evaporated without causing the liquid to boil. This positioning of the heaters also serves to heat the walls above the liquid, tending to keep them dry which minimizes creep of

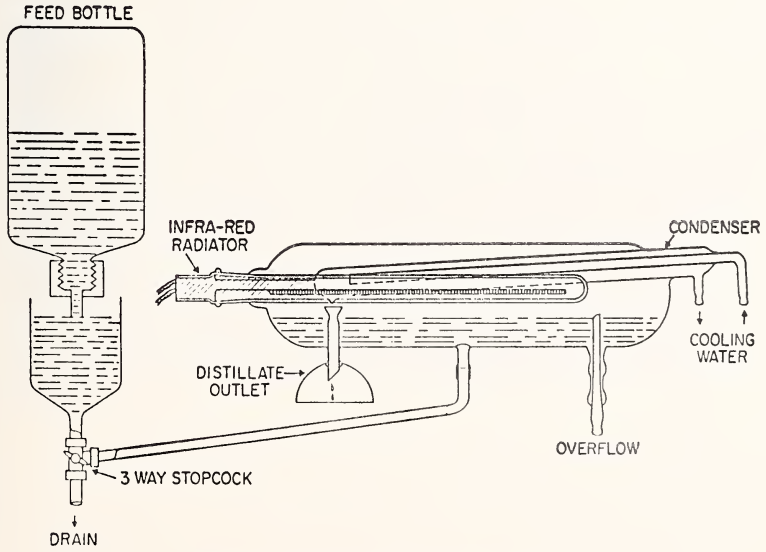

Figure 9. Pure quartz sub-boiling still.

liquid between the cold finger condenser and the liquid reservior. The cold finger condenser is tilted downward to allow the condensed liquid to flow to the tip above the outlet. The distillate is caught in Teflon FEP bottles which have been rigorously cleaned with nitric and hydrochloric acids.

Figure 10 is a schematic for the all Teflon sub-boiling still [11]. It was fabricated from a 2-liter TFE Teflon bottle and is designed on the same principal as the quartz still except that a trough of Teflon was hung from the cold finger to catch the drops of hydrofluoric acid dropping from the condenser.

Each "sub-boiling" still is housed in a clean air chamber, that is, a Class 100 hood, to protect the distillation process from external contamination. Figure 11 is a view of the laboratory for producing these high-purity acids. We produce enough high-purity acid in this facility to supply the entire Analytical Chemistry Division and some other laboratories with special needs.

Table 5 shows the results of the analysis of sub-boiling distilled hydrochloric acid, the starting ACS reagent grade acid, and a lot of commercial high-purity acid. A summation of these impurity elements shows that the sub-boiling distilled acid contained 6.2 ppb, the ACS reagent grade 820 ppb, and the commercial high purity, 70 ppb. The only element found in the sub-boiling distilled acid at a level higher than 1 ppb was iron

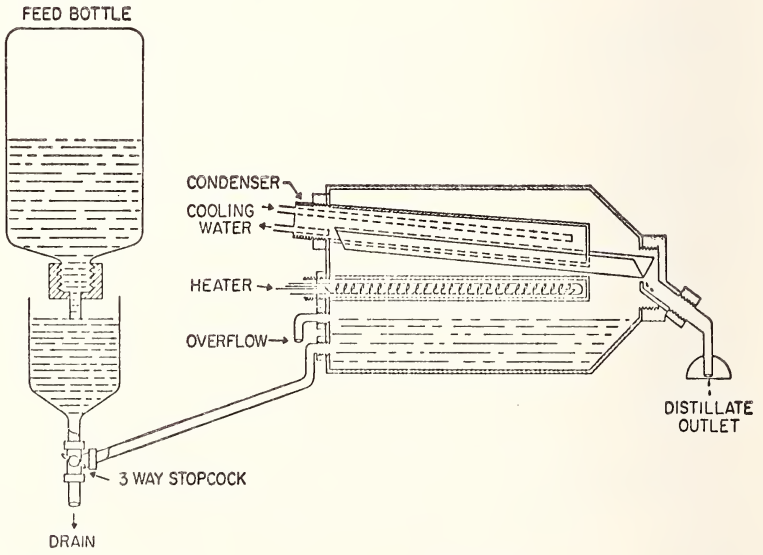

Figure 10. All-Teflon sub-boiling still.

Figure 11. Laboratory for production of high-purity acids.

TABLE 5. *Impurity concentration in hydrochloric acid*

	Sub-boiling distilled (ng/g)	ACS reagent grade acid (ng/g)	Commercial high purity (ng/g)
Pb	0.07	0.5	<1
Tl	.01	.1	—
Ba	.04	2	—
Te	.01	0.1	—
Sn	.05	.07	<6
In	.01	—	—
Cd	.02	.03	0.5
Ag	.03	.05	.2
Sr	.01	.05	—
Zn	.2	2	4
Cu	.1	4	1
Ni	.2	6	3
Fe	3	20	7
Cr	0.3	2	0.3
Ca	.06	70	24
K	.5	200	10
Mg	.6	10	20
Na	1	500	—
Total impurity	6.2 ppb	820 ppb	70 ppb

at 3 ppb. The other elements were at the sub-ppb level, generally lower than 0.1 ppb.

The results for the analyses of sub-boiling distilled nitric acid, the ACS reagent grade acid, and a lot of commercial high-purity acid are shown in table 6. The totals of the impurity elements determined were 2.3 ppb for the sub-boiling distilled acid, 220 ppb for the ACS starting acid, and 240 ppb for the commercial high-purity acid. No element was found in the sub-boiling distilled acid a concentration greater than 1 ppb and only sodium was detected at that level. Most of the elements were in the 0.05 to 0.01 ppb range.

Similar analyses for perchloric acid are shown in table 7. These analyses demonstrate the efficiency of sub-boiling distillation when the concentrations of impurity elements are compared to the starting acid. The concentration of barium which was greater than 1000 ppb was reduced to 0.1 ppb, a purification factor of greater than 10,000. Another example is calcium which was reduced to 0.2 ppb from 760 ppb in the starting acid, a factor of 3,800. It should also be noted that chromium which was present at 10 ppb in the starting acid was not significantly changed in the purified acid probably due to the formation of volatile

TABLE 6. *Impurity concentration in nitric acid*

	Sub-boiling distilled (ng/g)	ACS reagent grade acid (ng/g)	Commercial high purity (ng/g)
Pb	0.02	0.2	0.3
Tl	—	.2	—
Ba	.01	8	—
Te	.01	0.1	—
Sn	.01	.1	1
In	.01	—	—
Cd	.01	0.1	0.2
Ag	.1	.03	.1
Sr	.01	2	—
Se	.09	0.2	—
Zn	.04	4	8
Cu	.04	20	4
Ni	.05	20	3
Fe	.3	24	55
Cr	.05	6	130
Ca	.2	30	30
K	.2	10	11
Mg	.1	13	—
Na	1	80	—
Total impurity	2.3 ppb	220 ppb	240 ppb

TABLE 7. *Impurity concentration in perchloric acid*

	Sub-boiling distilled (ng/g)	ACS reagent grade acid (ng/g)	Commercial high purity (ng/g)
Pb	0.2	2	16
Tl	.1	0.1	—
Ba	.1	>1000	10
Te	.05	0.05	—
Sn	.3	.03	<1
Cd	.05	0.1	4
Ag	.1	0.1	0.5
Sr	.02	14	—
Zn	.1	7	17
Cu	.1	11	3
Ni	.5	8	0.5
Fe	2	330	10
Cr	9	10	18
Ca	0.2	760	7
K	.6	200	9
Mg	.2	500	4
Na	2	600	—
Total impurity	16 ppb	>3400 ppb	100 ppb

TABLE 8. *Impurity concentration in sulfuric acid*

	Sub-boiling distilled (ng/g)	ACS reagent grade acid (ng/g)
Pb	0.6	0.5
Tl	.1	.1
Ba	.3	.2
Te	.1	.1
Sn	.2	.6
Cd	.3	.2
Ag	.3	.6
Sr	.3	.4
Zn	.5	2
Cu	.2	6
Ni	.2	0.5
Fe	7	6
Cr	0.2	0.2
Ca	2	123
K	4	9
Mg	2	4
Na	9	50
Total impurity	27 ppb	200 ppb

chromyl chloride, CrO_2Cl_2. Chromium accounts for over half of the total impurities of 16 ppb found in the purified acid.

The analyses of sub-boiling distilled sulfuric acid, shown in table 8, showed that it contained 27 ppb of the elements determined. Sodium and iron were the principal contaminants, accounting for over half of the total impurities.

Hydrofluoric acid was purified using the all-Teflon still. Table 9 shows the results of the analyses of the starting ACS grade acid and the purified product. The total of the impurities in the sub-boiling distilled acid was 17 ppb compared to 320 ppb in the starting acid. The two principal impurities in the purified acid were calcium and chromium at 5 ppb, which accounted for over half the total impurities found.

The situation for salts and other reagents is not as favorable as for the acids. Again, there are special high-purity grades of some of these reagents available from commercial sources which are a major improvement over ACS reagent grade materials. However, there are relatively few of these reagents available and they often contain impurities at a level too high for use in low level trace analysis [22,23,37]. Hume [38] has stated in a recent article that natural sea water contains much lower concentrations of many heavy metals than artificial sea water made up from the

TABLE 9. *Impurity concentration in hydrofluoric acid*

	Sub-boiling distilled (ng/g)	ACS reagent grade acid (ng/g)
Pb	0.05	0.8
Tl	.1	.2
Ba	.1	.5
Te	.05	.1
Sn	.05	11
Cd	.03	2
Ag	.05	0.1
Sr	.1	.5
Zn	.2	4
Cu	.2	3
Ni	.3	12
Fe	.6	110
Cr	5	20
Ca	5	14
K	1	28
Mg	2	10
Na	2	100
Total impurity	17 ppb	320 ppb

purest available reagent chemicals. An example of a high-purity, commercially available reagent that has met a need is phosphorus pentoxide. The specifications for the ACS reagent grade would allow for up to 100 ppm of lead. One supplier of high purity reagents has a limit of 0.1 ppb for lead in this material and it has found wide use in the silica gel method for lead by isotope dilution mass spectrometry [39]. On the other hand, the specifications for high-purity sodium acetate allow a maximum of 0.05 ppm of lead. A total of 3 g of sodium acetate is sometimes used during the sample processing for the polarographic determination of lead, so the lead contamination could be as high as 150 ng Pb from this reagent alone. In cases like this the analyst will have to purify his reagents before use. The purification method used will depend on the nature of the reagent and the impurities to be removed. Mercury cathode electrolysis, extraction with dithizone or cupferron, ion-exchange, and crystallization have all found use in this regard [7,8,21,35].

C. APPARATUS

The third important source of contamination is that due to apparatus that comes into direct contact with the sample or sample solution, such as beakers, bottles, filters, mortars, *etc.*

Table 10 shows the results of Vasileyeskaya [23] for the analysis of high-purity hydrofluoric acid, hydrochloric acid, and nitric acid after evaporation in Teflon, platinum, and quartz containers. The results show higher trace element concentrations when the evaporations were performed in either platinum or quartz than in Teflon with the evaporation in quartz showing the highest values. Note the increase in aluminum, iron, calcium, and magnesium when the evaporations were performed in quartz compared to Teflon.

Table 11 shows the data compiled by Hetherington, Stephenson, and Witherburn [40] for the maximum recorded impurity levels in various types of fused silica or quartz. There are two main types of vitreous silica, translucent or opaque quartz, and transparent quartz. Translucent quartz has the highest trace element concentrations and should be avoided. Transparent Types I and II quartz are made from naturally occurring quartz crystals or sands. Type I is made by electric melting and Type II by flame melting. Type II quartz has a somewhat lower trace element content because some impurities are partially volatilized in the flame. Type III quartz is a synthetic quartz made by vapor phase hydrolysis of pure silicon compounds such as $SiCl_4$. It has a much lower trace element content than natural quartz with the exception of chloride which runs about 50 ppm and it contains about 0.1 percent hydroxide. There is yet another type of transparent quartz, Type IV, which is also a synthetic quartz made by the oxidation of $SiCl_4$ and electrical fusion. It has about the same trace element content as Type III, is virtually free from hydroxide but has a chloride content of several hundred ppm. Notice that the principal impurities in Types I and II quartz are aluminum, calcium, iron, and magnesium, the same elements that increased when the pure acids were evaporated in quartz. Obviously synthetic quartz should be used whenever possible. One problem is that it is usually not available in the form of laboratory ware such as beakers, dishes, or crucibles.

Table 12 shows the trace element concentrations of some container materials from the literature [7,48,53,54]. While variations of impurity content from batch to batch are to be expected, the compilation is useful for comparative purposes. Borosilicate glass can seriously contaminate solutions [8] and should be avoided unless the analyst is sure that the contamination of the element being determined is negligible such as for rhenium. In addition to these elements contamination from arsenic and lead have been reported. The quartz reported here is a Type 1 quartz made from the melting of pure domestic quartz sand which is claimed to be purer than the imported quartz crystal. Both polyethylene and Teflon are purer than natural quartz. The polyethylene reported here is produced by

TABLE 10. *Results of spectrochemical analysis of hydrogen fluoride, hydrogen chloride and nitric acid after evaporation in teflon, platinum and quartz dishes*[a]

Acid	Material	Elements determined, ng/g										
		Al	Fe	Ca	Cu	Mg	Mn	Ni	Pb	Ti	Cr	Sn
HF	Teflon	3	3	1	<0.04	<3	0.1	<0.4	<0.1	0.1	<0.4	ND[b]
	Platinum	10	10	10	.4	10	.2	.3	.5	1	0.5	ND
HCl	Teflon	<4	3	5	0.2	3	0.1	ND	<0.4	ND	ND	ND
	Platinum	2	2	10	1	6	.2	0.6	<.4	0.4	Tr[c]	<0.4
	Quartz	10	10	60	1	10	.4	2	.5	2	0.6	0.4
HNO_3	Teflon	2	8	4	<0.01	7	0.1	ND	ND	Tr	ND	ND
	Platinum	20	20	30	.4	20	.6	Tr	1	0.8	ND	ND
	Quartz	20	20	60	.1	10	.6	ND	1	0.3	ND	ND

[a] See reference [23].
[b] ND = not detected.
[c] Tr = trace, not evaluated quantitatively.

TABLE 11. *Maximum recorded impurity levels in various types of vitreous silica*[a]

Element	Translucent vitreous silica	Concentration (ppm) in transparent vitreous silica		
		Type I	Type II	Type III
Al	500	74	68	<0.25
Sb	ND[b]	0.3	0.1	.1
As	ND	ND	ND	<.02
B	9	4	.3	.1
Ca	200	16	0.4	<.1
Cr	ND	0.1	ND	.03
Co	ND	ND	ND	.0001
Cu	ND	1	1	<1
Ga	ND	ND	ND	<0.02
Au	ND	ND	ND	<.1
Fe	77	7	1.5	<.2
Li	3	7	1	ND
Mg	150	4	ND	ND
Mn	ND	1	0.2	<0.02
Hg	ND	ND	ND	<.1
P	ND	0.01	0.005	<.001
K	37	6	<1	.1
Na	60	9	5	<.1
Ti	120	3	2	ND
U	ND	ND	0.0006	ND
Zn	ND	ND	ND	<0.1
Zr	15	3	ND	ND

[a] See reference [40].
[b] ND = not detected.

TABLE 12. *Trace element concentration of container materials*

Element	Borosilicate glass (μg/g)	Quartz (μg/g)	Polyethylene (μg/g)	Teflon (μg/g)
Al	Major	50	0.3	
B	Major	0.5	.09	
Ca	1,000	12	.2	0.002
Cr			.015	<.030
Cu			.004	.022
Fe	3,000	5	.6	.035
K	3,000	4		
Mg	600	2	.08	
Mn	1,000		.01	
Na	Major	4	.17	
Sb	2.9		.005	.0004
Ti		2		
Zn	0.73			.093
Zr		<1	.09	

the high pressure noncatalyzed process which is superior in trace element content to the low-pressure process catalyzed by organic compounds of aluminum or oxides of transition metals [3,8,41].

Table 13 lists an order of preference for container materials based on the purity of the material. Teflon FEP bottles and beakers have been used at NBS for the past several years with favorable results after thorough cleaning with nitric and hydrochloric acids to remove contaminants introduced during fabrication. The high-purity acids described earlier were all stored in Teflon FEP bottles for at least 2 weeks and no significant levels of contamination were observed.

Filter paper is another source of trace element contamination. Quantitative ashless filter paper has been shown to contain 20 trace elements at greater than 1 ppm [42]. Membrane filters, while not free from trace element impurities are significantly better than paper [41]. All plastic filtration assemblies are available commercially which have been designed to minimize contamination. Glass filtration assemblies have been shown to contaminate pure water [43]. The trace element concentration of membrane filters which are fabricated from cellulose acetate, polypropylene, fluorocarbons, or polycarbonates can be lowered by acid leaching before use [41,44]. Maienthal [44], for example, has shown, table 14, that the concentrations of Fe, Cu, and Pb can be lowered by acid washing of cellulose ester filters, but the contamination is still too high for low level work. Whenever possible, centrifugation in plastic tubes should be substituted for filtration.

The grinding and sieving of samples prior to analysis is another serious contamination problem [7,41,45]. Mortars made from single crystal alumina, tungsten carbide or pure molybdenum have been used for this purpose since these mortars introduce significant contamination of only one

TABLE 13. *Container materials in order of increasing impurities*

1. Fluorocarbons Teflon TFE and FEP; Tefzel; Halar; Kel-F
2. Quartz—Synthetic
3. Polyethylene High Pressure—Conventional
4. Quartz—Natural
5. Platinum
6. Borosilicate

TABLE 14. *Reduction of contamination of celluolus acetate filters by acid leaching*[a]

	μg/47mm sheet		
	Fe	Cu	Pb
Unwashed	0.30	0.50	0.10
Acid washed	.036	.040	.041

[a] See reference [44].

or two elements which would be neglected in the analyses. Standard sieves made of metal should not be used for sieving [46]. Instead fine silk cloths have been recommended as a substitute. Whenever possible, it is best to avoid grinding and sieving altogether by dissolving large samples and aliquoting.

Rubber stoppers and tubing are to be avoided as they are extremely dirty materials with regards to trace element concentrations [48]. Likewise, polyvinyl chloride tubing is high in trace elements. Tygon tubing, for example, has been shown to contain lead and nickel in excess of 200 ppm and five other elements in excess of 10 ppm [48]. The choice of materials for stoppers or tubing would be Teflon or polyethylene, both of which are low in trace element content. Heating by gas using metallic burners is a possible source of contamination. Ceramic electric hot plates are the least contaminating heat source since metal hot plates corrode and are attacked by acids. Ovens and muffle furnaces are also possible sources of contamination. Fused silica liners are available for muffle furnaces which help to reduce contamination [7].

D. ANALYST

This brings us to the fourth source of contamination, the analyst. Careless manipulations can cause serious contamination. The touching of surfaces that will come into contact with either the sample or a solution of the sample can cause contamination with a number of elements besides sodium and chlorine. Sweat has been shown to contain potassium, lead, ammonium, calcium, magnesium, sulfate and phosphate ions [49].

Table 15 shows the results of a test for lead from the fingers of 16 individuals. Two fingers were soaked for two minutes in 15 ml of $1N$ HNO_3 and the lead determined by isotope dilution mass spectrometry. The results, which showed an average 3.1 μg of lead, indicate that one must be very careful in handling solutions containing trace amounts of lead.

TABLE 15. *Lead leached from fingers of 16 individuals by dilute nitric acid*

	Lead found (μg)
High reading	13.1
Low reading	0.8
Average	3.1

The use of cosmetics by analysts can cause serious trace element contamination since they can contain high concentrations of Al, Be, Ca, Cu, Cr, K, Fe, Mn, Ti, and Zn [23,50]. Some hair dyes contain lead acetate and eye make-up may contain mercury as a preservative. Trace analysts would be well advised to avoid the use of such products. Even medications can be a problem. Calamine lotion, for example, which is used to treat skin irritations such as poison ivy, is practically pure zinc oxide when dry and has been known to cause contaminations. Watches and jewelry such as rings and bracelets should not be worn in the trace laboratory because of contamination hazards.

To keep blank levels as low as possible the analyst must exercise care in keeping the volume of reagents to a minimum, and the time or temperature of evaporations or reactions to a minimum. Excess of reagents, time, or temperature, all cause higher and more variable blanks.

Unless great care is exercised, cross-contamination from other work in the laboratory can contaminate a sample. The analyst must be aware of the history of beakers and other containers so that vessels that have been exposed to major concentrations of one element are not used for trace determinations of that element even after rigorous cleaning treatment. Great care must also be exercised in the handling of ultra-pure reagents least they become contaminated due to careless acts such as opening a container in a unclean atmosphere or pouring a liquid from the container into another solution.

And, finally, the analyst must "think blank"; that is, he must be aware as to the effect on the blank of every step of the procedure. He must ask himself "If I do this, what is the effect on the blank?" and avoid those operations which tend to increase the blank or whose effect is not known, whenever possible.

The importance of the analyst in this regard has also been noted by others. Burriel-Marti [51] has stated that the analyst must really work under "chemically aseptic" conditions. Libby [21] has stated that "low level radiochemistry (and low level trace analysis as well) is something like the discipline of surgery—cleanliness, care, seriousness, and prac-

tice." Alimarin [52], has written that "a major factor is also the experience and the skill of the analyst, which obviously cannot be described statistically." One last quote is from Ruzicka and Stary [21], "It is interesting to note that often an analytical chemist trained in trace determinations by spectrophotometry or spectrography is more successful in this field (substoichiometry) than a radiochemist who is used to working on neutron-activation analysis where the value of the reagent blank can be neglected."

III. Conclusion

In order to decrease the variability of the analytical blank and increase the accuracy of the blank correction, the analyst must reduce the contamination from each source, (1) by working in a Class 100 clean air environment, (2) by using specially purified reagents, (3) by selecting apparatus materials with the lowest possible impurities, and (4) by being constantly aware of his own effect on the blank.

The methods discussed above for the control of the analytical blank have been applied at NBS for the reduction of blank values and hence the variability of the blank correction. Table 16 shows our experience with lead in the Trace Elements in Glass (TEG) Standard Reference Materials over a period of 4 years. Since the lead blanks have been reduced to about 2 ng with an uncertainty of ± 1 ng, we have been able to analyze lunar rocks and other materials containing sub-ppm amounts of lead with less than 1 percent uncertainty. Table 17 shows our experience with silver. Again by lowering the silver blank from 970 ng to 3 ng, the variability was also reduced. These two examples illustrate the fact that control of contamination is not a hopeless task but does take special care, patience, and financial support.

TABLE 16. *Example of lead blank reduction*

	Lead found (μg)
Initial analysis of TEG[a] standard	330 ± 250
TEG analysis using selected acids	260 ± 200
TEG analysis in Class 100 hood	20 ± 8
TEG analysis using special acids in Clean Room (Lunar also)	2 ± 1

[a] TEG = Trace element in glass, SRM's 610 through 619.

TABLE 17. *Example of silver blank reduction*

	Silver found (μg)
Initial analysis of TEG[a] standard	970 ± 500
TEG analysis using Class 100 hoods	207 ± 200
TEG analysis using special acids in Clean Room	3 ± 2

[a] TEG = Trace element in glass, SRM's 610 through 619.

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THE ANALYSIS OF SURFACES AND MICROSTRUCTURAL DETAIL

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The spatial distribution of trace elements detected by bulk chemical analysis can be as important a factor in determining the properties of any material as its overall composition. In structural materials such as steel; for example, the presence of certain impurities below the 100 parts per million level, segregated to grain boundaries, can cause intergranular failure while a uniform distribution of the same concentration might have a negligible effect on mechanical properties. Similar examples can readily be cited regarding the influence of minor or trace constituents on electrical properties, corrosion, oxidation, adhesion and catalysis behavior.

New instruments for chemical analysis of fine structure and surfaces include electron and ion microanalyzers, as well as Auger, photoelectron and ion scattering spectrometers. It will be the purpose of this paper to review the present status and limitations of these techniques for local chemical analysis and discuss how they complement each other and more traditional methods of bulk chemical analysis.

Keywords: Auger spectrometry; electron microprobe analysis; ESCA; ion scattering; secondary ion mass spectrometry; surface analysis.

I. Introduction

A wide variety of analytical instrumentation for the examination of surfaces and fine structural detail is presently available to researchers. Morphology can be examined by transmission and scanning electron microscopy while chemical analysis can be performed by electron microprobe, Auger, ESCA (electron spectroscopy for chemical analysis), ion scattering and ion microprobe methods. The need for these techniques follows from the realization that the spatial distribution of even trace ele-

ments within a material can significantly alter its properties. For example, in certain steels the presence of impurities below the 100 parts per million level, segregated to grain boundaries, can cause intergranular failure while a uniform distribution of the same concentration may only have a negligible effect on mechanical properties. Similar examples can readily be found regarding the influence of minor or trace constituents on electrical properties, corrosion, oxidation, adhesion and catalysis behavior. It is the purpose of this paper to introduce selected methods of chemical analysis which can be applied to these problems, discuss their specific limitations and illustrate how they can be used to complement each other. The theories of these techniques are very well treated in the literature, and will only be summarized here. The reader interested in more detailed descriptions of surface analysis instrumentation should consult the books listed in references [1-3] as well as those cited in each section.

Table 1 gives a brief comparison of the sampled depth, excited area, elemental sensitivity and detection limits. The statements on elemental sensitivities were drawn from available investigations [4-6] and from general trends within series of elements; a rigorous definition of elemental sensitivity is not implied for all the methods. The detection limits are the minimum detectable quantities of the material defined [7] as the quantity necessary to give a signal twice the background. Since the data acquisition times vary for each method, meaningful comparison of detection levels for different methods are not always possible. The excited area figures quoted refer to the typical diameter of an excitation source whose incident flux is approximately circular in shape on the surface of the sample.

From the sampled depths, it is clear that all of the methods to be described are appropriate for surface examination, with the possible exception of electron probe microanalysis which typically samples a depth of several thousand angstroms within a specimen. This technique is included, however, because its lateral spatial resolution is generally superior to that of the other methods, making it the most effective way to examine micrometer dimension structural detail or mapping elemental concentration distributions.

II. Electron Microprobe Analysis

Electron microprobe analysis (EMA), first developed by Castaing [8] in 1951 is now a well-established method for qualitative and quantitative chemical analysis on a micrometer scale. The basis for electron-excited x-ray emission is illustrated in figure 1. A focused beam of monoenergetic

TABLE 1. *Comparison of sampled depth, excited area, elemental sensitivity and detection limits*

Method	EMA	ISS	SIMS	AES	ESCA
Sampled depth	2000–20000 Å	one atom layer to ~10000 Å	3–100 atom layers	3–20 Å	5–20 Å
Minimum irradiated region	~1 μm	0.5–3 mm	2–500 μm	2–25 μm	mm
Relative elemental sensitivity	moderate variation	moderate variation	wide variation	less than factor of 10	less than factor of 10
Detection limits (minimum detectable quantity)	10^{-14} g[a]	10^{-15} g[b]	10^{-16} g	10^{-14} g	10^{-7} g[b]

[a] W. Reuter, *Surface Sci.* **25,** 80 (1971).
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Notes: EMA = electron microprobe analysis
ISS = ion scattering spectrometry
SIMS = secondary ion mass spectrometry
AES = auger electron spectrometry
ESCA = electron spectroscopy for chemical analysis.

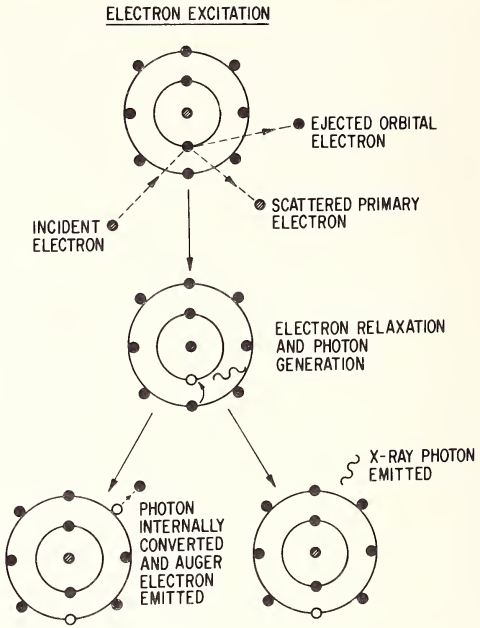

Figure 1. Principles of electron excited x-ray production.

electrons impinges on a specimen creating a number of orbital vacancies. The vacancies are filled by electronic relaxation from higher orbitals followed immediately by a release of energy, equal to the electron transition energy, either in the form of x-ray or Auger electron emission. The relative amounts of these two competing processes are described by a quantity known as the fluorescent yield, which is the fraction of core shell ionizations followed by x-ray emission. From figure 2, it can be seen that for a light element, like carbon, only one ionization in a thousand results in x-ray production, but that x-ray emission becomes more favorable with increasing atomic number. The ability to do chemical analysis based on x-ray spectroscopy follows directly from Moseley's law which states that the wavelength of x-ray emission, λ, for a given spectral line can be simply related to atomic number, Z, as follows:

$$1/\lambda = K[Z - \delta]^2 \qquad (1)$$

where δ is a screening constant and the value of K is fixed for each spectral series. The operating principles of the EMA are shown in figure 3. A beam of 2 to 50 kV electrons is focused to a fine spot on the surface of the sample by means of two or more magnetic lenses, causing x-ray excitation. Measurement of x-ray wavelengths and intensities has traditionally

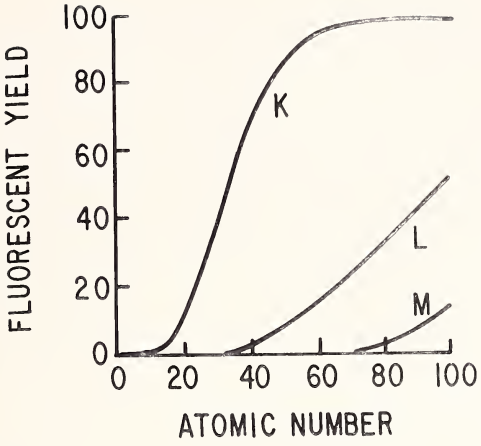

Figure 2. The fluorescent yield as a function of atomic number.

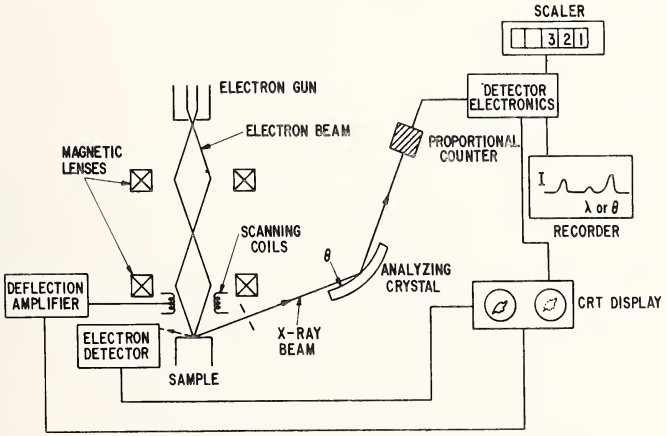

Figure 3. A schematic representation of an electron microprobe analyzer.

been performed with crystal diffraction spectrometers. A collimated x-ray beam from the specimen is reflected from an analyzing crystal and then detected with a proportional counter. The reflected x-ray beam will have a maximum intensity when Bragg's law:

$$n\lambda = 2d \sin \theta \tag{2}$$

is obeyed, where d is the interplanar spacing of the analyzing crystal, θ is the angle of incidence and n is an integer. A typical qualitative scan of a high temperature alloy sample is shown in figure 4. It was obtained by observing the x-ray intensity as a function of θ on a strip chart recorder. In

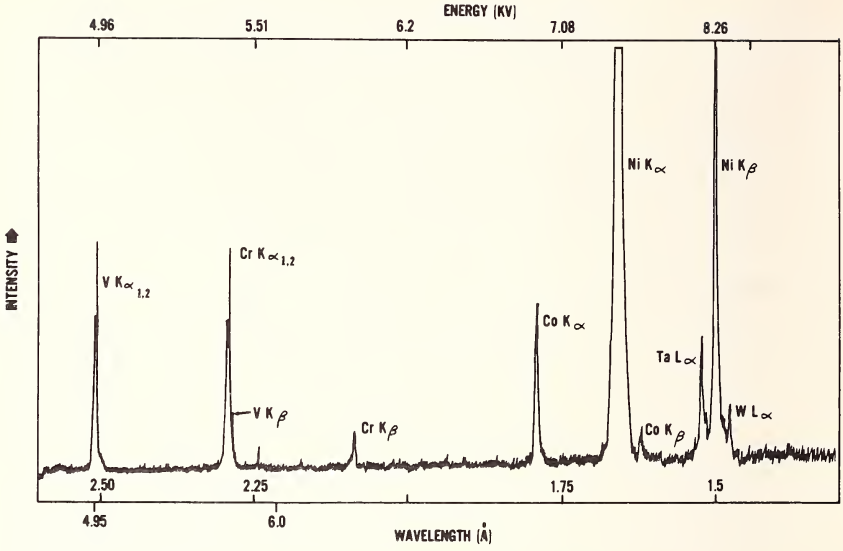

Figure 4. A qualitative EMA scan of a nickel base high temperature alloy.

addition to crystal spectrometers solid state x-ray detectors are now widely used with EMA's and scanning electron microscopes [9]. These detectors are totally electronic in nature, have high collection efficiency, and are used in conjunction with rapid methods of data presentation and evaluation. Their principal limitations are significantly poorer resolution than crystal spectrometers and lower maximum count rate capability due to pulse pileup and dead time problems [10].

Elemental sensitivity is strongly dependent on operating conditions and the particular characteristic line (K, L or M) chosen. Serious difficulties are encountered in the light element region ($Z < Mg$) due to absorption effects and low x-ray production efficiencies. In addition to the x-ray yield and a variety of operating parameters the minimum detectability limit is strongly influenced by instrumental stability since x-ray emission is statistical in nature and sufficient counting times are required to distinguish the characteristic line intensity from the white radiation background. In the shorter wavelength region practical detection limits range from 100 to 500 parts per million.

When applicable, the qualitative capabilities of EMA are used in many areas of research requiring elemental compositions on a micrometer scale including studies of inclusions, segregation and diffusion [11]. Furthermore, since the energy of the emitted x-rays depends on the orbital energy levels, which can shift with chemical bonding, this method has been used

to gain information about elemental oxidation states [12,13]. Such measurements, however, are not routinely done because of special resolution requirements of the spectrometers.

Figure 3 also illustrates the components necessary to use the EMA in a scanning electron microscope (SEM) mode [14]. A deflection amplifier synchronously scans the focused electron beam across the sample and a second electron beam in a cathode ray display tube (CRT). Electrons emitted or reflected from the sample are detected by a scintillation detector and the resulting signal used to modulate the brightness of the CRT display. If secondary electrons are detected then the major source of contrast will be variations in surface topography. If high-energy backscattered electrons are used, then the contrast will be due to both atomic number effects and surface topography. The magnification of the SEM is the ratio of the distance scanned on the CRT to that scanned on the sample. The resolution is determined by the electron beam size and the volume of sample-electron beam interaction. In the secondary electron mode it can be less than 100 Å while in the backscatter mode it is typically about 0.5 μm. Since the x-ray signal can be displayed as well, it is also possible to obtain elemental distribution maps of the type shown in figure 5 which contains a secondary electron image and x-ray distribution maps for Cr, S and Ni. The sample is a polished section of a Ni-5Cr alloy ex-

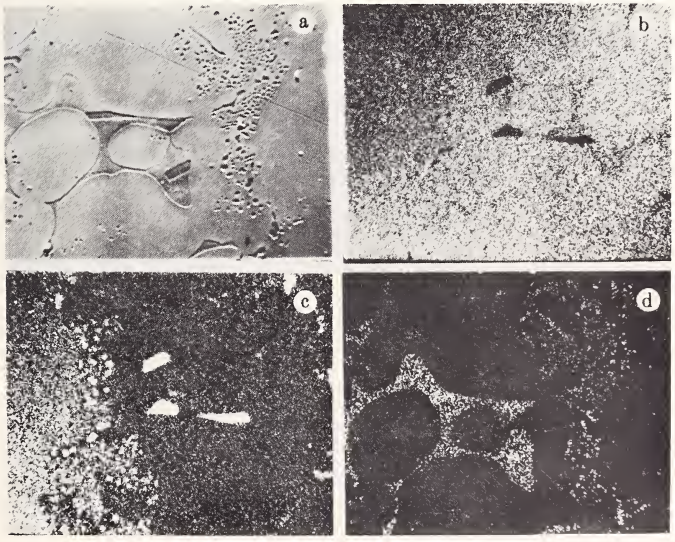

Figure 5. EMA images of Ni-5Cr alloy exposed to 2% SO_2 – 5% CH_4-N_2 gas mixture for 5 hours at 850 °C (from McKee *et al.* [15]). a. Secondary electron image; b. Ni K_α x-ray image; c. Cr K_α x-ray image; and d. S K_α x-ray image.

posed to a 2% SO_2-5% CH_4 balance N_2 gas mixture for 5 hours at 850 °C studied by McKee *et al.* [15] in an investigation of hot corrosion.

The common task in quantitative analysis of all of the analytical methods to be discussed is the correlation of the observed signal intensity—x-ray, electron or atomic masses—with the number of atoms present in the detected volume. For the EMA there are a variety of approaches to this problem including the use of theoretical models and calibration standards of known stoichiometry [16]. In all of these methods the x-ray intensities of specific spectral lines for each element in a sample are carefully measured and then normalized by values obtained from pure elemental or simple binary standards collected under identical operating conditions. Compared to the other techniques quantitative EMA using theoretical models is probably the most effective since relative accuracies of 2 to 5 percent are common even for complex multicomponent systems. The accuracy of the models is principally limited by the physical constants used in them such as mass absorption coefficients and fluorescent yields [17]. Other sources of error are associated with sample preparation, since rough surface topography, smearing during polishing and deviations from normal beam incidence can all contribute to poor experimental results.

Lateral spatial resolution in the EMA is principally limited by electron beam penetration and scattering which can be several micrometers even though the electron beam striking the sample may only be several hundred angstroms in diameter [18]. This effect arises because electron penetration is determined by the operating voltage, which must be sufficiently in excess of the x-ray excitation potential to obtain adequate sensitivity. Other factors affecting spatial resolution include indirect x-ray fluorescence and x-ray production by electrons scattered within the instrument.

III. Secondary Ion Mass Spectrometry

Secondary ion mass spectrometry (SIMS) is the ion analog of electron microprobe analysis [19,20]. A beam of primary ions is focused onto a selected area of a specimen causing the ejection of electrons, atoms and ions. The secondary ions, which constitute a small fraction (10^{-2} or less) of the atoms removed, are then identified by mass spectrometry. The technique has a number of advantages over EMA including generally higher sensitivity, often less than 1 ppm; complete coverage of the periodic table, including H, He and Li; a smaller sampling depth, on the

order of 100 Å; the ability to measure relative isotopic abundance; and the capability for in-depth analysis as a natural consequence of the sputtering process. The principal disadvantages of SIMS include widely varying sensitivities for different elements due to large variations in ion yields; high cost, greater than any of the other instruments described in this article; difficulties in spectral interpretation due to limited mass resolution; restricted quantitative capability, and its inherently destructive nature, which can consume a sample before it can be completely analyzed.

The basic operating principles of SIMS are shown in figure 6 which is a schematic representation of the instrument designed by Liebl [21]. A duoplasmatron gun serves as a high brightness source of either positive or negative ions with energies up to about 20 kV. Specific ions are then selected by a magnetic prism and focused to a fine spot on the sample by two electrostatic lenses. Positive or negative secondary ions are attracted by a "pick-up" electrode held at ± 1.5 kV and passed through the mass spectrometer section consisting of a retrofocal lens, an electrostatic analyzer and a 90° magnetic prism. Selected ions of a given mass to charge ratio then strike a conversion electrode (target) liberating secondary electrons which are detected with a photomultiplier tube (PMT). Individual ions can be counted with a scaler or integrated to give a ratemeter output on a strip chart recording of ion intensity *versus* charge to mass ratio. In

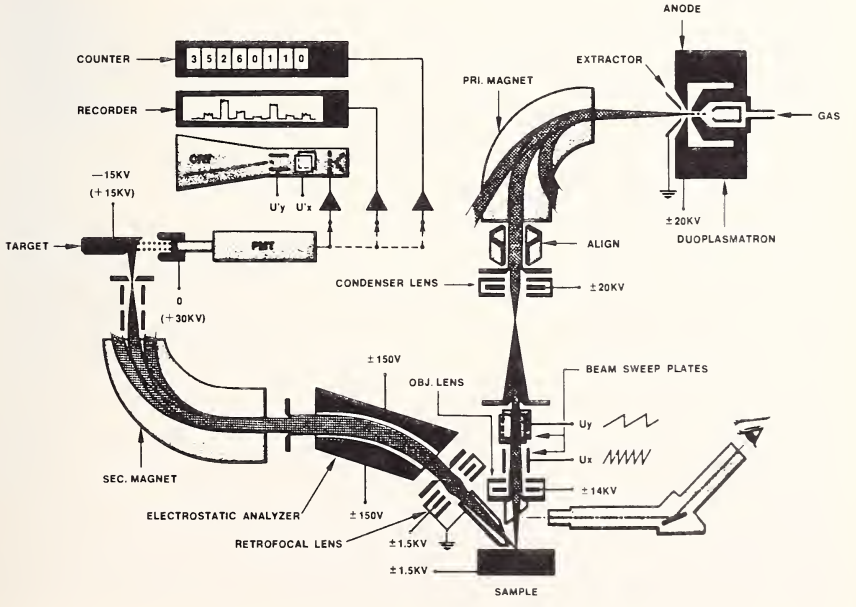

Figure 6. Schematic representation of an ARL ion microprobe.

addition to the static or point mode of analysis the ion beam can be scanned across the surface of a sample and the intensity of a selected ion displayed on a synchronously scanned cathode ray tube (CRT) forming a scanning ion image similar to x-ray distribution maps formed with an EMA. The CRT display can also be used to form a specimen current image of the sample useful in locating the region to be analyzed. Figure 7 shows a set of ion maps and an optical micrograph of a stringer encountered in the study of a high temperature alloy [22]. It appears to contain titanium, tungsten, carbon, and possibly boron. Neither of the latter two elements were detected by an EMA examination of the sample.

The spatial resolution of SIMS is limited by the probe size itself rather than by penetration and scattering effects as is the case with the EMA. While ion optical systems exist which are capable of producing 1 micrometer probes, the total sputtered volume may be insufficient to provide enough ions to yield the desired precision for the analysis of low concentration levels. For example, Morabito and Lewis [23] calculated that in order to detect 10 ppm of aluminum with a precision of 3 percent a microvolume of about 100 μm^3 is required, and therefore, for a 100 μm beam 128 Å of material must be removed.

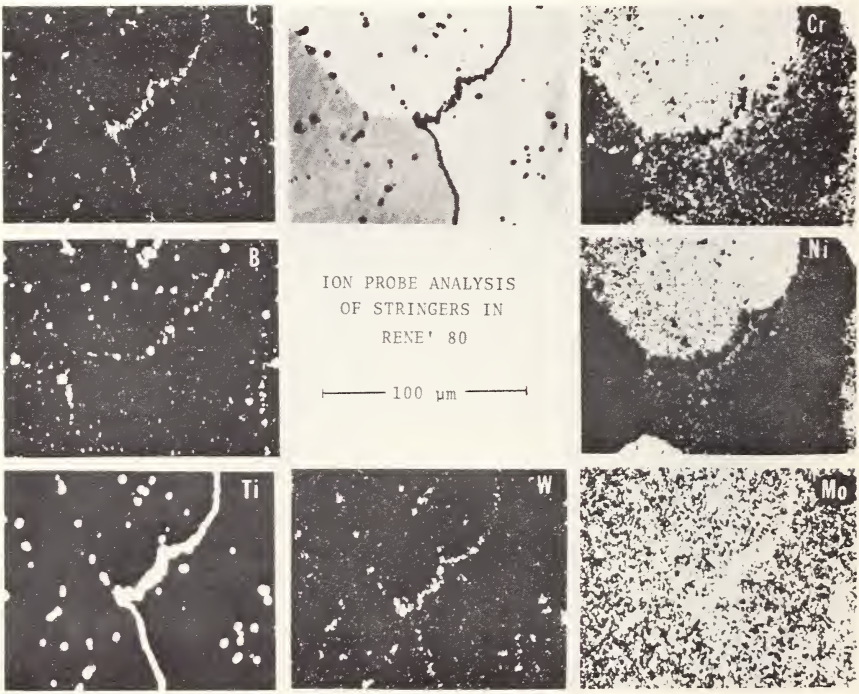

Figure 7. Ion probe analysis of stringers in a high temperature alloy (from Bolon [18]).

Closely related to the ion microprobe is the ion microscope of a type similar to that designed by Castaing and Slodzian [24] shown schematically in figure 8. An area of the sample of from 20 to 300 μm is bombarded with ions from a duoplasmatron source. Secondary ions leaving the sample are accelerated into a uniform electrostatic field by an immersion lens, thus forming a series of superimposed ion images. Separation of these ion images based on mass to charge ratio is then accomplished by the first magnetic sector of the mass spectrometer. An electrostatic mirror used as an energy filter reflects only those ions with energy below a predetermined value and a second deflection through a magnetic sector reduces optical aberrations. Finally the focused ions are passed through an image converter and an electronic image is directly photographed or viewed on a fluorescent screen. Selected areas of the sample can be analyzed in detail by replacing the fluorescent screen with an adjustable aperture.

The nature of secondary ion emission varies greatly with a number of parameters including matrix composition, the type of primary ion beam used, and crystal orientation, thus making quantitative analysis extremely difficult. Figure 9 taken from work published by Anderson and Hinthorne [25] shows the differences observed with time in the $^{27}Al^{+}$ ion intensity obtained from aluminum using neutral and electronegative gas sputtering. The enormous drop in positive ion yield when argon is used as the prima-

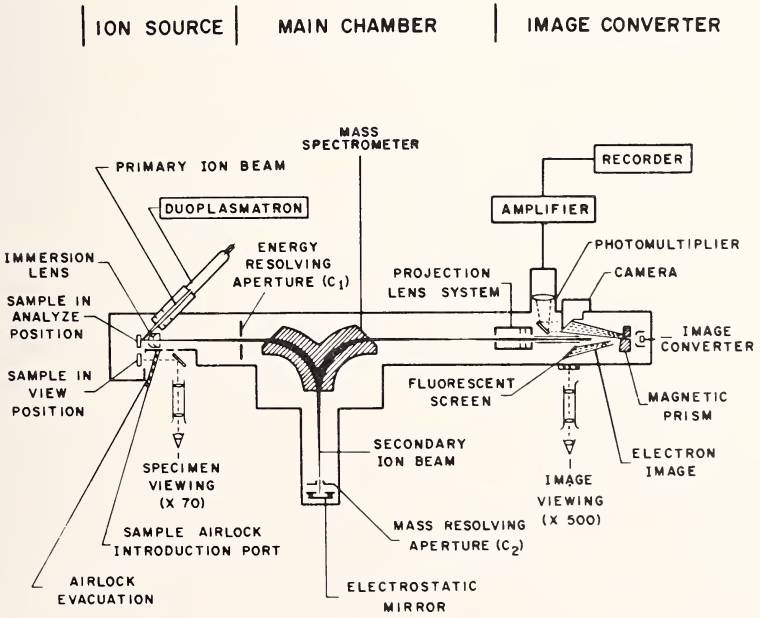

Figure 8. Schematic representation of a Cameca ion microscope.

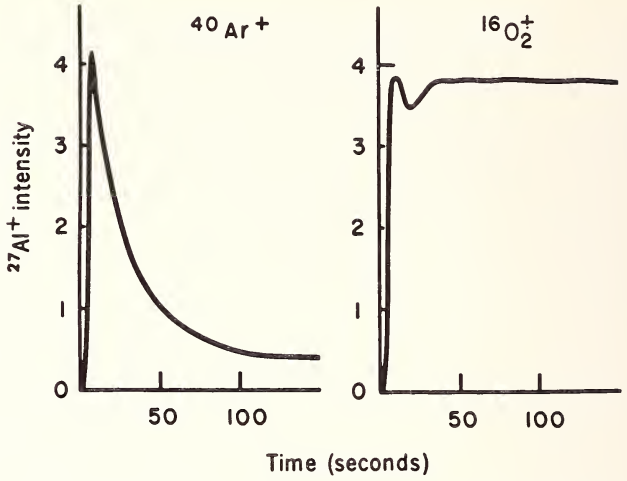

Figure 9. $^{27}Al^+$ intensity as a function of time for $^{40}Ar^+$ and $^{16}O_2^+$ ion bombardment (from Anderson and Hinthorne [25]).

ry ion source is believed to be associated with the removal of a thin oxide film, since electrons capable of neutralizing the sputtered ions are held more strongly by the film than the underlying metallic aluminum revealed by sputtering. The use of a primary oxygen beam, however, appears to reconstitute the surface oxide and maintain a more constant emission. Even more dramatic variations of ion yield are noted with atomic number as shown in figure 10 where the yields of aluminum and gold are separated by four orders of magnitude. McHugh [20] has studied the effect of crystal orientation on the ion yield and found, in the examination of polycrystalline Inconel, that a variation of as much as a factor of three can occur. This effect is further illustrated in the nickel ion image shown in figure 7.

Because of its high elemental sensitivity, the ion microprobe is considered one of the most powerful methods for in-depth chemical analysis and has been widely used in the study of thin films particularly in the examination of semiconductor devices [26]. Data obtained in this mode are not totally unambiguous, however, and considerable care must be exercised in recognizing artifacts due to ion recoil (knock-on) and crater build-up effects. Experiments by McHugh [20] on a Ta_2O_5 sample containing a sub-phosphorus layer located 230 Å below the surface showed that the shape of the "in-depth" phosphorus profile varied with the primary ion energy, indicating that significant atom mixing effects were caused at 18.5 kV compared to 1.75 kV. Crater effects can arise from differences in the sputtering rate across the area being analyzed, due to the nonuniform cur-

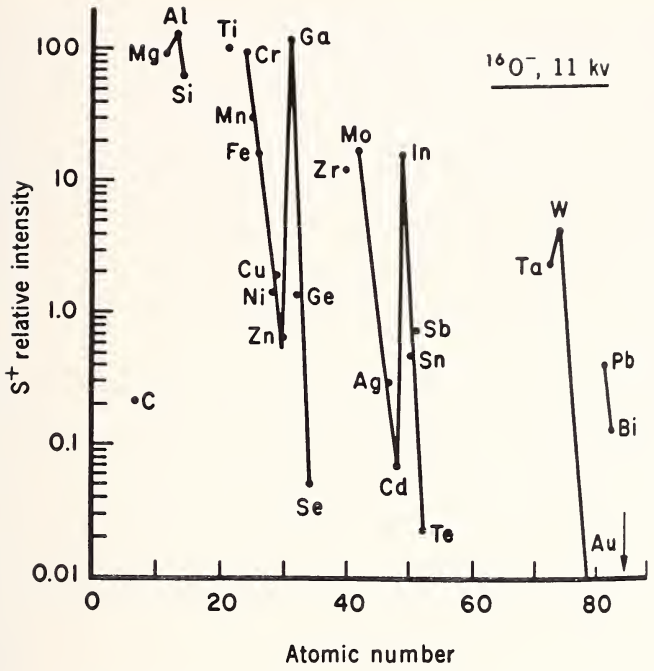

Figure 10. Positive ion yield as a function of atomic number.

rent density of the primary ion beam. In the ion microprobe they can be significantly reduced by scanning the primary beam over the sample and collecting data only when the beam is in the center of the region being analyzed (electronic aperturing), or in the case of the ion microscope by mechanically aperturing the center of the crater while a much larger area is sputtered.

A number of successful applications of SIMS to practical problems in materials science can be found in the literature [27,28] particularly in the study of gases such as oxygen, hydrogen and nitrogen dissolved in metals at concentration levels below 100 ppm. An example is a recent study by Walsh [29] of the solubility of oxygen and nitrogen in selected titanium alloys. Large concentrations of these gases can diffuse to interstitial sites in an alloy during ingot production causing the formation of alpha phase stabilizing defects which can degrade mechanical properties [30]. SIMS techniques were used to establish that nitrogen rather than oxygen was segregated to the defects. The ratio of $^{14}N^+$ to $^{47}Ti^+$ was measured as a function of distance from the defect and is shown in figure 11 as well as hardness profiles taken in the same area.

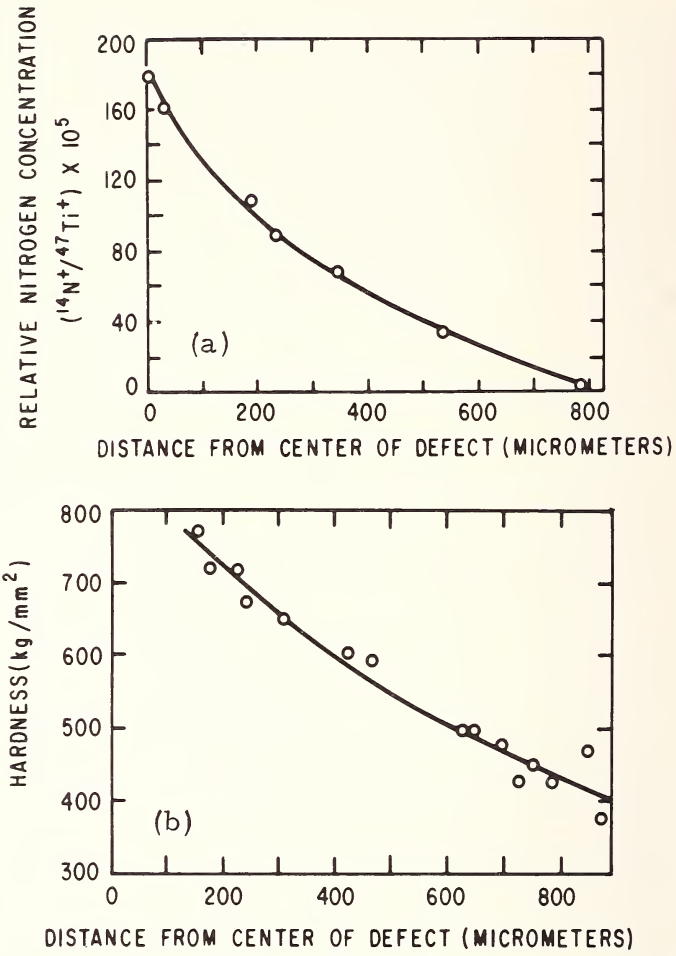

Figure 11. Nitrogen segregation adjacent to alpha stabilizing defects in titanium (from Walsh [29]). a. relative nitrogen concentration *versus* distance; b. hardness *versus* distance.

IV. Ion Scattering Spectrometry

Ion scattering spectrometry (ISS) can be divided into three categories depending on the energy of the incident ion beam: high energy (1-2 MeV), medium energy (100-400 keV) and low energy (0.5-10 keV). Together these three ranges are capable of providing information about specimen surfaces at depths ranging from the outermost atomic layers to a few micrometers. Measurements are performed by bombarding the target with

a monoenergetic beam of collimated noble gas ions and then determining the energy spectrum of the ions scattered at a fixed angle, usually greater than 90°, as shown in figure 12. Since the scattering process can be treated as a simple binary collision it can be shown from conservation of energy and momentum considerations that the relationship between the mass of an elastically scattered ion, M_1, and the mass of a target atom, M_2, for a scattering angle of 90° is given by:

$$\frac{E_1}{E_0} = \frac{M_2 - M_1}{M_2 + M_1} \tag{3}$$

where E_1 and E_0 are the energies of the scattered and incident ions respectively. Thus, for low energy noble gas scattering, the energy spectrum becomes a mass scale, making it possible to identify all elements except hydrogen and helium. For low energy ISS the variation of sensitivity with atomic number is only about a factor of 10, while detection limits are on the order of 10^{-2} to 10^{-3} monolayers. In the high energy case, however, sensitivity varies as the second power of the target's mass. Therefore, detection levels of 10^{-2} to 10^{-3} monolayers are only observed for the heavy elements.

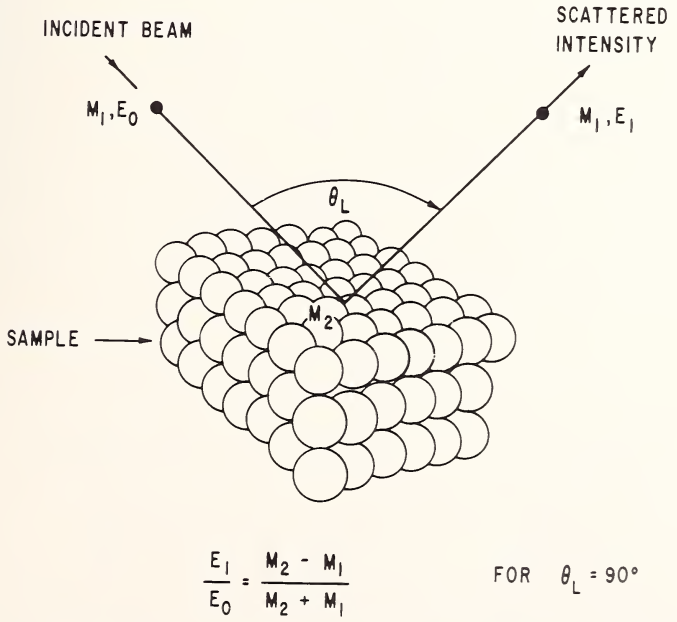

Figure 12. Basic principles of ion scattering spectrometry.

From table 2, which compares the effective sampling depths for the three categories of ion scattering, it is clear that only the low energy scattering method is strictly a surface tool, whereas the high energy method probes depths of about the same order as the electron microprobe. The sampled surface area is large compared to other methods because fairly large beam diameters, typically 0.5 mm to 3 mm are used to enhance the yield. Depth profiles can be obtained with low energy ISS if it is combined with more active sputtering. However, sputtering is not necessary with high energy scattering because of the increased penetration which makes it possible to use the energy loss of the scattered ions to establish a depth scale. For 180° scattering the relationship between energy loss, ΔE, and depth, Δt, is given by:

$$\Delta t \cong \Delta E \left[\frac{dE}{dx_{in}} \left[\frac{M_2 - M_1}{M_2 + M_1} \right]^2 + \frac{dE}{dx_{out}} \right]^{-1} \tag{4}$$

where values of dE/dx are calculated or measured ion stopping powers. The most serious drawback to determining depth profiles by this method is the interpretation of the observed scattering spectrum, since the depth scale is also the mass scale. In other words, a light element located on the surface may give a signal which cannot be readily distinguished from that of a heavy atom located beneath the surface. Other complications arise in the examination of multicomponent systems where limited mass resolution and a large background may complicate spectral interpretation.

Quantitative analyses have been done [31,32] for low energy ISS giving quoted accuracies in the neighborhood of 10 percent. A major uncertainty in quantitative analyses, as with perhaps any surface analytical technique, is the lack of true "surface" standards of known stoichiometry. Because of the limitations of high energy scattering mentioned above, it appears that quantitative high energy scattering work is still very limited.

The essential components used to measure the scattering of low energy ions from target surfaces include an ion gun, an energy analyzer, a detec-

TABLE 2. *Comparison of the effective sampling depths for the three categories of ion scattering*

Energy range (keV)	Particle	Sampled depths Å
0.5–2.0	He^+, Ne^+	Surface[a]
20–200	H^+, He^{2+}	~1000[b]
1000–3000	He^+	~10,000[c]

[a] D. P. Smith, *Surface Sci.* **25,** 171 (1971).
[b] A. Van Wijngarrden, B. Miremadi, W. D. Baylis, *Can. J. Phys.* **49,** 2440 (1971).
[c] O. Meyer, J. Gyulai, J. W. Mayer, *Surface Sci.* **22,** 263 (1970).

tor, an accurately positioned sample, and associated recording equipment. The 90° ion scattering instrument of Goff and Smith [33] is shown in figure 13. The ion gun for this instrument is a cylindrical grid with an external filament. Ions formed in the grid are taken out axially and focused onto the target by an electrostatic lens system. A 127° electrostatic analyzer is used for energy analysis and a continuous electron multiplier is the ion detector. The entire system can be fitted onto an 8-inch outer diameter ultra-high vacuum flange. The analysis chamber is first pumped down to a pressure of 10^{-10} and then, during scattering, the noble gas to be ionized is admitted into the system at a pressure of 10^{-5} torr. Figure 14 gives a typical low energy ion scattering spectrum of 1.5 keV helium ions scattered from bulk polycrystalline nickel contaminated with carbon and oxygen.

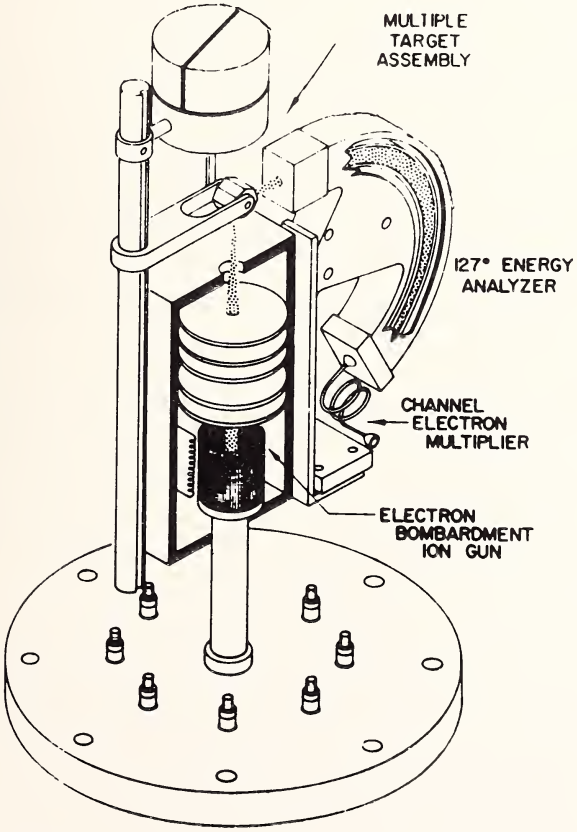

Figure 13. Low energy ion scattering apparatus (from Goff and Smith [33]).

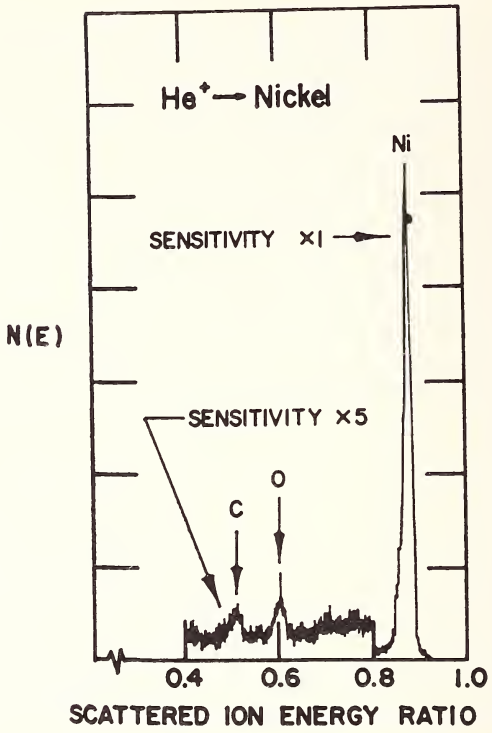

Figure 14. Low energy ion scattering spectrum of polycrystalline nickel (from Goff and Smith [33]).

The basic components of high energy scattering instrumentation, with the exception of an MeV range accelerator, are shown schematically in figure 15 [34]. The ion beam is adjusted to strike the sample housed in a vacuum chamber. Backscattered ions are detected with a solid state detector interfaced to a multi-channel pulse height analyzer. The energy spectrum of the reflected ions can be printed out, displayed on a CRT, or transmitted to a computer for further processing.

ISS has proven useful in a number of electronic applications such as the study of interdiffusion in thin metallic films at relatively low temperatures (< 500 °C). One system of interest is gold-chromium-aluminum oxide associated with electrical interconnections in which chromium serves as an intermediate bonding layer between the ceramic and gold. For electrical applications diffusion of chromium can have an adverse effect on the adherence and electrical conductivity of the gold film. Hirvonen *et al.* [35] have used ion scattering results with 3 MeV helium ions to show significant migration of chromium into and through the gold films at tempera-

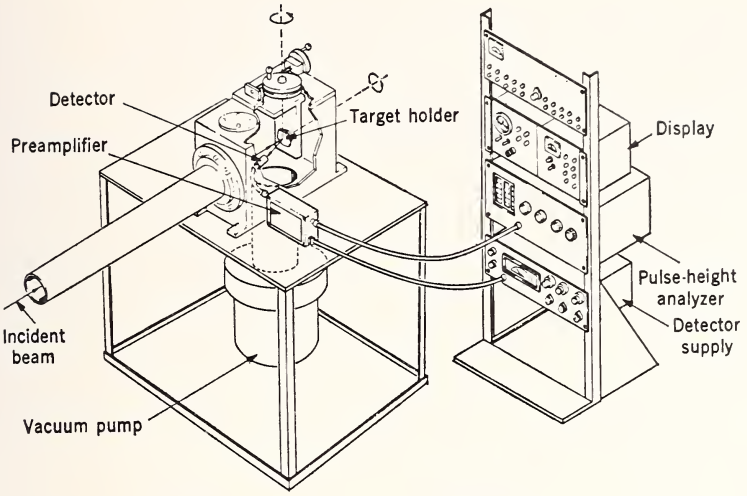

Figure 15. High energy ion scattering apparatus (from Nicolet *et al.* [34]).

tures as low as 250 °C and 450 °C. Film thicknesses were about 100 Å and 200 Å. Figure 16 gives the scattering yields *versus* energy of an annealed sample (annealing temperature of 450 °C for 0.5 h) and an unannealed sample. The symmetric shapes of the chromium and gold scattering peaks suggest no migration of chromium or gold for the unannealed ("as deposited") sample whereas the broadened chromium and gold scattering peaks for the annealed sample indicate significant migration of these elements. These counts correspond both to scattering from chromium and the outer surface of the gold layer. These data complemented other studies of the Au-Cr systems; *e.g.*, electron microscopy [36] and resistivity measurements [37] which were unable to confirm the location of chromium on the surface.

Other applications of ISS include studies of the growth of oxides on compound semiconductors. Such studies aid in developing a knowledge of surface passivation and the formation of insulating or masking films. An example is work by Poate *et al.* [38] who used 1.7 to 2.0 MeV helium in examining the anodization of (111)-oriented gallium-phosphide crystals under varying hydrogen ion concentrations, since evidence suggested a pH dependence of the dissolution of gallium oxide. The scattering results showed two distinct types of film depending on the hydrogen ion concentration of the electrolyte. One type, the uniform film, has a constant composition throughout its thickness. The other type, a nonuniform film, shows a gallium-rich and phosphorus-deficient layer near the film electrolyte interface. The depth profiles obtained for gallium, phosphorus and

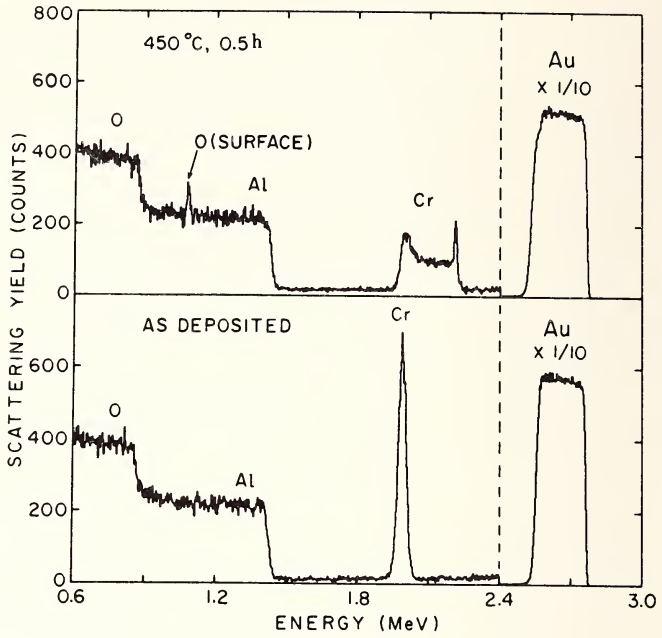

Figure 16. High energy scattering spectra from gold-chromium-aluminum oxide contact (from Hirvonen *et al.* [35]).

oxygen are shown in figure 17. The depth resolution is about 100 Å. The ISS results seem to suggest the existence of competing factors—*e.g.*, varying cationic mobilities and dissolution rates—in the formation of complex film structures.

V. Auger Electron Spectrometry

Practical Auger electron spectrometry (AES) has been used extensively since 1968 largely due to improved methods of spectral measurement developed by Harris [39]. The principles of the Auger process, which have already been discussed in connection with figure 1, are further illustrated for Auger KLL emission in figure 18. A focused monoenergetic electron beam of energy sufficient to cause ionization of a core level electron of energy E_K impinges on a target which has the energy level diagram shown. The vacancy created by the ionization is filled by an electron from a higher orbital of energy E_{L_1} and the energy released dissipated by one of two competing processes: x-ray production or the ejection of an Auger

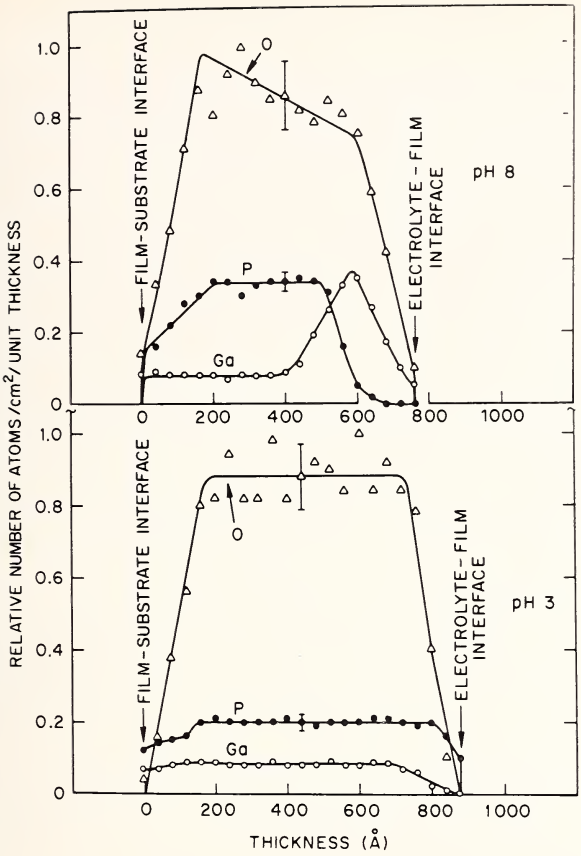

Figure 17. Depth profiles obtained from anodized gallium phosphide crystals (from Poate *et al.* [38]).

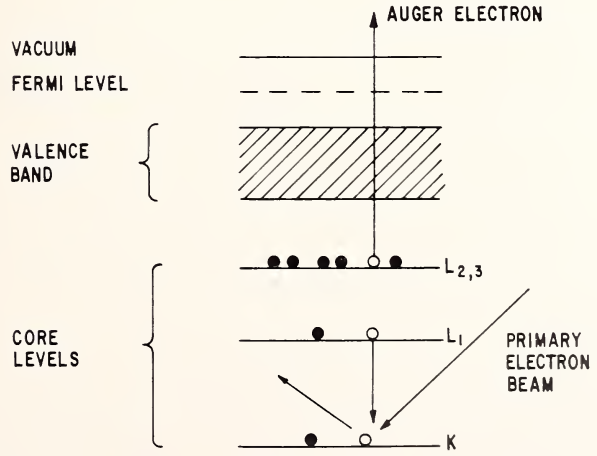

Figure 18. Auger KLL emission process.

electron. As shown in figure 2, x-ray production is less likely for low atomic numbers and small transitional energies in which case the Auger process predominates. From energy conservation considerations, the Auger electron will have a characteristic energy approximated by

$$E_{KLL} = E_K - E_{L_1} - E_{L_2}(Z+\Delta) - \Phi \tag{5}$$

where E_{L_2} is the energy of the orbital from which the Auger electron was ejected. The term E_{L_2} $(Z+\Delta)$ is the ionization energy for the initial ion; Z is the atomic number and Δ accounts for the extra positive charge of the atom resulting from the first ionization. The Φ term is the surface work function of the sample. Similar expressions can be derived for other Auger transitions such as the LMM or MNN. The actual kinetic energy of the Auger electron measured by an energy analyzer for a generalized Auger process involving the initially ionized level W, the level of the relaxing electron X and the level of the ejected Auger electron Y is given by

$$E = E_W - E_X - E_Y(Z+\Delta) - \Phi_{\text{spec}} \tag{6}$$

where Φ_{spec} is the spectrometer work function which is constant. Note that the work function term of the sample drops out since the actual measured energy will have an additional term$(\Phi - \Phi_{\text{spec}})$ added onto equation 5.

The equation for the characteristic energy of Auger electrons is obeyed only by those Auger electrons which escape from the sample without being inelastically scattered. The inelastic mean free path depends on the Auger electron energy and substrate material. Experimental measurements indicate that the range is about 3 to 20 Å [40,41]. Accordingly, Auger electron spectrometry is truly a surface technique capable of uniquely identifying elements above lithium. The variation in sensitivity for elements through thorium is less than a factor of 10 with detection limits reported as low as 10^{-3} of a monolayer [42]. The major limitation of the technique is found in the examination of high Z elements due to increased spectral complexity. In addition to elemental analyses, it is possible to observe the chemical environment of atoms by means of chemical shifts. The results are not as easily interpreted for Auger work as similar measurements are for photoelectron spectroscopy because in AES the shifts are the results of changes in three levels (K, L_1, L_2 for the example shown in fig. 18).

Quantitative Auger analysis is still in the developmental stage; nonetheless, analyses are routinely done by using standard specimens of known concentrations in a fashion similar to electron microprobe analysis [43]. More theoretical methods based on the calculations of expected Auger

currents [44] are complicated by backscattering effects and the limited availability of data on the cross section for electron impact ionization, the inelastic mean free path and the backscattered fraction. Therefore, generally quoted accuracies of Auger quantitative work are in the region of 20 to 50 percent.

The basic components required for AES measurements include an ultra-high vacuum system, an electron gun for sample excitation and an electron energy analyzer. Depth profile measurements are also possible by means of sputter etching the sample with an auxiliary ion gun. The system shown in figure 19 uses an electron gun placed in the center of a coaxial energy analyzer. An electron energy spectrum can be determined by scanning the voltage applied to the outer cylinder and monitoring the number of electrons striking an electron multiplier which serves as the detector. Since the Auger signal is relatively weak compared to the background, electronic differentiation is used to improve sensitivity. Therefore, Auger spectra are typically presented as derivatives of the electron energy distribution. The differentiated spectrum is obtained by superimposing a small ac voltage on the dc sweep voltage of the analyzer while synchronously detecting the ac output with a lock-in amplifier. A

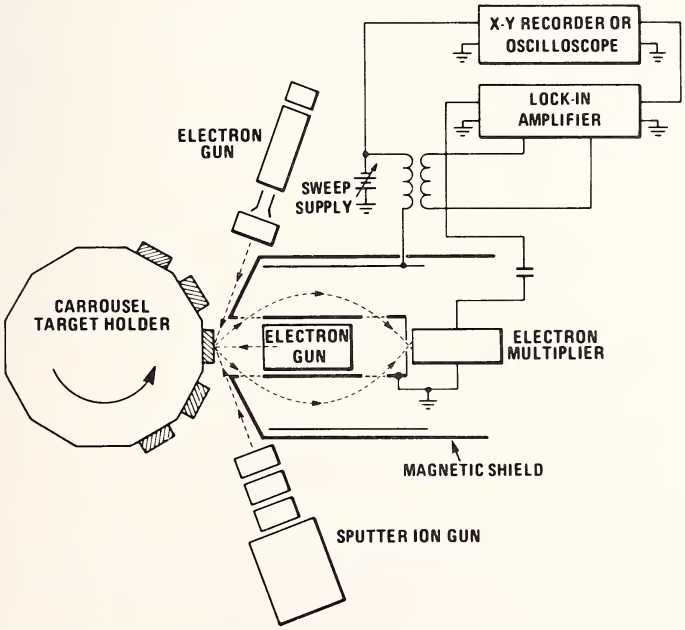

Figure 19. Schematic representation of an Auger electron analysis system (courtesy of Physical Electronics).

recent variation of the instrument shown in figure 19 is the scanning Auger microscope. It uses an electrostatic lens system to produce a focused spot less than 5 μm in diameter which can be scanned over the sample. Both secondary and Auger electron images can then be displayed on a CRT in a manner similar to the SEM.

The applications of AES combined with sputter etching are numerous. Johnson and Stein [45], for example, have determined the cause of thermal embrittlement in 250 series maraging steel. It was found that improper heat treatment resulted in severe fracture toughness degradation. Evidence further suggested that the material becomes thermally embrittled over certain temperatures (1090 °C) and slowly cooled. Under these conditions fractures occurred and the fracture toughness (Charpy impact energy) decreased by an order of magnitude. Other investigations did not identify the cause of embrittlement, but the AES results demonstrated that the cause of thermal embrittlement was the segregation of titanium to the grain boundary and, presumably, the subsequent formation of TiC. The Auger spectrum of a sample fractured intergranularly under UHV conditions is shown in figure 20. Figure 21 shows the depth profile for titanium.

Morris and Cahn [46] have used Auger spectroscopy to elucidate the role of Bi_2O_3 in the non-ohmic properties of zinc oxide ceramics. Addition of small amounts of Bi_2O_3 to the microstructure of sintered polycrystalline zinc oxide results in an increase in the electrical resistivity of the ceramic by a factor of about 10^8 when a small voltage is applied. The amount of

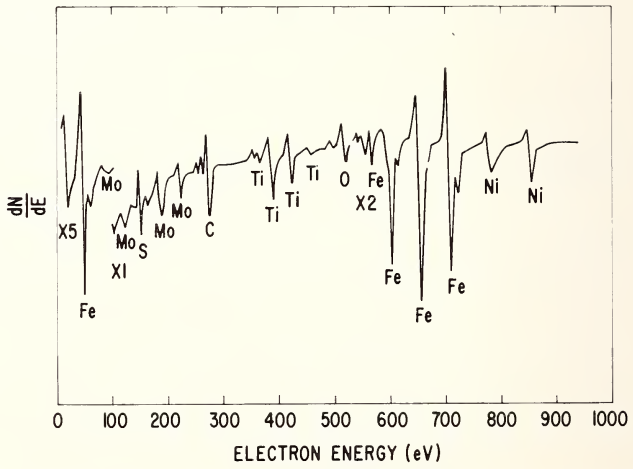

Figure 20. The Auger spectrum of a fracture surface of a 250 series maraging steel (from Johnson and Stein [45]).

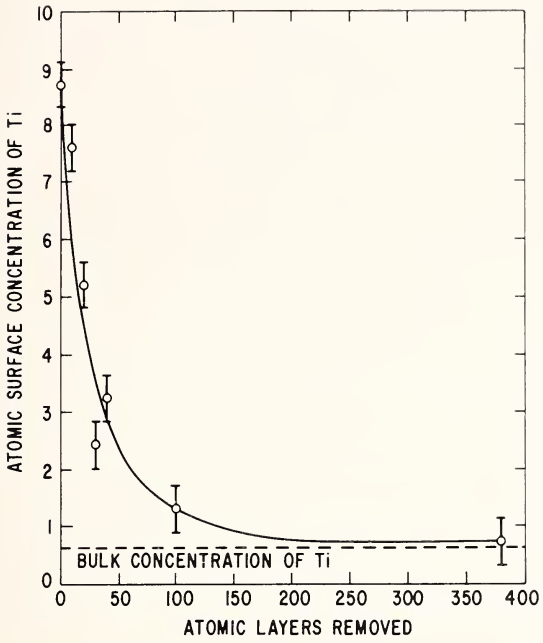

Figure 21. Titanium depth profile obtained a fracture surface of a 250 series maraging steel (from Johnson and Stein [45]).

Bi_2O_3 added exceeds its solubility in ZnO at the sintering temperature and it was assumed that the excess Bi_2O_3 solidified out into an intergranular phase on cooling. Interpretation of AES work, however, suggested the formation of a grain boundary film as opposed to an intergranular phase. Figure 22 is the depth profile for bismuth showing that the concentration drops rapidly over a distance much smaller than the size of typical grain phases. The Auger work agrees qualitatively with similar ion scattering work.

VI. Electron Spectroscopy for Chemical Analysis

Electron spectroscopy for chemical analysis [47-49], (ESCA), has experienced considerable growth in the past few years, as evidenced by the large number of recent books, articles and conferences on the subject. The basic principles of ESCA are illustrated in figure 23. X-rays of known energy strike the surface of the sample. If the x-ray energy exceeds the binding energy E_B of a core level electron, then that core level electron can be excited into a vacant state above the Fermi level. Furthermore, if

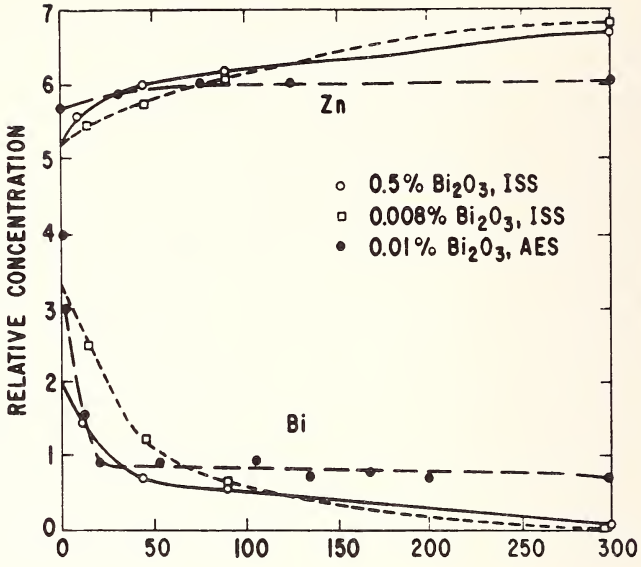

Figure 22. Bismuth depth profiles obtained by AES and ISS examination of fractured zinc oxide (from Morris and Cahn [46]).

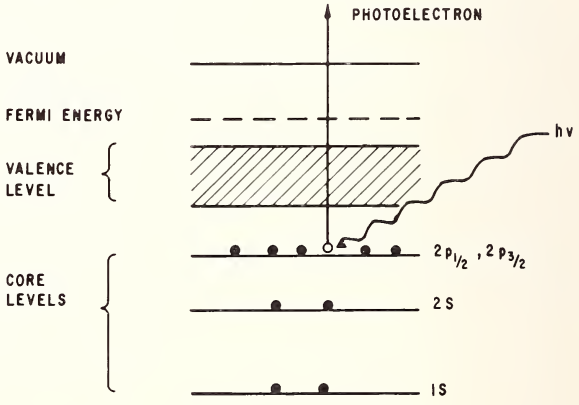

Figure 23. Basic principles of ESCA.

the excited electron has sufficient energy to overcome the surface work function of the sample then it will be emitted into the vacuum with a characteristic energy measured by an electron spectrometer of:

$$E = h\nu - E_B - \Phi_{spec} \tag{7}$$

where Φ_{spec} is the work function of the spectrometer which is a constant. Because electronic binding energies as calculated by equation 7 are

characteristic of a particular chemical environment, ESCA can be used both to obtain elemental compositions and also information about chemical bonding. As in the case of AES, ESCA is a surface technique since only those atoms located within a distance comparable to the mean free path of the generated photoelectrons, about 5 to 20 Å, will contribute to the useful signal. However, the ESCA detection volume is somewhat larger than that of AES, because unlike electron beams the incident x-rays cannot be focused. The minimum sample area illuminated is on the order of square millimeters compared to tens of square micrometers for the scanning Auger microscope. Detection levels as low as 0.1 to 0.01 monolayer [50], make ESCA well suited to such problems as catalysis, corrosion studies and fiber finishes. Furthermore, the less destructive nature of x rays compared with electron bombardment is important since it minimizes sample degradation in the study of organic compounds.

Although some quantitative analysis is possible, basic data on inelastic mean free paths, photon impact ionization cross sections and the exact shape of the background are still badly needed for the development of a satisfactory theoretical model. There have been quantitative measurements based on standards [47,51,52], however, such as the determination of the concentrations of each oxide in MoO_2-MoO_3 mixtures measured by Swartz *et al.* [53]. These measurements illustrate the advantage of the chemical bonding information which can be readily gained from ESCA compared to ISS, AES and SIMS.

Figure 24 shows the basic components of an ESCA spectrometer which includes an x-ray source, sample chamber, an electron energy analyzer, and a detector together with data acquisition devices. As in the case of the other methods of surface analysis already described to prevent contamination and to minimize electron scattering the sample chamber must operate at pressures of 10^{-7} torr or better. There exists a number of different versions of ESCA instrumentation. The type shown in figure 24 uses polychromatic x rays taken directly from an x-ray tube having either an aluminum or magnesium target. Photoelectrons ejected from the sample are focused onto the entrance slit of a hemispherical analyzer (shown in cross-section). Electrons which have appropriate energy for a given potential applied to the analyzer plates will follow a variety of trajectories to the exit slit and be detected by a continuous spiral electron multiplier. Individual pulses can be counted or an integrated signal displayed on a strip chart recorder. The choice of polychromatic radiation, which consists of a strong characteristic peak superimposed on a continuous background, is indicative of the need to maximize the x-ray flux striking the sample. Monochromating the x-ray beam will improve the observed

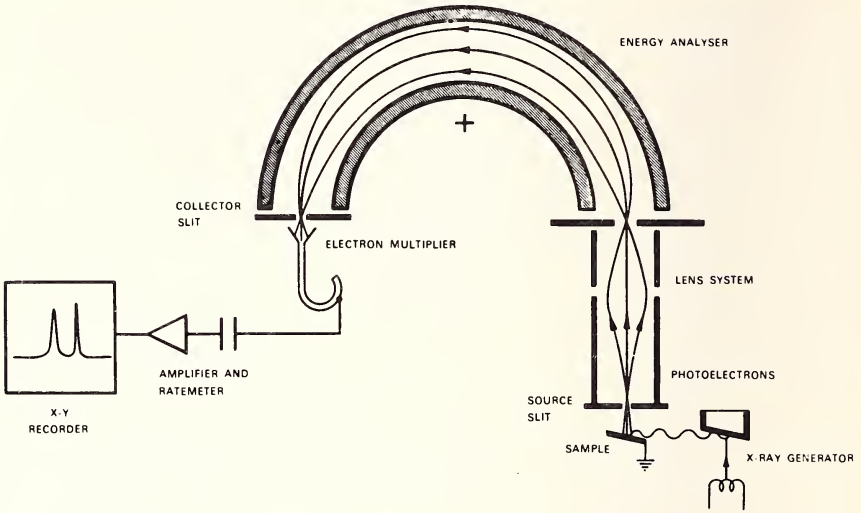

Figure 24. The basic components of an AEI ESCA spectrometer.

signal to noise ratio in an ESCA spectrum but at the cost of considerable intensity, increasing data acquisition time. Nevertheless, if maximum energy resolution is required, systems can be employed (see Siegbahn ref. 47) which use a monochromator and analyzer combination in such a way as to obtain a resolution better than the natural width of the characteristic source line (about 1 eV).

The obvious similarities between AES and ESCA have recently prompted the development of equipment which combines the two techniques. Figure 25 illustrates an instrument which uses a high resolution double pass coaxial cylindrical analyzer with a choice between x-ray and electron excitation. In the ESCA mode an x-ray source is used in combination with retarding grids in the front of the analyzers to decrease the energy of the incoming electrons. In the Auger mode a coaxial electron gun similar to that shown in figure 19 is used in combination with a different detection system for derivative processing. Data collection times in the Auger mode are typically 10 times shorter than with ESCA.

A typical ESCA spectrum is a presentation of the photoelectron intensity *versus* the electron binding energy. The example shown in figure 26 gives the spectra of elemental copper and its two oxides [54]. The differences in spectra reflect the oxidation states of copper in each of the three different chemical environments.

ESCA has been applied to a variety of practical problems. Brinen and Melera [55] used ESCA to study catalytic activity with respect to the ef-

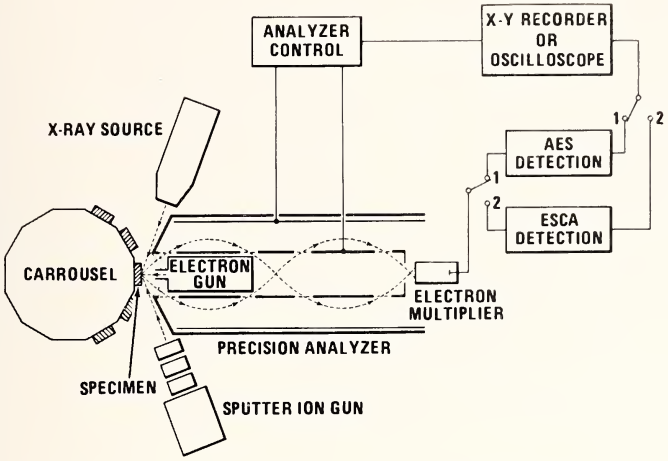

Figure 25. The basic component of a PHI ESCA – Auger analysis system.

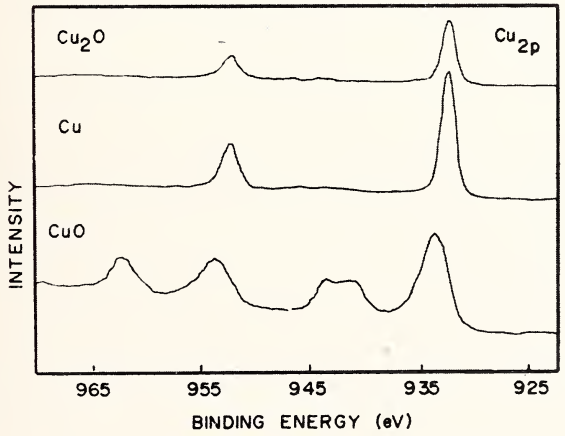

Figure 26. ESCA spectra of copper and copper oxides (from Baitinger and Amy[54]).

ficiency of selective hydrogenation of rhodium on charcoal. Although the absolute binding energies were not measured, the results suggested differences between catalysts having high and low activity. Figure 27 shows the 3d electronic orbital ESCA spectrum of three catalyst systems of similar bulk chemical compositions. These catalyst systems are the precursors of actual catalysts in their working states. Catalyst A and B show similar spectra, but the spectrum of catalyst C shows a higher intensity of metallic rhodium than of the oxide. The improved performance of catalyst A and B may be attributable to higher oxide surface concentration.

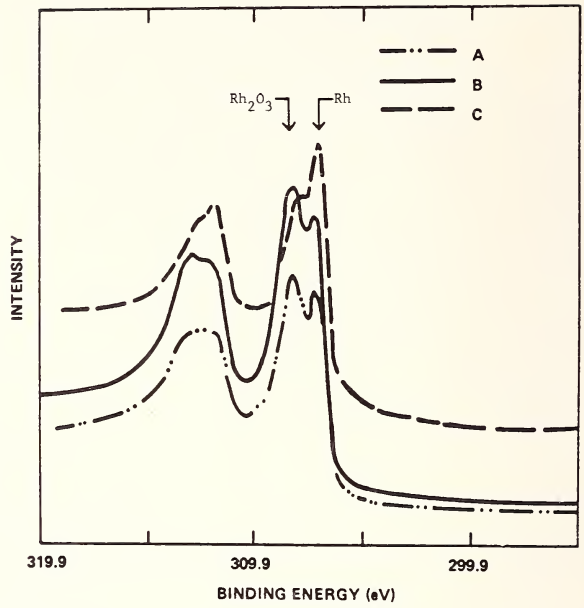

Figure 27. ESCA spectra obtained rhodium catalysts (from Brinen and Melera [55]).

As another example, Millard [56] used ESCA to determine the affect of corona discharge and low temperature discharge plasma treatment as a method of improving wool yarn. The ESCA spectra of the wool components – sulfur, nitrogen, carbon and oxygen – were observed before and after the treatments. The most significant changes were observed for sulfur. Figure 28a shows the spectrum of the sulfur 2p electronic orbital from an untreated sample which closely resembles that of sulfur from cystine samples. A shift toward higher electronic binding energies is observed after exposure to a corona discharge as shown in figure 28b. Low temperature plasma discharge treatment results in a decrease in intensity for the shifted peak (fig. 28c). From these observations, it can be concluded that varying amounts of sulfur had been oxidized. This is consistent with the fact that ozone and atomic oxygen are known to exist in discharge systems and both species readily oxidize sulfur.

VII. Summary

A variety of analytical instrumentations for the chemical analysis of surfaces and fine microstructural detail has been introduced. The list is by

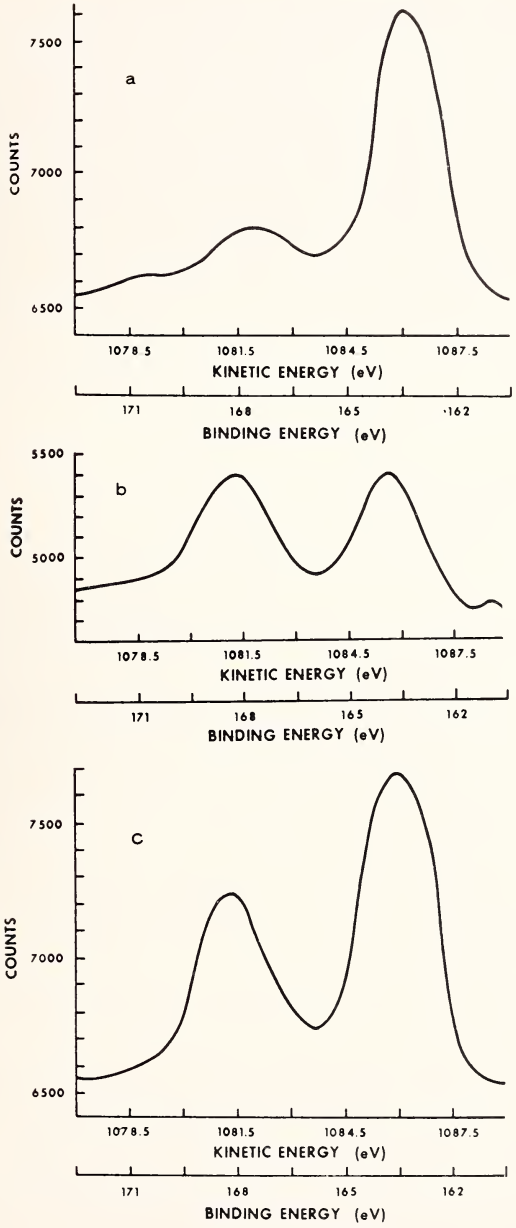

Figure 28. ESCA spectra of wool treated by low temperature corona discharge (from Millard [56]). a. sulfur 2p spectrum of untreated sample; b. sulfur 2p spectrum sample exposed to corona discharge; c. sulfur 2p spectrum of sample treated by low temperature plasma discharge.

no means complete since other new techniques like proton excited x-ray spectroscopy and x-ray appearance potential spectroscopy are also finding increased applications. Clearly no single technique combines all of the requirements for sensitivity, resolution and structural information that arise in the diversity of problems encountered by material characterization laboratories throughout the country. All of the methods discussed have common requirements of meticulous sample handling and high vacuum systems to avoid contamination. If the increasing number of publications and conferences on surface analysis is any indication, we can expect to see continued growth in this area over the coming years.

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THE EFFECTS OF CONTAINER COMPOSITION, STORAGE DURATION, AND TEMPERATURE ON SERUM MINERAL LEVELS

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To evaluate the effects of various storage parameters on serum mineral levels, blood-bank pooled, human serum was stored in Erlenmeyer flasks of five different compositions at three temperatures. The samples were analyzed by atomic absorption techniques for calcium, magnesium, copper, zinc, sodium, and potassium at 0, 1, 2, 4, 8, 16, 32, and 50 days of storage. Container effects were highly significant for Zn, K, and Na, but were small with maximal container differences of 2 to 3 percent. To 16 days of storage, temperature effects were not significant, and at later times temperature was highly significant at room temperature for Ca, Mg, and Zn. The effect of storage time was highly significant for all elements: Zn showed a 20 percent decrease after 2 days; Ca, Mg, and Cu had 10 percent decreases at 2, 4, and 8 days, respectively; K and Na showed maximal decreases of 2 percent and 3.5 percent with storage time. For the elements Zn, Ca, Mg, and Na, decreases were observed after 4 days of storage with near return to the original values on day 8. The patterns of mineral change in serum suggested at least two mechanisms: (1) pH change and (2) at later times, bacterial growth. To test these hypotheses, two subsequent studies were initiated. In one study, pH of freshly pooled human serum was measured, and streak plates were counted for bacteria at the time of each elemental analysis under various storage conditions. In another study, serum pH changes were accelerated by physical techniques that accelerated the loss of CO_2. The data indicate that factors that influence serum pH levels appear to affect elemental concentrations.

Keywords: Atomic absorption spectrophotometry; calcium; container effects; copper; magnesium; potassium; serum minerals; serum storage effects; sodium; temperature effects; trace elements; zinc.

I. Introduction

With the recent advances in trace element analysis and the accumulating evidence that changes in the levels of biologic trace elements do have clinical significance, greater emphasis has been placed on sample handling and preparation. This report describes the effects of storage parameters on serum mineral levels in human blood serum, as determined by atomic absorption spectroscopy. Containers of similar geometries and different compositions containing the same volume of pooled human serum were stored at three temperatures. The elements Cu, Zn, K, Na, Mg, and Ca were analyzed at approximately a geometric progression of storage times to 50 days of storage.

II. Methods and Materials

All serum analyses were performed with a Perkin-Elmer Model 306 atomic absorption spectrophotometer utilizing the 10-second integration mode, a Bohling burner and air-acetylene flames. Calcium, magnesium, potassium, and sodium levels were determined by the method of Paschen and Fuchs [1]. In this technique, 0.1 ml serum is diluted in 9.9 ml of 0.25 percent strontium (as $SrCl_2 \cdot 6H_2O$) to avoid chemical interferences. Calcium and magnesium were determined at the commonly used resonance lines of 422.7 nm and 285.2 nm, respectively. Sodium, because of its presence at higher concentrations, was determined at a less sensitive resonance line, 330.3 nm, and potassium at 769.9 nm using the order-separating filter as described by Paschen and Fuchs. Serum copper and zinc were determined in dilutions of 1 ml of serum in 4 ml of doubly distilled water. This dilution technique has been successfully used by Pekarek *et al.* [2] for zinc and by Tessmer *et al.* [3] for copper analyses. Copper and zinc analyses were performed as recommended in the Perkin-Elmer users' manual. Our choice of methodology was dictated by the simplicity and accuracy of the analytical techniques since many samples had to be processed in a short time.

The main storage study was performed utilizing pooled human serum from approximately 1,000 normal donors, 78 percent of whom were men 18 to 65 years of age (mean age, 38 years). Serum samples were added to a frozen pool at the time of harvesting at the Sacramento Medical Foundation Blood Bank. The pool was allowed to thaw only when we pipetted aliquots into the experimental storage containers at zero experimental time. In a later experiment to study freshly pooled serum and to evaluate

pH changes that could have been associated with the trace element levels observed in the main experiment, serum from 16 donors (14 males) 20 to 45 years of age (mean age, 28 years) was harvested, pooled, and pipetted directly into the storage containers. Also, pooled serum samples from the SMF Blood Bank were subjected to treatments that accelerated pH changes by displacing carbon dioxide from the samples.

All glassware was rinsed three times with solutions of 1:19 concentrated nitric acid: doubly distilled water (from a glass still) followed by three rinsings with doubly distilled water. On the morning of each analysis, new standard solutions were prepared from 1,000 ppm Techtron standard stock solutions. Before use, the stock solutions were compared to laboratory-prepared solutions. The diluents, either doubly distilled water or 0.25 percent Sr solution, were used as blanks. The standard for each element was prepared separately to prevent contamination. The Erlenmeyer flasks were covered with Parafilm, stored on Teflon sheets, and housed under plastic shields. The serum samples were pipetted with Eppendorf pipets (100 λ and 1,000 λ) using disposable plastic tips. At no time did the sample or storage container come into contact with metal; dust contamination was minimized by the described storage precautions.

In the second study, at the time of elemental analysis, serum aliquots were transferred to tightly capped containers for pH measurement using a Duo-matic M 123 pH meter with an Instrument Laboratory Model 127 constant temperature bath. Bacterial counts in the second study were determined on 10 μl serum by streaking onto sheep's blood agar plates and incubating at 37 °C for 2 days.

In the main study, 50 ml of pooled human serum was pipetted into 250-ml Erlenmeyer flasks of (1) Pyrex, (2) polypropylene, (3) polycarbonate, (4) Teflon, and (5) Vycor, all of similar geometries to ensure similar surface-to-volume ratios. The flasks were stored at various temperatures: room temperature (about 25 °C), refrigeration (8 °C), and freezing (− 15 °C). Duplicate containers were maintained at each temperature. The samples were analyzed at 0, 1, 2, 4, 8, 16, 32, and 50 days of storage. At the time of analysis, each container was equilibrated at room temperature to allow accurate pipetting of aliquots. Data from the main study were analyzed by a three-way analysis of variance, fixed effects model.

In the second study, only Pyrex containers were used. Aliquots of 25 ml of freshly pooled serum were pipetted into 125-ml Erlenmeyer flasks and 25-ml glass-stoppered volumetric flasks which were stored at room and refrigerator temperatures. The elements Cu, Zn, Ca, and Mg were analyzed at storage days 0, 1, 2, 4, 8, and 16 as described for the main study.

III. Discussion and Results

Serum mineral data from the main study were statistically analyzed for container composition, storage duration, and storage temperature effects as well as for container-duration, container-temperature, and duration-temperature interactions.

A. Temperature and Duration-Temperature Effects

Regardless of the container, no significant effects of storage temperature were noted for any minerals studied up to 16 days of storage. At days 32 and 50 the effects of temperature were highly significant ($p < 0.001$) for Ca, Mg, and Zn, due to the effects of storage at room temperature. Calcium levels for containers at room temperature declined from an initial value of 10.7 mg/100 ml to 6.1 mg/100 ml at day 50 (fig. 1). For calcium, the effect of storage temperature became evident only after day 16 at room temperature; no effect was observed in either the refrigerated or frozen samples. Similarly, the magnesium concentration at room temperature declined from an initial value of 2.12 mg/100 ml to 1.81 mg/100 ml at

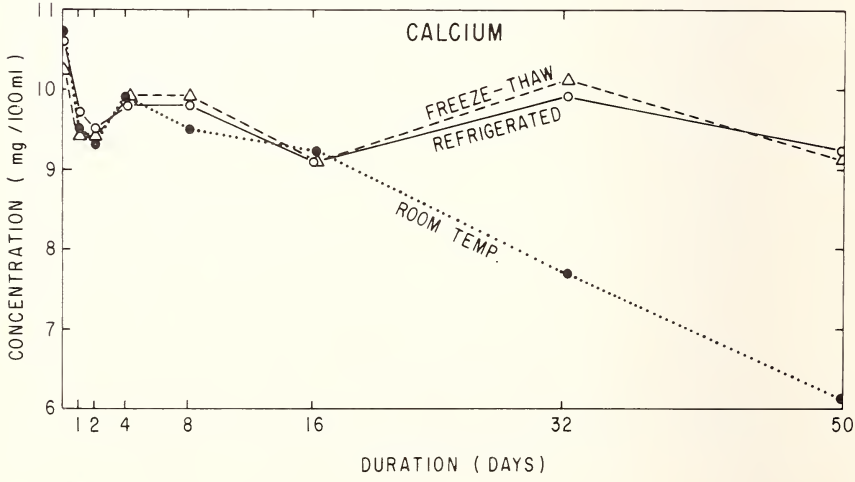

Figure 1. Effects of storage duration and temperature on serum calcium levels in the main study.

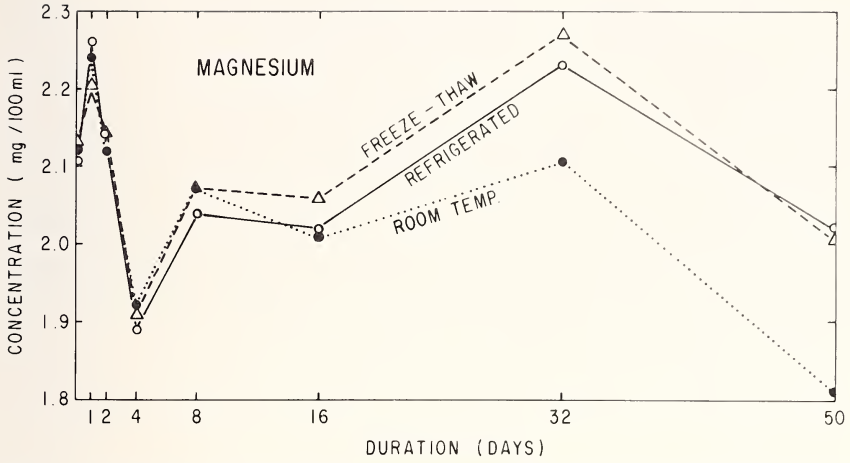

Figure 2. Effects of storage duration and temperature on serum magnesium levels in the main study.

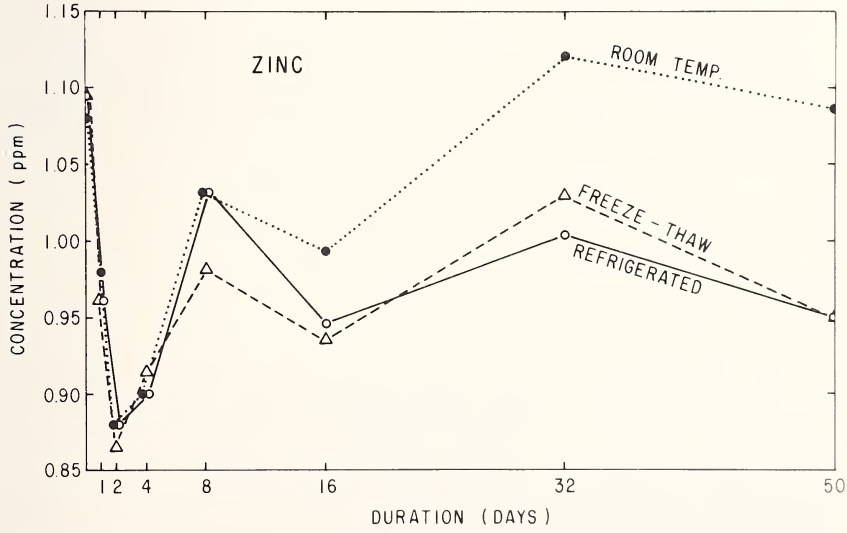

Figure 3. Effects of storage duration and temperature on serum zinc levels in the main study.

day 50 (fig. 2). Again, this effect became evident only after day 16 and only at room temperature. The room temperature effect on zinc levels likewise appeared to begin at day 16, at which time the concentration of Zn was higher than it was at the other temperatures (fig. 3). At day 32, the Zn levels approximated their initial values; at day 50, the concentration of Zn in samples at room temperature was 1.10 ppm and that in refrigerated and frozen samples was 0.95 ppm. The effects of temperature on Ca, Mg, and Zn concentration in serum samples in all containers stored to 50 days are summarized in figure 4. The only significant temperature effect was at room temperature after 16 days of storage. The temperature effects were not significant for Na, K, and Cu.

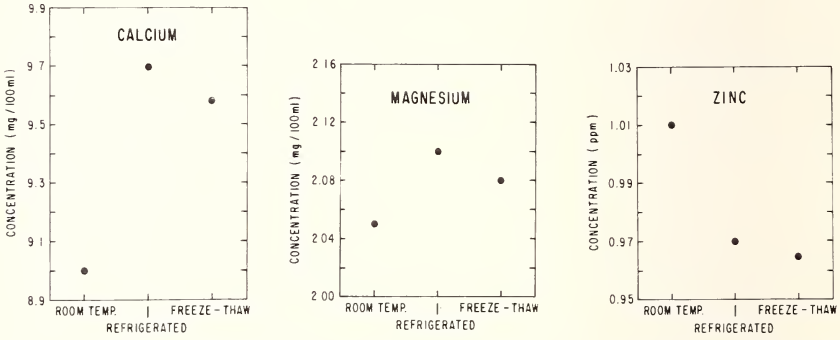

Figure 4. Effects of storage temperature on serum calcium, magnesium, and zinc levels in the main study.

B. Container, Container-Duration, and Container-Temperature Effects

The effect of container composition for all days at the three storage temperatures was significant ($p < 0.01$) only for K, Na, and Zn. In each case, however, the container effect was small, with maximal differences of 2 to 3 percent. For zinc, the difference was mainly due to a lower concentration in Pyrex (fig. 5). For potassium, the difference was primarily due to a higher value in polypropylene and a lower value in Pyrex (fig. 5).

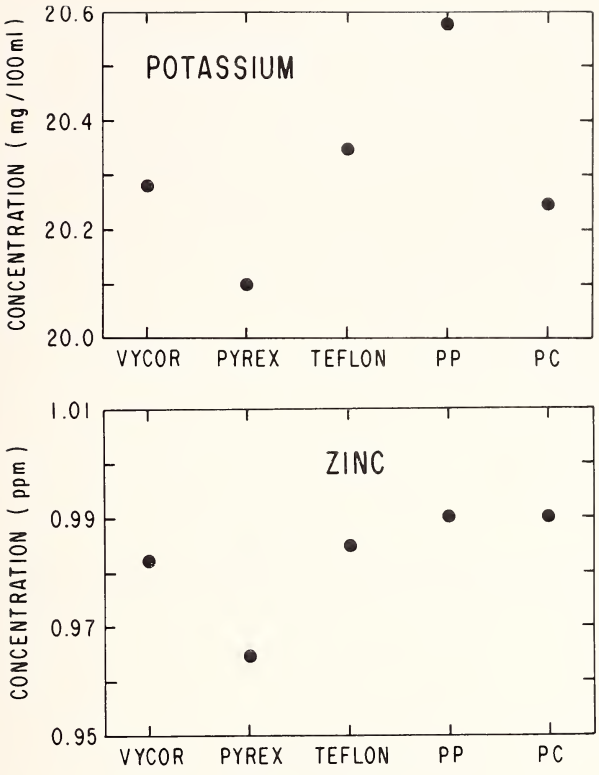

Figure 5. Effects of container composition on serum zinc and potassium levels in the main study.

Both polypropylene and Vycor contributed equally to the slight but significant container effect observed for sodium; the polypropylene values were slightly higher and the Vycor values were slightly lower than the grand mean. Container-duration effects were significant ($p < 0.05$) only for zinc, primarily due to the low zinc levels in the Pyrex containers measured at day 8, and to a lesser extent at day 32 (fig. 6). As noted above, the container effect was due to an overall low mean Zn concentration in samples stored in Pyrex, but was not consistent over time. The temperature-container interactions were not significant for any element, indicating that all containers were similarly affected by the three temperatures.

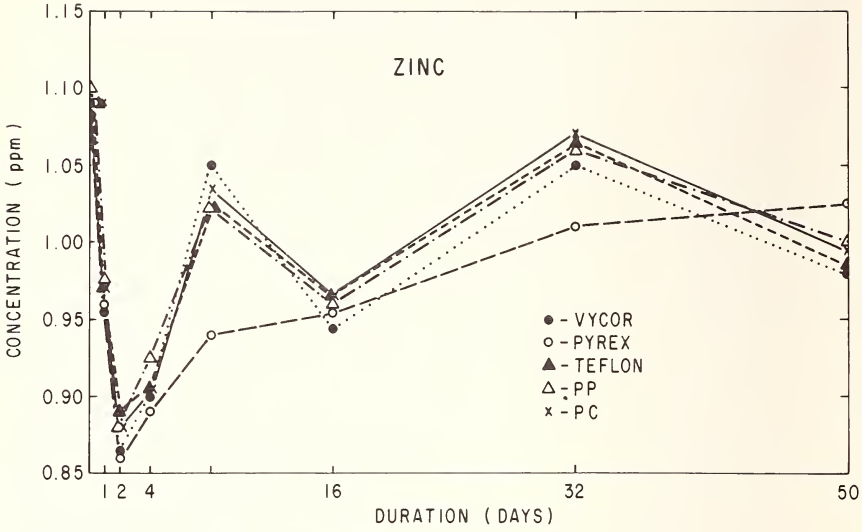

Figure 6. Effects of container composition and storage duration on serum zinc levels in the main study.

C. Storage Duration Effects

The effect of storage duration was significant for all elements ($p < 0.001$ for Cu, Zn, Ca, Mg, and Na and $p < 0.01$ for K). Zinc levels showed a rapid decline of approximately 20 percent from day 0 to day 2 (fig. 7), followed by a return to near-initial values after day 4. As previously discussed, the room temperature effect contributed significantly to this finding. Copper levels decreased by approximately 10 percent at day 8, followed by a rise to near-initial values at day 16 and a return to the depressed level of day 8 at days 32 and 50 (fig. 8). Calcium levels decreased approximately 10 percent at days 1 and 2, followed by a slight rise and then a continual decrease to approximately 80 percent of the day 0 value (fig. 9). The decrease after 16 days was primarily due to storage at room temperature. Magnesium showed an increase of about 5 percent at day 1, followed by a decrease to 10 percent of the original level at day 4, nearly returning to original values at day 32 and dropping to day 4 level at day 50 (fig. 10). For calcium, the decline at day 50 was mainly due to the room temperature effect. For copper, zinc, calcium, and magnesium the variations observed in the measured concentrations were due primarily to the effect of duration of storage. Sodium and potassium showed maximal

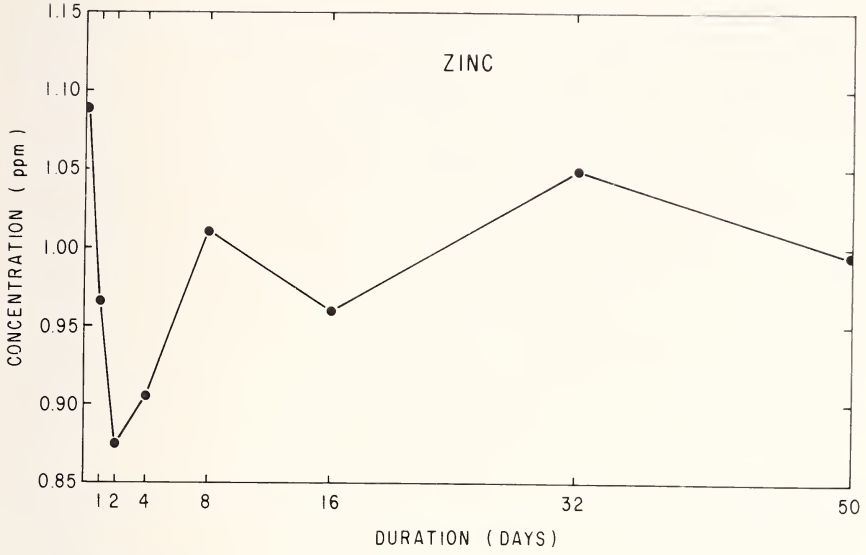

Figure 7. Effects of storage duration on serum zinc levels in the main study.

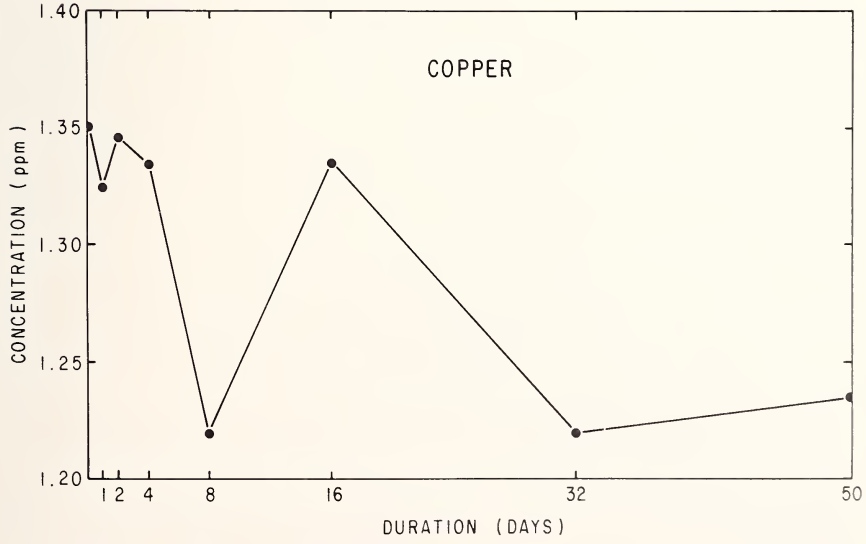

Figure 8. Effects of storage duration on serum copper levels in the main study.

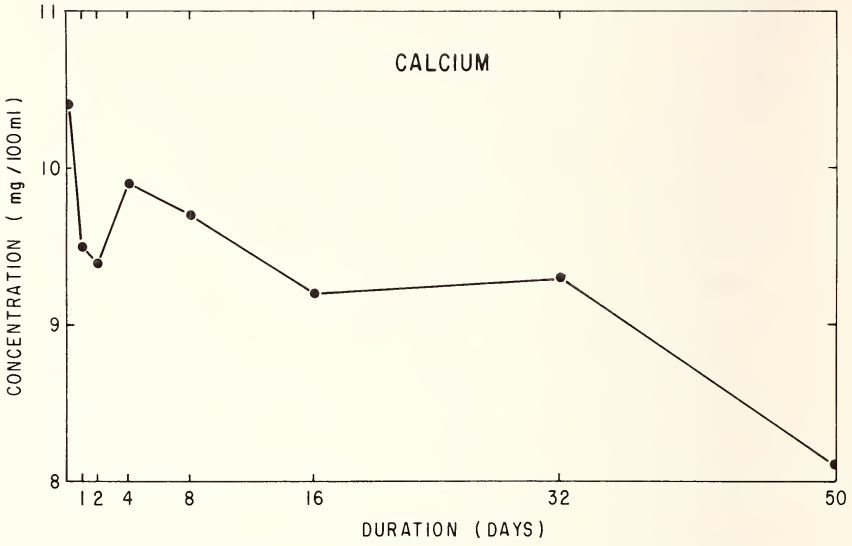

Figure 9. Effects of storage duration on serum calcium levels in the main study.

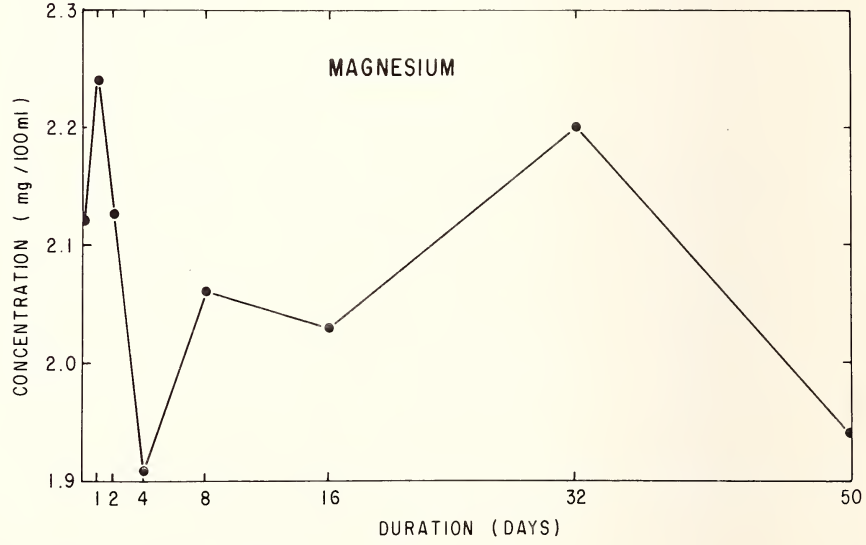

Figure 10. Effects of storage duration on serum magnesium levels in the main study.

changes of 2 percent and 3.5 percent, respectively, with storage duration. The changes in sodium levels occurred predominantly at the early time periods. The statistical findings of the main study are summarized in table 1.

TABLE 1. *Summary of significant effects observed in the main serum storage study*

Main effects	
Containers (C)	Zn,[a] Na,[b] K[b]
Temperature (T)	Ca,[a] Mg,[a] Zn[a]
Duration (D)	Ca,[a] Mg,[a] Cu,[a] Zn,[a] Na,[a] K[b]
Interactions	
C × T	None
C × D	Zn[c]
T × D	Ca,[a] Mg,[a] Zn[a]

[a] $p < 0.001$
[b] $p < 0.01$
[c] $p < 0.05$

The initial decrease in serum calcium concentration was similar to that reported by Hall and Whitehead [4], who studied the effects of overnight storage at room temperature on serum calcium. They ascribed the observed changes to loss of carbon dioxide from the serum, followed by a pH increase and protein denaturation and wall absorption leading to calcium adsorption on the proteins. Our results agreed in magnitude and direction with those of Hall and Whitehead, although the near return to initial concentrations observed in many samples suggested that CO_2 loss was not the only factor involved. Since we observed gross microbial growth in our containers at approximately day 10 of storage, we postulated the initial changes to be due to CO_2 loss, and the near return to be due to microbial respiration leading to CO_2 production and/or microbial metabolism leading to acid intermediates such as those found in the "TCA cycle." The subsequent drop in concentration at 32 and 50 days of room temperature storage was thought to be due to the sequestering of elements by massive growth of microorganisms that adhered to the container walls.

To test these hypotheses, a second study was initiated using freshly pooled human serum from volunteers in our laboratory. In this study pH of each serum sample was measured, and streak plates were counted for bacteria at the time of each elemental analysis. Since the first study indicated few container composition effects and no difference between refrigerated and freeze-thaw, only Pyrex containers were used at room and refrigerator temperatures. The elements Na and K were not analyzed in the follow-up, since the magnitude of the observed changes was within the range of error of the analytical techniques. In this study 25 ml of freshly pooled serum was added in duplicate to either 125-ml Erlenmeyer

flasks covered with Parafilm or to 25-ml volumetric flasks with glass stoppers. The volumetric flasks were chosen because of the relatively small air space above the serum and because they accommodate ground glass stoppers which prevent CO_2 loss.

The results of the pH analysis are presented in figure 11. Even at day zero of storage (actually 1 hour), serum samples in glass-stoppered volumetric flasks had lower pH than those in the Erlenmeyers; this difference was consistent throughout the study. Also, for each container, the pH rise was greatest at room temperature storage. A drop in pH was observed from day 8 to day 16 for the room temperature Erlenmeyers, due mainly to the pH change of 8.58 to 8.35 in one flask which had massive bacterial growth. One of the room temperature volumetrics also had massive bacterial growth and displayed a pH change from 8.52 to 8.42. Concomitant with the pH decreases was an increase in calcium concentration from 9.2 mg/100 ml to 9.8 (zero treatment value was 1.01) and from 9.6 to 9.9 (zero treatment value was 9.9) in the Erlenmeyer and volumetric flasks, respectively. The calcium data are presented in figure 12. Maximal decrease in calcium occurred in the room temperature Erlenmeyers at day 8, at the time of the highest measured pH. Similar results were found for the volumetric flasks at room temperature. No changes were observed in the Mg and Cu levels for the storage period. Zinc (fig. 13) showed a maximal decrease at days 1 and 2, followed by a return nearly to initial values at days 4, 8, and 16. The zinc results are similar to the changes observed in the main study.

In another study, we manipulated the pH of the serum by physical techniques that accelerated the loss of CO_2. For these studies, 25-ml aliquots of Blood Bank frozen pooled serum were pipetted into 125-ml Pyrex Erlenmeyer flasks. CO_2 was displaced by bubbling with N_2, by aspirating, by oven heating, or by leaving the containers open to the atmosphere. The control values are the pH and elemental levels at 3 hours of storage in sealed flasks at room temperature. Nitrogen and oven treatment (75 °C) was maintained for 3 hours, after which the samples were stored at room temperature in Parafilm-sealed flasks for the remainder of the experiment. Aspirated samples were treated for 24 hours and then maintained at room temperature. The open containers were left open throughout the experiment. The pH changes are presented in figure 14. As expected, oven treatment gave the most radical pH increase. The pH changes in open and aspirated containers were similar from days 1 to 5, although the aspiration was terminated at day 1. The open, nitrogen-bubbled, and aspirated samples all showed similar pH at day 5. After 3 hours for all treatments but oven heating, Ca, Mg, and Cu did not show a change

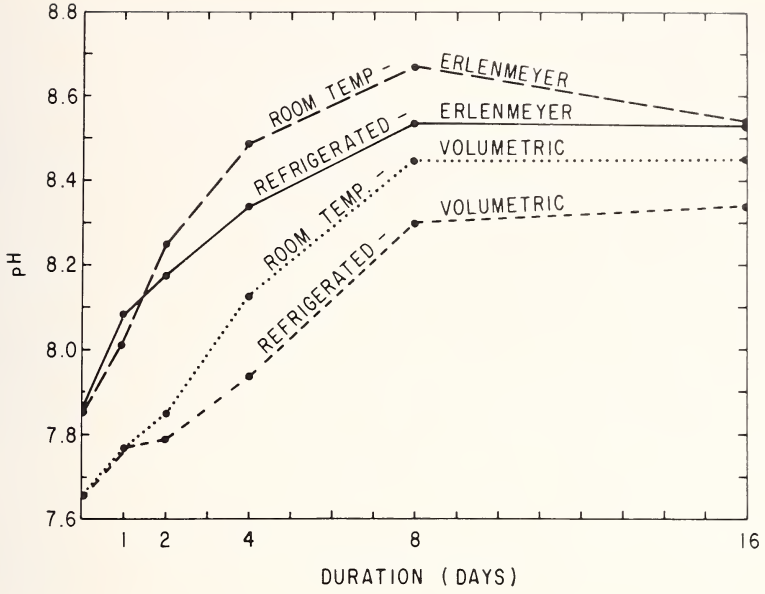

Figure 11. Effects of storage duration, temperature, and container type on serum pH levels in the second study.

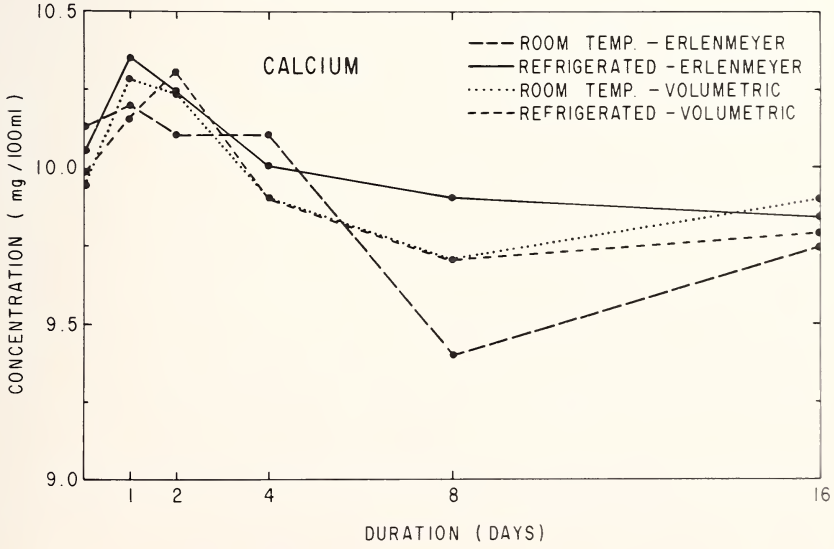

Figure 12. Effects of storage duration, temperature, and container type on serum calcium levels in the second study.

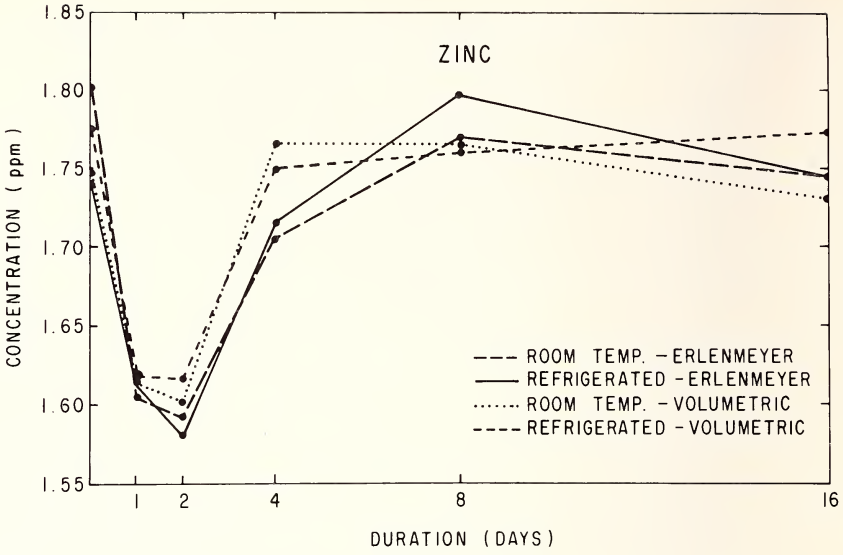

Figure 13. Effects of storage duration, temperature, and container type on serum zinc levels in the second study.

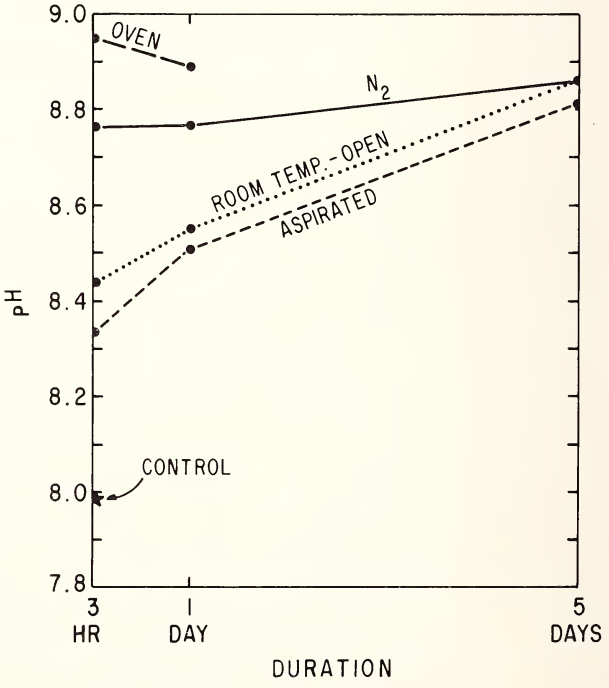

Figure 14. Effects of physical treatments that accelerated the rate of CO_2 loss on serum pH levels.

from the control value (fig. 15). Zinc did drop in the N_2 and open containers, but not in the aspirated. Oven treatment resulted in evaporation of 7.6 ml of sera and a cloudy, opaque suspension. All elements but copper showed marked decreases after heat treatment (fig. 15). Little change was observed in pH or elemental concentration (except zinc) 1 day after heat treatment. The effects of the other treatments were similar. Calcium declined after the first day by about 10 percent and continued to decline at day 5 (fig. 15). Magnesium declined by about 5 percent and remained constant at day 5 (fig. 15). No effect was observed on copper. No correction for evaporation was made since maximal evaporation was only 2 to 3 percent.

Our results indicate, in agreement with Hall and Whitehead, that the measured levels of trace elements in stored serum samples are pH-dependent. Factors that influence serum pH levels appear to affect elemental concentrations. In the storage studies, increases in pH were associated with CO_2 loss due to exposure to the atmosphere, and pH changes were accelerated by physical techniques that displaced CO_2—namely, N_2 bubbling, aspiration, heating, and constant exposure to the atmosphere.

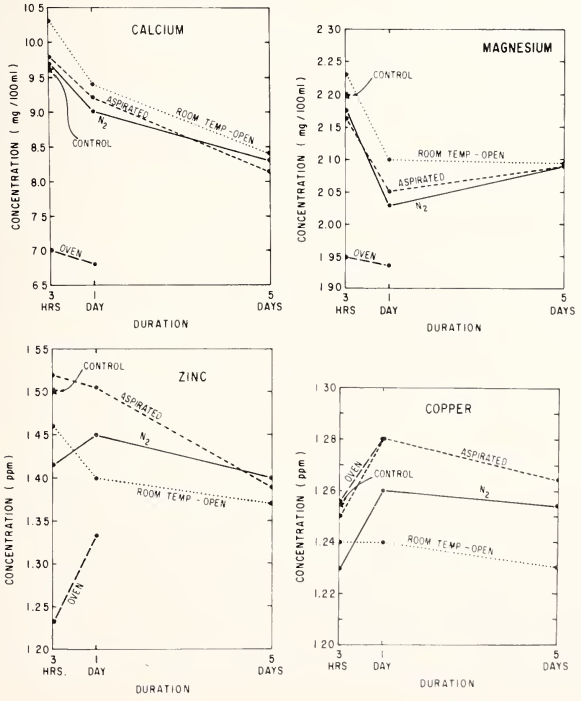

Figure 15. Effects of physical treatments that accelerated the rate of CO_2 loss on serum calcium, magnesium, copper, and zinc levels.

The main study indicated that container composition had little effect on mineral levels of serum stored in Pyrex, polypropylene, polycarbonate, Vycor, or Teflon Erlenmeyer flasks. Container effects were significant only for Na, K, and Zn and are probably not of importance to the clinical chemist. The fact that no difference in serum minerals was observed between refrigerated and frozen samples may be due to equilibration of serum pools to room temperature before aliquots were taken for daily analysis. In the second storage study no changes in Cu and Mg were observed and Ca levels fell only at day 8. However, the main study showed depressions of Ca at day 1, of Mg at day 4, and of Cu at day 8. In both studies, zinc decreased rapidly, followed by a return nearly to initial values. The difference in results of the two studies is probably due to the histories of the pooled samples and the handling of the storage containers. The large volume of serum required in the main study was provided by a blood bank. The serum was provided by many donors and continuously pooled for 3 weeks. We received the serum frozen and thawed it in open containers in warm tap water. After thawing and equilibrating to room temperature, the 30 aliquots of 50 ml were pipetted into the appropriate storage containers. The serum was left uncapped at room temperature for approximately 5 hours. Throughout the main study, major efforts were directed at avoiding contamination but not CO_2 loss. In the second study serum was pooled immediately after harvesting, and exposure to the atmosphere was held to a minimum. The changes observed in Ca at day 8 in the second study may have been due to the same causes as those observed in the main study, but offset temporally because of the precautions used to avoid CO_2 loss. The lack of changes in Mg may have been due to a similar delay. Changes in copper levels were not observed in the second study or in any of the pH alteration studies. Thus, no explanation for the copper changes in the main study is available. The zinc decrease in all studies was rapid, occurring even after exposure to the atmosphere at room temperature for only 3 hours. These results suggest that the mechanism of serum zinc level depression in stored samples is different than that for calcium.

With regard to proper storage of serum for mineral analysis, our findings imply that container composition is not an important factor, but that conditions that might increase pH should be avoided. If serum samples are to be stored, they should be quickly frozen after harvesting in tightly capped containers with a minimum of air space above the serum. We also found that acidification with dilute HCl could increase calcium values in containers which presumably had calcium adsorbed to the walls due to prolonged storage.

We suggest that further work is necessary to explain the mechanism of serum mineral loss in storage and to explain the differences observed in the behavior of mineral levels in freshly pooled and frozen pooled serum.

IV. Acknowledgements

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BIOLOGICAL SAMPLE CONTAMINATION DUE TO QUARTZ CONTAINER IN NEUTRON ACTIVATION ANALYSIS

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Activation analysis in not dependent on the purity of chemical reagents because these are used only after irradiation. However, in the analysis of biological samples a new source of "contamination" appears, due to the container in which the sample is irradiated. For this reason it is often necessary to separate the irradiated sample from its irradiation container before recording the activity. Since the quartz of the container is partly dissolved, it is necessary to eliminate contamination due to impurities adsorbed on the surface of the quartz ampoule. The values of the so-called "analytical blank" are given for some 15 elements, certain of which have a recognized importance in biology.

Keywords: Biological analysis; container blank; neutron activation analysis; wet-ashing blank.

I. Introduction

The accuracy of an analysis is always limited by the "analytical blank," which in standard methods is chiefly due to mineral impurities present in the reagents. Activation analysis is not dependent on the purity of the chemical reagents because if these are used it is only after irradiation, the analysis proper being the subsequent measurement of the radioactive isotopes formed during this phase.

However, when samples are analyzed whether in liquid, powdered or thermodegradable form, like biological samples, an irradiation container becomes necessary. This container is a source of contamination and may

introduce an "analytical blank." For intense neutron irradiations containers of fused quartz or fused silica are used.

II. Direct γ-Ray Spectrometry of the Sample in the Container – Quartz Container Blank

After variable irradiation and decay times, the sealed quartz containers, holding the samples and the standards, are cleaned by boiling in nitric acid, rinsed carefully in water and placed in front of a semiconductor detector.

Radioactivity of the samples is then recorded directly through the walls of the ampoule; in which case, it is the quartz glass impurities becoming radioactive during irradiation which are responsible for the "analytical blank," henceforward called "quartz container blank."

To minimize as much as possible the importance of this "blank," it is necessary to use a rigorously clean container; for this purpose, empty quartz ampoules are successively treated with EDTA, distilled water, boiling nitric acid and finally, distilled water.

Figure 1 shows the γ-ray spectra of a fused quartz container (2.2 g) irradiated for 2 days in a thermal neutron flux of 2.5×10^{14} n $\cdot$ cm^{-2} $\cdot$ s^{-1}, recorded for 1 hour with a Ge(Li) detector of 40 cm^3, after decay of 2 days (1a) and 11 days (1b).

The mass of some elements present in one gram of several kinds of silica glass, and the mass of the same elements contained in 100 μl of blood serum (volume usually put in a 1 g quartz ampoule) are presented in table 1.

The serum was chosen to represent a typical biological sample because of its low concentration of trace elements which renders it particularly sensitive to the influence of the "analytical blank." The determination of trace elements by direct γ-ray spectrometry of blood serum, through the quartz container, is not possible, except for selenium and potassium, whatever the kind of quartz used; in fact, the mass of each of the elements present in the quartz is always at least the same order of magnitude (and often 10 or 100 times larger) as the mass of these same elements in the serum.

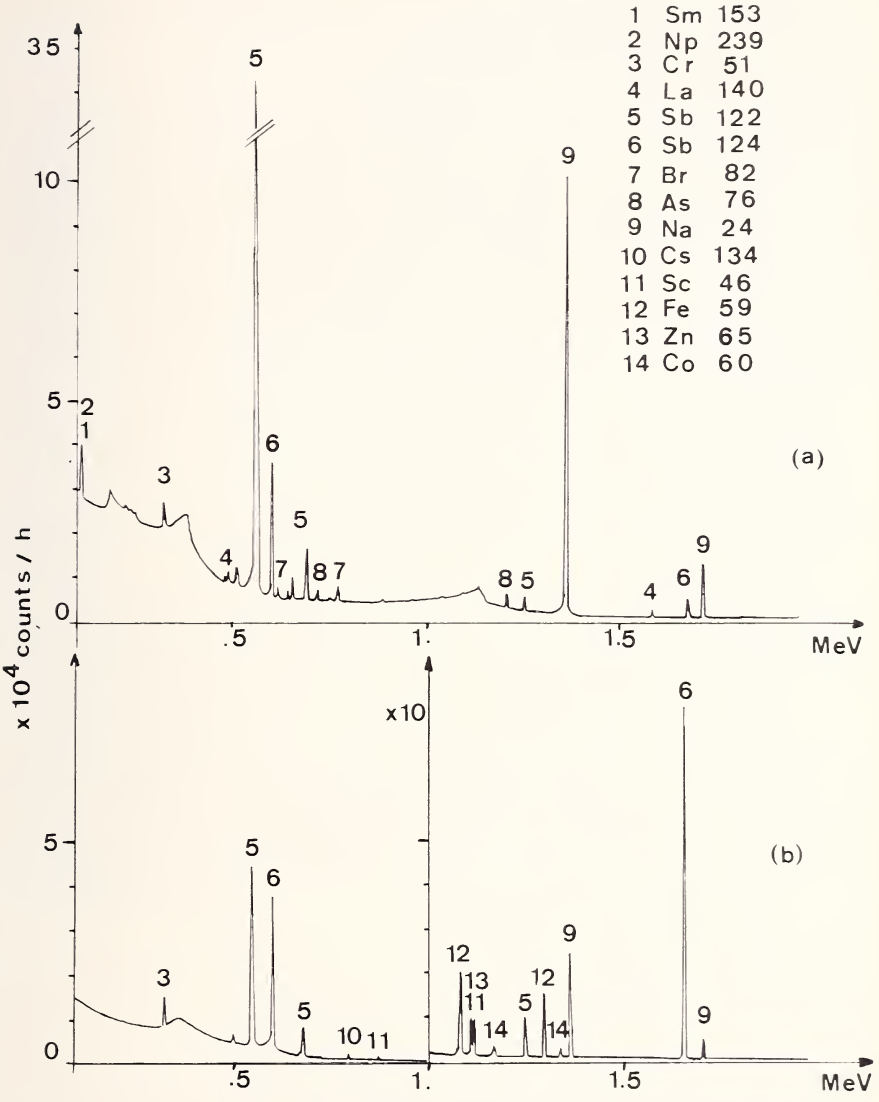

Figure 1. γ-ray spectra of a fused quartz container (2.2 g), irradiated for 2 days in a thermal neutron flux of 2.5×10^{14} n · cm^{-2} · s^{-1}, recorded for 1 hour with a Ge(Li) detector of 40 cm^3. a) after 2 days decay, b) after 11 days decay.

TABLE 1. *Comparison of the amount of elements contained in 1 g of various kinds of silica glass and in 100 μl of serum*

Element	Silica glass[a]				Serum μg/100 μl
	Fused quartz		Fused silica		
	A μg/g	B μg/g	C μg/g	D μg/g	
Au	1×10^{-2}	8.2×10^{-2}	1.3×10^{-4}	1×10^{-4}	2×10^{-5}
Cl	<0.1	<0.15	3.7	2.8	0.37
Co	1.3×10^{-3}	1.8×10^{-3}	$<10^{-3}$	1.8×10^{-3}	1.2×10^{-4}
Fe	1.44	<1	<0.5	<0.5	0.11
K	<1	1.7	<0.5	1.1	17
La	1.6×10^{-3}	2.3×10^{-3}	2.2×10^{-3}	4.5×10^{-3}	$<6 \times 10^{-4}$
Mn	2.7×10^{-2}	1.9×10^{-2}	3.2×10^{-2}	0.7×10^{-2}	0.5×10^{-4}
Sb	0.33	0.11	4×10^{-4}	4.1×10^{-4}	2.5×10^{-4}
Sc	1.5×10^{-4}	1.8×10^{-3}	10^{-3}	1.8×10^{-3}	1.5×10^{-5}
Se	$<9.2 \times 10^{-4}$				8.3×10^{-3}
Sm	3×10^{-4}	8.5×10^{-4}	3.7×10^{-4}	3.9×10^{-4}	2×10^{-4}
Zn	7.2×10^{-2}	$<5 \times 10^{-2}$	$<10^{-2}$	$<10^{-2}$	0.1

[a] A = Quartex (blown).
B = Quartex (drawn).
C = Spectrosil (T.Q.S.).
D = Electroquartz.

III. γ-Ray Spectrometry of the Sample Separated from Irradiation Container—Wet-Ashing Blank

Owing to the relative importance of the "quartz container blank" (whatever kind) compared with the amounts of trace elements in the biological reference sample, it is necessary, in order to obtain accurate quantitative results, to separate the sample from its holder before any measurement. Biological samples always suffer thermal degradation, to varying degrees, during intense reactor irradiation; therefore this separation can only be done by "wet-ashing" the samples.

In spite of the chemical inertia, well known in quartz, with respect to the reagents traditionally used during this "wet-ashing" process, some impurities coming from the quartz container are dissolved, forming a new kind of analytical blank: the "wet-ashing blank."

From the analytical point of view, this quartz-sample separation produces a spectacular drop of the analytical blank, the "wet-ashing blank" (Ao) being much weaker than the "quartz blank," as indicated in figure 2.

The mass of the elements dissolved during the "wet-ashing" process from the fused quartz ampoule by the mixture $HNO_3 - H_2O_2$ (normalized to 1 g of silica) is compared, in table 2, with the mass of the same elements present in 100 μl of serum.

In spite of the relative importance and the fluctuating character of the "wet-ashing blank," some serum trace elements can be accurately measured, for among these elements the influence of the "blank" on the results stays always less or equal to 1 percent; these are, besides of course selenium and potassium, barium, bromine, sodium, phosphorous, rubidium and strontium.

IV. Origin of "Wet-Ashing Blank"

This blank is probably produced at the moment when the extremity of the quartz ampoule, in order to be sealed, is heated in the flame of a gas-burner at a temperature between 1500 and 1900 °C.

The semi-liquid fusion of quartz is always accompanied with a significant volatilization. A part of the volatilized silica condenses on the cold interior and exterior surfaces of the tube. It is almost certain that the impurities contained in the volatilized silica are deposited on the walls of the tube. Whereas the impurities condensed on the exterior of the tube are eliminated during the cleaning of the ampoule after irradiation, the impurities condensed on the interior are only dissolved once the ampoule is

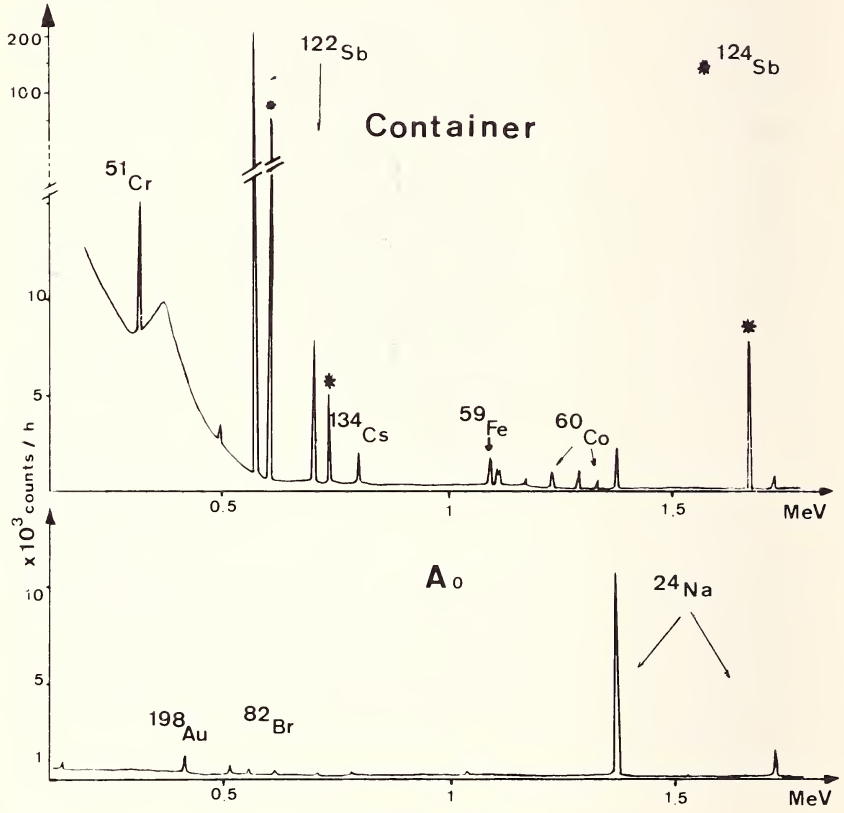

Figure 2. γ-ray spectra of a fused quartz container, irradiated for 24 h in a thermal neutron flux of 2.5×10^{14} n · cm^{-2} · s^{-1} (decay time: 11 days) and of its "wet-ashing" solution (decay time: 3 days), recorded for 1 hour with the 40 cm^3 Ge(Li) detector.

TABLE 2. *Comparison of the amount of elements contained in the "wet-ashing" solution of a 1 g ampoule of fused quartz and in 100 µl of serum*

Element	Wet-ashing blank µg/g quartz A	Serum µg/100 µl	Element	Wet-ashing blank µg/g quartz A	Serum µg/100 µl
Ag	$<10^{-2}$	8.5×10^{-4}	K	~0.7	17
Au	2.1×10^{-4}	2×10^{-5}	La	$0.15 - 3.4 \times 10^{-5}$	$<6 \times 10^{-4}$
Ba	$2.5 - 8.2 \times 10^{-5}$	7.2×10^{-3}	Lu	$<10^{-7}$	?
Br	$2.8 - 3.3 \times 10^{-4}$	0.33	Mn	$0.7 - 2 \times 10^{-4}$	5×10^{-5}
Cd	$0.6 - 3.3 \times 10^{-4}$	3×10^{-4}	Mo	$<3 \times 10^{-2}$	3×10^{-4}
Ce	$0.6 - 3.8 \times 10^{-4}$	$<2 \times 10^{-4}$	Na	$2 - 3 \times 10^{-2}$	325
Co	$0.2 - 1.7 \times 10^{-4}$	1.2×10^{-4}	Nd	$1.5 - 1.7 \times 10^{-3}$	?
Cr	$0.1 - 1.4 \times 10^{-3}$	1.9×10^{-3}	P	<0.1	13.7
Cs	$0.1 - 2.8 \times 10^{-5}$	1.8×10^{-4}	Pr	$<3.5 \times 10^{-6}$	$<5 \times 10^{-3}$
Cu	$0.2 - 7.5 \times 10^{-3}$	0.11	Rb	$<3 \times 10^{-4}$	2×10^{-2}
Dy	$<1 - 8 \times 10^{-6}$	$<2 \times 10^{-4}$	Sb	$<1 - 4 \times 10^{-5}$	2.5×10^{-4}
Er	$<1 \times 10^{-4}$	?	Sc	$0.3 - 1.7 \times 10^{-5}$	1.5×10^{-5}
			Se	$<1.8 \times 10^{-4}$	8.3×10^{-3}
Eu	$0.2 - 2.7 \times 10^{-8}$	$<4 \times 10^{-4}$	Sm	$0.7 - 2.7 \times 10^{-7}$	$<2 \times 10^{-4}$
Fe	$3.1 - 4.4 \times 10^{-2}$	0.11	Sr	$<2.5 \times 10^{-5}$	3.8×10^{-3}
Gd	$<2.8 \times 10^{-4}$	?	Tb	$<10^{-6}$	$<6.10^{-5}$
Hg	$3.7 - 7 \times 10^{-5}$	5×10^{-4}	W	$1 - 3 \times 10^{-5}$	$<7 \times 10^{-3}$
Ho	$<1.9 \times 10^{-4}$	?	Yb	$<1 \times 10^{-5}$	$<2 \times 10^{-4}$
Ir	$<10^{-6}$	?	Zn	$0.7 - 2.9 \times 10^{-2}$	0.1

opened for "wet-ashing"; and these are certainly at the origin of what is called the "wet-ashing blank."

V. Localization of Impurities in Silica Glass

To study the distribution in depth of impurities in silica glass, fused quartz ampoules are first irradiated then etched (for 10 μm of thickness) by hydrofluoric acid, three or four times.

Figure 3 represents the variations of activity, and therefore concentration, of various elements in silica glass in function of the depth of the layer studied.

These elements can be classed in two categories according to the homogeneity or heterogeneity of their distribution. For the elements of the second category, the observed concentration "gradient" probably results from an impurity migration towards the surface, during the fusion of quartz when the tube was produced.

Figure 4 represents the γ-ray spectra of the hydrofluoric etching solutions corresponding to layers 0-10 μm (HF 1) and 10-20 μm (HF 2), which illustrates very well this partial heterogeneity of distribution.

VI. Treatment of Container to Decrease the "Wet-Ashing Blank"

If, before sealing the ampoule, the superficial layer of quartz, which is the most contaminated, were eliminated, a very much lower quantity of impurities would be volatilized during the sealing of the tube, and thus the importance of the "wet-ashing blank" would be diminished.

To verify this hypothesis, an intact quartz tube and an etched quartz tube (20 μm depth) were sealed, irradiated and, after a 3-day decay, treated by the classical "wet-ashing" process: $HNO_3 - H_2O_2$.

In table 3, we have grouped the ratio values of ("blank" of intact tube)/("blank" of etched tube), (Ao/A2), which were calculated for some elements.

This table, and figure 5 which shows the γ-ray spectra of the "wet-ashing blank" Ao and A2, demonstrate very well the need for pre-irradiation etching of the quartz ampoule.

Evidently, this treatment lowers only the "wet-ashing blank" values of surface-localized elements such as Cr, La, Co, Zn, Fe, Au, *etc.* For elements uniformly distributed in the whole mass of the quartz (Sb, As, Mo, Sn) the superficial etching has no effect.

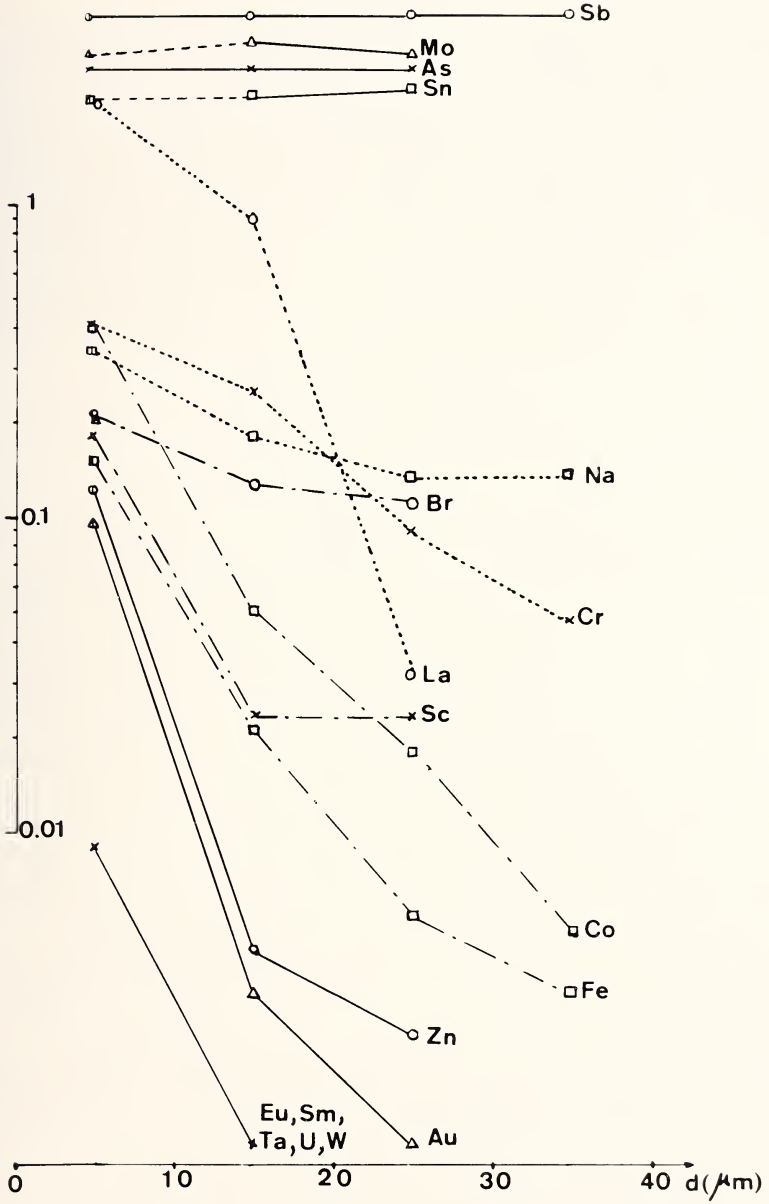

Figure 3. Radioactivity variations of several elements in silica glass, in function of depth-layer studied. The ordinates are arbitrary and do correspond to real level of concentration.

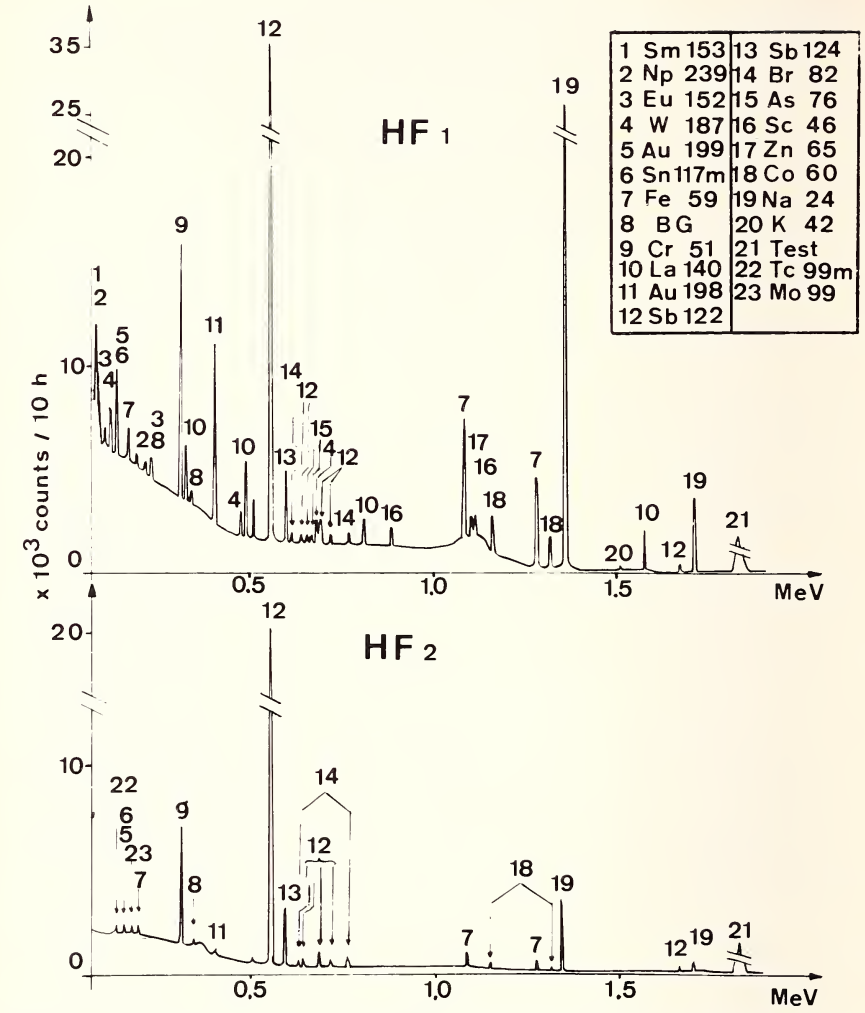

Figure 4. γ-ray spectra of HF-etching solutions corresponding to 2 successive layers of 10 μm thickness (HF 1: 0-10 μm and HF_2: 10-20 μm) of a fused quartz ampoule (~ 2g) irradiated for 48 hours in a thermal neutron flux of 2.5×10^{14} n · cm^{-2} · s^{-1} and recorded during 10 hours with a 40 cm³ Ge(Li) detector.

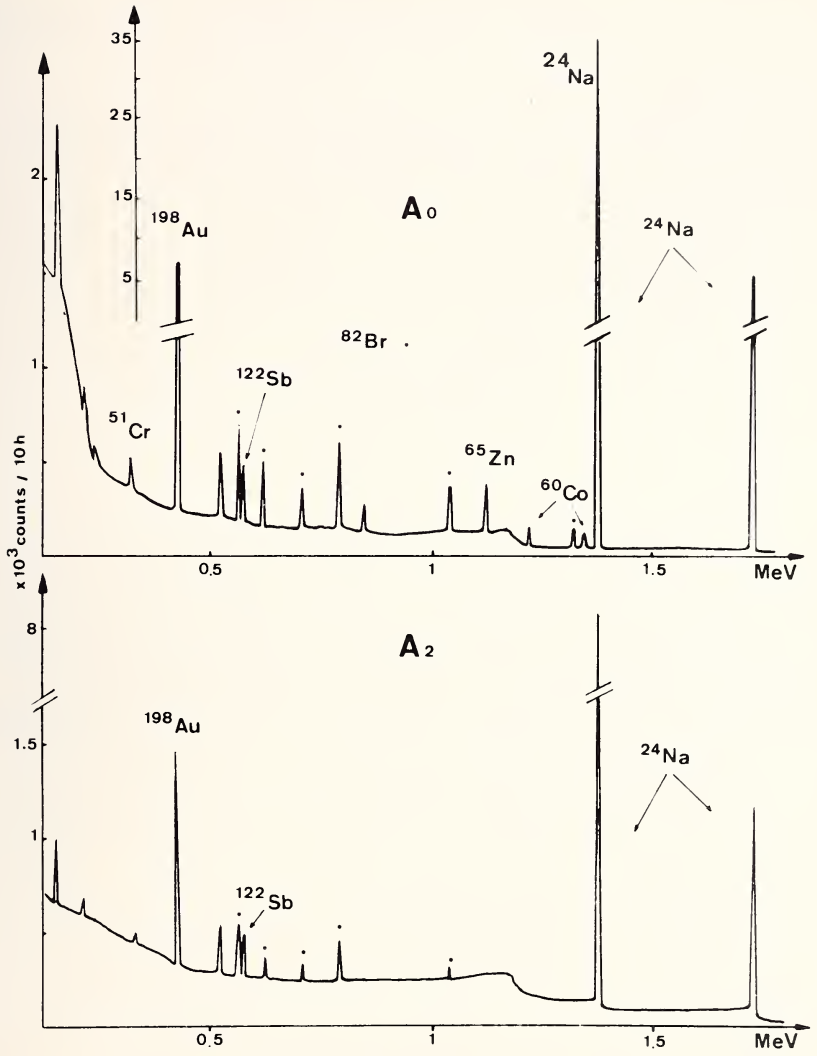

Figure 5. γ-ray spectra of "wet-ashing" solutions of fused quartz containers, one intact (Ao), the other etched to 20 μm depth (A2), irradiated for 24 hours in a thermal neutron flux of 2.5×10^{14} n · cm^{-2} · s^{-1} and recorded during 10 h with a 40 cm^3 Ge(Li) detector.

TABLE 3. *Improvement factors due to a 20 μm etching of fused quartz irradiation containers*

Element	A_0/A_2
Sb	1
Sn	1
Na	2.5
Fe	>3
K	4
La, Sm	5
Au	6
Co	>6
Cr	>7
Zn	>22

VII. Conclusion

The surface etching of a fused quartz container, of 20 μm depth, modified neither the mechanical properties of the ampoule, nor its macroscopical aspect; the silica glass remains polished and transparent.

However, it decreases, in a significant way, the level of the "wet-ashing blank" for many elements.

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PREPARATION OF BIOLOGICAL MATERIALS FOR CHROMIUM ANALYSIS

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In flameless atomic absorption spectrometry, introducing biological materials directly into a graphite furnace leads to a lower response than if the samples are pre-ashed. The decrease is due to organically bound chromium. Since few biological standards are available with reliable data as to chromium content, it is necessary to use inorganic standards of known chromium content and prepare the biological materials in such a way as to destroy the chemical history of the chromium. Studies were carried out on the NBS Standard Reference Material SRM-1577 (Bovine liver), looking at a variety of sample digestion and ashing procedures. An order of magnitude variation in chromium content was seen depending upon the type of sample preparation procedure used. The existence of volatile compounds of chromium that are lost during ashing or digestion steps has been postulated for an explanation of these different results.

Keywords: Biological materials; biological standards; chromium; flameless atomic absorption; volatile chromium compounds.

I. Introduction

Chromium is an essential element involved, as a co-factor with insulin, in carbohydrate metabolism [1]. Chromium exists in biological materials in different forms whose chemical, biological and analytical characteristics are not well defined, and at physiological levels that are too low, generally parts per billion, to be analyzed by classic methods of analysis. Therefore, highly sensitive instrumental methods must be used. Because calibration and quantitation of these methods depend upon the comparison of an instrumental response of the sample to some known stan-

dard, the unknowns and the standard must be shown to give the same relative response, and recovery of the different forms of chromium must be quantitative for any type of destructive sample preparation.

Accuracy of a method of analysis is best shown by use of a carefully defined reference material of known content. For chromium, however, very few biological standards are available with even preliminary data as to chromium content, and attempts to define chromium values in biological materials have led to reports of wide variations [2]. In order to develop reliable instrumental analytical methods for chromium therefore, it is necessary to use inorganic standards of known chromium content and to prepare the biological materials so that the chemical history of the chromium is destroyed and the element can be presented to the instrument in the same form as in the standards.

II. Experimental

Our work [3] with flameless atomic absorption spectrometry showed that sugar samples introduced directly into the graphite furnace had lower apparent chromium content than samples that were pre-ashed before analysis (table 1). Inorganic chromium added to the samples did not show this decrease, which was attributed to the organically bound fraction of the chromium.

TABLE 1. *Mean apparent chromium content in sugars by different procedures* (3)

Type	Number of samples	Sample, ng/g		
		Oxygen plasma ashing (150 °C)	Muffle furnace ashing (450 °C)	Graphite furnace ashing 1000 °C (direct analysis)
Molasses	3	266 ± 50	129 ± 54	29 ± 5
Unrefined	8	162 ± 36	88 ± 20	37 ± 13
Brown	5	64 ± 5	53 ± 8	31 ± 2
Refined	7	20 ± 3	25 ± 3	<10

[a] Values listed are averages of means of multiple determinations of each type ± standard mean error of this average.

This same type of behavior was observed for urine samples [4]. Chromium content was higher in samples that had been low-temperature ashed than samples analyzed directly by injection into the graphite furnace (table 2). The difference between the direct analysis and low-temperature pre-ashing analysis varied among samples and subjects and indicated that two fractions of chromium are present in urine. One fraction

TABLE 2. *Chromium concentration in urine analyzed by different procedures*

Procedure	Subject A (ng/ml ± SD)	Subject B (ng/ml ± SD)
Unashed, direct analysis	2.0 ± 0.5 (6)[a]	2.2 ± 0.5 (6)
Method of additions	1.8	2.5
Oxygen plasma ashing	2.7 ± 1.0 (6)	10.4 ± 1.9 (17)[b]

[a] Number of aliquots analyzed in parenthesis.
[b] Significantly different from unashed ($P < 0.01$).

can be detected by the direct method or by standard method of additions of inorganic chromium and the other fraction detected only by pre-ashing the sample before introduction into and analysis by graphite furnace atomic absorption.

In light of these findings we have studied extensively the effects of sample digestion and ashing procedures upon the analysis of chromium in different biological materials. All analyses were done by graphite furnace atomic absorption (Perkin Elmer 503 AA Spectrophotometer with HGA–2000 graphite furnace) and the sample response was compared to that of inorganic chromium as chromium chloride.

Samples of bovine liver (NBS SRM 1577) were prepared for analysis by several procedures and apparent chromium content varied widely among the methods of sample preparation (table 3). Low-temperature ashing and direct analysis gave low chromium values and muffle furnace ashing and acid digestions gave high values. Acid digestion with only HNO_3 gave lower values than HNO_3/H_2SO_4, in both open and closed digestion systems, and digestion with $HNO_3/HClO_4$ gave still lower values. Since these values were corrected for contamination and reagent blanks and analysis of copper content showed no significant physical loss of sample, these data led to the hypothesis that, depending upon the digestion or sample destruction system, chromium can be either lost or incompletely decomposed to a form which is not detectable by flameless atomic absorption.

In order to test this hypothesis, other different biological materials were studied (table 4). The data in tables 3 and 4 show that Brewer's Yeast and Bovine Liver samples showed much lower values by low-temperature ashing and direct analysis than by muffle furnace ashing. These materials showed values comparable to muffle furnace ashing when they were acid digested in a closed system with a HNO_3/H_2SO_4 digestion mixture. Analysis of NBS SRM 1571-Orchard Leaves by these procedures showed only a slight decrease in low-temperature and muffle furnace ashing *vs.* direct analysis and acid digestion. Our findings indicate that the form of

TABLE 3. *Effect of sample preparation on apparent chromium content of NBS Bovine Liver (SRM 1577)*

Procedure	Samples analyzed	Cr (ng/g ± SD)	Cu (μg/g ± SD)[a]
Direct Analysis	6	53 ± 9	—
Low Temperature Ashing			
200 *w* power	12	45 ± 6	140 ± 8
400 *w* power (porcelain)	12	68 ± 8	133 ± 4
400 *w* power (Pt)	12	67 ± 14	
Max power (≃ 450 *w*)	11	93 ± 32	136 ± 22
New porcelain dishes	6	197 ± 56	
Muffle Ash–unshielded			
500 °C	5	623 ± 418[b]	138 ± 11
3*N* HNO_3 treated	4	408 ± 126[b]	
600 °C (porcelain)	4	463 ± 20	
Ether extracted	8	466 ± 110	
(Quartz)	5	412 ± 132	
Muffle Ash—shielded	4	213 ± 28	
Acid Digestion—open flask			
HNO_3	5	114 ± 31	
To dryness	5	182 ± 93	
No heat	3	41 ± 31	
Heat	3	100 ± 46	
HNO_3-H_2SO_4—sit overnight	3	569 ± 183	
HNO_3-$HClO_4$	9	54 ± 6	
Acid Digestion—Parr bomb			
HNO_3 alone	6	268 ± 67	162 ± 26
HNO_3 + H_2SO_4	2	765 ± 240	167 ± 13
	3	619 ± 189	—
	3	424 ± 87	141 ± 13

[a] NBS value (193 ± 10).
[b] Blank correction high for these samples.

TABLE 4. *Effects of sample preparation on apparent chromium content of Brewers yeast and NBS Orchard Leaves (SRM 1571)*

Method	Brewers Yeast (ng/g ± SD)	NBS SRM 1571 Orchard Leaves (μg/g ± SD) (NBS, ± 2.3)
Direct analysis	78 ± 26 (7)[a]	2.8 ± 0.6 (7)
Muffle furnace		
Unshielded[c]	1230 ± 160 (8)[b,e]	1.75 ± 0.2 (10)[b,e]
Shielded[d]	529 ± 85 (10)[e]	2.7 ± 0.4 (4)[e]
	241 ± 66 (8)[f]	
Low temperature ashing	472 ± 67 (8)	1.52 ± 0.23 (10)
Acid digestion		
HNO_3/H_2SO_4		
Parr bomb	1030 ± 100 (3)	2.29 ± 0.43 (7)
HNO_3 only		
Open flask, no heat	—	1.25 ± 0.25 (6)
Open flask, heat	85 ± 60 (8)	2.25 ± 0.25 (5)
		2.37 ± 0.07 (7)[g]

[a] Number of samples in parenthesis.
[b] Muffle 450—500 °C.
[c] Open dish.
[d] Shielded quartz combustion tube.
[e] Porcelain ashing dish.
[f] Platinum ashing dish.
[g] Cu = 12.5 ± 0.7 (12.0 ± 1, NBS).

chromium in liver and yeast differs analytically from that in orchard leaves. We know that the chromium content of liver and yeast is more biologically active than that of orchard leaves [5].

III. Interpretation

At present we attribute the variations in analytical results to one or more of the following processes:

(1) The naturally occurring forms of chromium might have different relative responses to the instrumentation than inorganic or simple forms of chromium.

(2) Decomposition products or intermediates might be sufficiently volatile to be lost during sample preparation or non-atomic volatile species might be formed in the graphite furnace.
(3) Naturally occurring volatile chromium compounds would be susceptible to loss upon heating, especially in a vacuum.
(4) Muffle furnace ashing can lead to high values due to adsorption of airborne chromium on the ash; this adsorption would not be observed in the blanks and would not be corrected for.
(5) There may be leaching of chromium from the ashing dishes by a component of the biological material.
(6) Different forms of chromium can be adsorbed differently on the surface of ashing and digestion systems.
(7) Incomplete digestion and ashing will lead to apparent losses through processes (1) and (2) above.

IV. Conclusion

We are attempting to define the extent and significance of each of these processes at physiological levels of chromium for different biological materials. Only when these analytical variations are understood and accurate, reliable values are defined for chromium in biological materials can we fully proceed with the important task of putting the biological role of chromium into its proper perspective.

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STABILITY OF CHROMIUM IONS AT LOW CONCENTRATIONS IN AQUEOUS AND BIOLOGICAL MATRICES STORED IN GLASS, POLYETHYLENE, AND POLYCARBONATE CONTAINERS

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Low-concentration solutions of chromium (< 50 μg/dl), in either aqueous or biological matrices, frequently need to be stored for short or long term studies. Storage at low temperatures is usually considered the best available precaution against changes in concentration through adsorption or leaching or evaporation of water. Polyethylene containers are not appropriate for storage of aqueous chromium standards of low concentration. Borosilicate glass containers are preferred for the storage of low-concentration aqueous standards for up to 6 months. The stability of serum and plasma chromium concentration (< 0.2 – 0.5 μg/dl) is maintained in polyethylene vessels, if stored frozen at below – 10 °C. Polycarbonate tubes were found adequate for storage of serum and plasma for up to 2 weeks, under refrigeration at 4 °C.

Keywords: Adsorption losses; biological systems; chromium; stability; storage containers.

I. Introduction

The importance and necessity for utmost accuracy in trace element analysis has been increasingly recognized in recent years. The most significant role played by essential trace elements in health and nutrition has further stressed and emphasized this requirement for high accuracy in the determination of trace elements. Precise and accurate analysis is essential if meaningful results are to be obtained at low concentrational or absolute

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levels at which most essential trace elements are present in biological specimens [1]. The reliability of any analyte determination is, in turn, a direct function of the dependability, accuracy, and stability of concentration of the standard solutions against which the analyte concentrations are measured. The integrity and stability of the standards, thus become extremely important in trace element analysis.

II. Discussion and Results

The loss of trace amounts of metallic ions on container walls during sample collection, handling, and storage of aqueous solutions and biological fluids has been recognized for some time. Trace element analysis and problems of contamination are firmly and unavoidably linked. Contamination of the specimen through leaching or chemical interactions with container material must, therefore, be considered as a relatively significant source of error. In table 1 are presented some significant considerations in regard to storage of specimens. As technology has advanced to achieve lower detection limits, the need to prevent adsorption losses or leaching gains of ions at the solution-container interface has become more significant. Such changes in specimen concentration appear to vary with pH, contact time with container, type of container, and solution concentrations [2-8].

TABLE 1. *Some important aspects of storage of specimens*

Storage Container Material
Temperature of Storage
Duration of Storage
Contamination
External: handling, processing, airborne, evaporation, etc.
Internal: absorption, leaching, chemical interactions

In studies involving biological fluids, it is frequently impractical to analyze the sample immediately following collection of the specimen. It is thus most important to ensure that there be no changes in the composition of the specimen arising from either long or short term container storage. Adsorption losses or leaching gains from the container surface may alter the composition of the solutions that are otherwise stable. Appropriate initial treatment of the sample and the container are, therefore, as important as the sensitivity and accuracy of the method of analysis, if the results are to be of any significance for a meaningful interpretation.

Freezing of the sample immediately following collection can be helpful in diminishing contamination of the metallic ions on container walls [3]; however, this method is not always practical and alternate procedures must be sought in many instances.

A study of the correlation between chromium levels in different body fluids and the incidence of cardiovascular disease (CVD) initiated under the auspices of the National Research Council at the Clinical Sciences Division of the USAF School of Aerospace Medicine, required the acquisition of systematic information on the stability of low-concentration solutions of chromium ions stored in glass, polyethylene and polycarbonate containers. The role of contamination during specimen collection was also considered sufficiently significant to merit investigation.

Most previous studies reported in the literature [2-4,7-12] have been limited to the investigation of the role of adsorption at the container walls during short term storage (< 3 weeks). None of these investigations reported on the long term losses from adsorption at the container surface, nor was any effort made at investigating the effect of container pretreatment on the stability of solutions stored in the various types of containers used.

The results of a study of long term storage of low and very low concentration aqueous solutions of chromium ions stored in either glass or polyethylene containers are presented. The effects of a variety of washing and pretreatment procedures on the surface adsorption by such containers was also investigated. Changes in chromium concentration were followed by assaying the solution at various lengths of storage time by flame and flameless atomic absorption spectrometry. These data are presented in tables 2, 3, and 4. The results of a study of the stability of pooled blood serum stored in polyethylene and polycarbonate containers at ambient temperature, +4 °C, and − 10 °C are shown in table 5. Chromium contamination during specimen collection and processing was also investigated. The levels of contamination in water or saline wash solutions following passage through or contact with plastic and glass syringes, polycarbonate tubes, polyethylene beakers, stainless steel or titanium blood collecting needles, HNO_3 and Eppendorf tips are presented in table 6.

The data represent the average of at least two atomizations of each test solution which was also container duplicated. Furthermore, the study was repeated at varying storage times extending up to 48 weeks. Peak absorbance on the recorder was used as a measure of the concentration of the chromium ion. In the heated graphite flameless determinations, measurement of peak height, rather than integrated absorbance, proved to be a more sensitive measure of concentration. Steady and consistent

TABLE 2. *Stability of low concentration chromium solutions (50μg% Cr^{+6}) in glass and polyethylene containers*

(Acetylene-Air Flame AAS)

Prior treatment	Stability (in weeks)			
	Glass		Polyethylene	
	Unused	Used	Unused	Used
Water washed	48	48	24	36
Acid washed $HCl + HNO_3$	48	48	1	36
Water washed siliconized	15	48	1	40
Acid washed siliconized	<1	48	1	36
Acid washed rinsed in NH_4OH (1N) and siliconized	48	40	3	16
Soaked in chromic acid and water washed	—	48	—	3

decrease in concentration greater than 2 standard deviations at each concentration level was used as the yardstick for establishing the maximum period of stable storage in any container (see individual tables).

Stability of low concentration aqueous solutions of trace elements of biologic interest during long periods of storage has not been investigated previously. In as much as it is inconceivable that there could be any alterations in analyte concentration through evaporation, under the strictly controlled experimental conditions of the present study, other possible causes of changes in concentration must obviously be considered. Reactivity of the container and the prior treatment to which it has been subjected, the pH of the solution, the adsorption characteristic of the container surface, and contamination from dissolution of metallic ions from the container wall into the specimen are some of the more important possible causes and sources of observed changes in solution concentration. For aqueous metal solutions of low concentration, possibilities of gain through dissolution from the container or loss through adsorption onto the container surface obviously assume great significance. The fact that it is generally inadvisable, or in many instances impractical, to alter

TABLE 3. *Stability of very low concentration chromium solutions (2μg% Cr^{+6}) in glass and polyethylene containers*

Prior treatment	Stability (in weeks)			
	Glass		Polyethylene	
	Unused	Used	Unused	Used
Water washed	12	12	2	4
Acid washed $HCl + HNO_3$ (6*N*)	12	12	2	3
Acid washed soaked in NH_4OH (1*N*) and siliconized	12	12	2	2
Soaked in chromic acid and water washed	<24 h[a]	<24 h[a]	<1 h[b]	<1 h[b]

[a] Determined concentrations elevated above base value.
[b] Determined concentrations below base value with a tendency to equilibrate to original levels following 96 hours of storage.

TABLE 4. *Stability of very low concentration chromium solutions (1μg% Cr^{+6}) in glass and polyethylene containers*

Prior treatment	Stability (in weeks)			
	Glass		Polyethylene	
	New Unused	Used	New Unused	Used
Water washed	2	4	2	72 h
Acid washed $HCl + HNO_3$ (6*N*)	4	1	1	24 h[a]
Acid washed soaked in NH_4OH (1*N*) and siliconized	1	8	<1 h[a]	<1 h[a]
Soaked in chromic acid and water washed	8	8	<1 h[a]	<1 h[a]

[a] Uneven fluctuations, low initial concentrations, tending to equilibrate to base values within 72 hours and subsequently decreasing steadily.

TABLE 5. *Storage characteristics of serum (plasma) in polyethylene and polycarbonate containers at different temperatures*

Temperature of storage	Polyethylene	Polycarbonate
Ambient 25–28 °C	<48 hours	<96 hours
Refrigerated 4 °C	<1 week	<3 weeks
Frozen −10 °C	>18 months	>18 months

[a] Stabilized serum pool (5.0 μg%) and unprocessed pooled patient specimens.

TABLE 6. *Chromium contamination during specimen collection and processing*

Test material (washed and unwashed)	Level found in H_2O or saline extract Cr^{+6} concentration pg/ml[a]
10 and 20 ml plastic syringes	<4
10 and 20 ml glass syringes	<4
15 ml PC tubes	<4
Polyethylene beakers	<4
18-8 SS. needles	<4
Titanium needles	<4
Ultrex HNO3	<20
Eppendorf tips for micropipette	occasionally <20

[a] Minimum detection limit for chromium $= 4 \times 10^{-12}$g.

the pH of the sample during storage of body fluid specimens obtained for trace metal analyses is equally important. Thus, the aqueous standards employed in such studies must be maintained at their normal pH (determined to be between 8 to 8.5 in most instances in our study).

There are very few references in the literature reporting on the stability of metal ion solutions of low or very low concentrations. Most previous studies [2,4,7,11,12] have dealt with investigation of the changes in concentration of trace elements by adsorption at the container surface or through evaporation. The effect of pH on adsorptive losses has also been reported. A recent investigation [3] has dealt with the effects of pH on the stability and solubility of a number of metal ion solutions of low concentration. No data are available which describe the effects of different surface treatments of the container walls prior to storage of the specimen or of the effects of the different materials commonly used in fabricating laboratory containers (glass, polyethylene, polypropylene, and polycarbonate). We fill this gap in regard to the stability of chromium solution.

Contamination of the specimen in trace element analysis has recently been receiving considerable attention [1,13-15]. Contamination of chromium solutions through leaching from glass or polyethylene containers should not be a serious problem since both these materials have negligible chromium content. Consequently, adsorption losses are the primary hazard to the stability of chromium ion solutions of low concentration stored in glass or polyethylene containers. Borosilicate glass, with its low porosity surface, should obviously be preferred over soft, soda glass. Although polyethylene has generally been preferred for long term storage of aqueous metal ion standards, several investigators have cautioned against the use of acid washed polyethylene [16-21]. Our data unequivocally establish borosilicate glass as the material of choice for the storage of low and very low concentration (> 1 μg/dl $=> 0.01$ μg/g) chromium ion solutions (tables 2, 3, and 4). The superiority of borosilicate glass over polyethylene is demonstrated in both unused virgin containers and those with prior use history, having been subjected to detergents, machine washing, acid corrosion of the surface, and adsorption-desorption of various contaminant. The data for the containers soaked in chromic acid adduces strong evidence to the hypothesis of surface adsorption as a major cause of the loss of metal ion concentration. It may be postulated that soaking in chromic acid saturates all the adsorption sites on the container wall and that a steady state of equilibrium is rapidly attained between the specimen solution and the container surface. In case of the polyethylene and polypropylene containers, the surface etching is a strong possibility. This would increase the surface area and thus the adsorption sites. Our data support this line of reasoning and chromic-acid soaked polyethylene containers were the worst vehicles for long or short term storage of chromium ion solutions.

Siliconizing of a glass surface has long been accepted as an effective solution to the problem of surface activity, resulting in adsorptive losses at the container surface. Siliconizing of both glass and polyethylene containers according to the procedure recommended by the manufacturers of SILICLAD, did not prevent adsorption losses of Cr for either material. However, if the surface of glass containers was soaked in $1N$ NH_4OH prior to siliconizing, the adsorptivity was significantly diminished, and chromium ion solutions stored in glass containers subjected to such treatment maintained their original concentrations for up to 48 weeks. Similar treatment was not as effective in case of polyethylene containers, as is shown in tables 2, 3, and 4.

Storage of serum specimens at various temperatures in a variety of containers was also investigated. The data in table 5 indicate that polycar-

bonate is the best material, ordinarily in use, for the storage of serum and plasma specimens. It shows superior performance at all temperatures investigated. However, polyethylene containers are equally efficient if the specimens are stored frozen at − 10 °C.

Contamination of blood during specimen collection has been assumed to be an unavoidable hazard. Some investigators have reportedly used aluminum needles in preference to the stainless steel blood collecting needles [22,23]. We have investigated the effects of contamination from a variety of materials commonly utilized in the collection of blood specimens. The data show no measurable contamination from chromium leaching into water and saline even following repeated contacts with these various materials. However, this is not conclusive evidence for the absence of measurable contamination of blood or serum and it remains to be seen if the above observations can be extended to these biological specimens as well.

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A POSSIBILITY OF STATE ANALYSIS OF PLASMA SPECTROMETRY

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This report describes a new method of state analysis by plasma spectrometry which is found to be very useful for preliminary analysis of natural samples. An induction furnace or an electric heater is used for the vaporization of this solid sample and the resulting gas is introduced into the plasma torch. The vaporized sample enters the plasma flame in the order of height of boiling point and depends upon the temperature gradient of the furnace.

Keywords: Plasma spectrometry; preliminary analysis; specimen decomposition; specimen evaporation.

Radio frequency discharges produced at atmospheric pressure and having a flame-like geometry can be used as excitation sources in optical emission spectrometry. This report describes such a discharge which operates in a capacitive coupled mode at a frequency of 2450 MHz and is powered by a 350 W magnetron. The analytical sample initially in solid form is supplied to the discharge as a vapor obtained through a thermal generator external to the discharge. The vapor was produced in this arc by an electrically heated oven-like unit or by an induction furnace. This last mode of operation is illustrated in figure 1. The discharge is produced in argon while the carrier gas used to entrain the analytical sample vapor is nitrogen.

The source was used to determine mercury, arsenic, and silica in organic (bovine liver, SRM-1577) and inorganic (coal SRM-1632) samples with good sensitivity.

As a result of the thermal vaporization process of the analytical sample, it was possible to identify the molecular species from which Hg, As, and Si originated by operating the sample oven at a predetermined temperature. Figures 2, 3, and 4 illustrate the results obtained for the molecules

$HgCl_2$ and Hg_2Cl_2, As_2O_3 and As_2O_5, and Si and SiO_2 respectively. In this last case a low boiling Si compound was observed in bovine liver (SRM-1577).

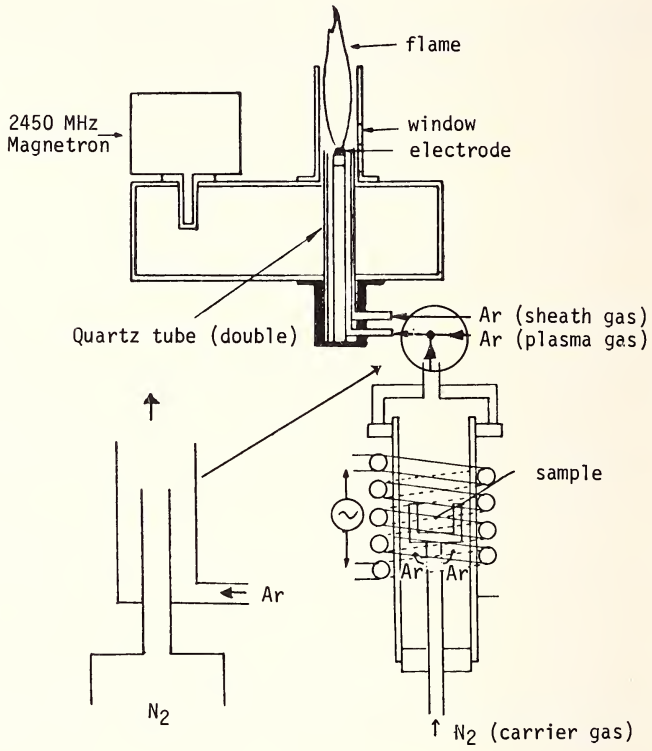

Figure 1. Schematic illustration of a capacitive coupled 2450 MHz RF-discharge operated by a 350 W magnetron. The flame-like discharge which is produced at the extremity of the electrode is supplied with the analytical sample vaporized in an induction oven external to the discharge.

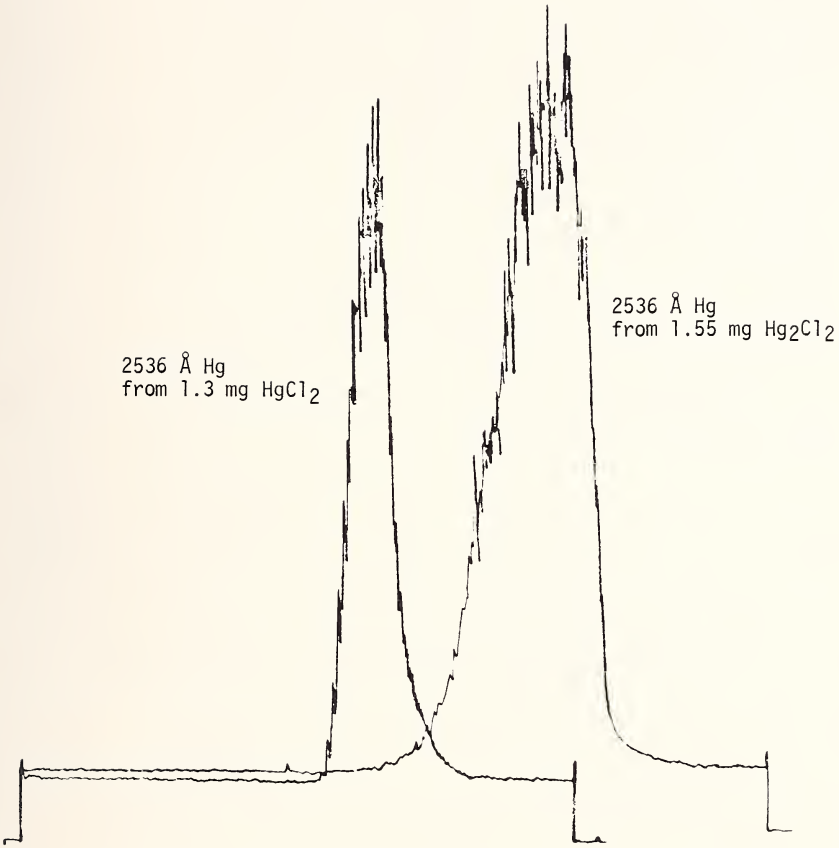

Figure 2. Fractional vaporization of Hg from $HgCl_2$ and Hg_2Cl_2 and excitation of Hg.

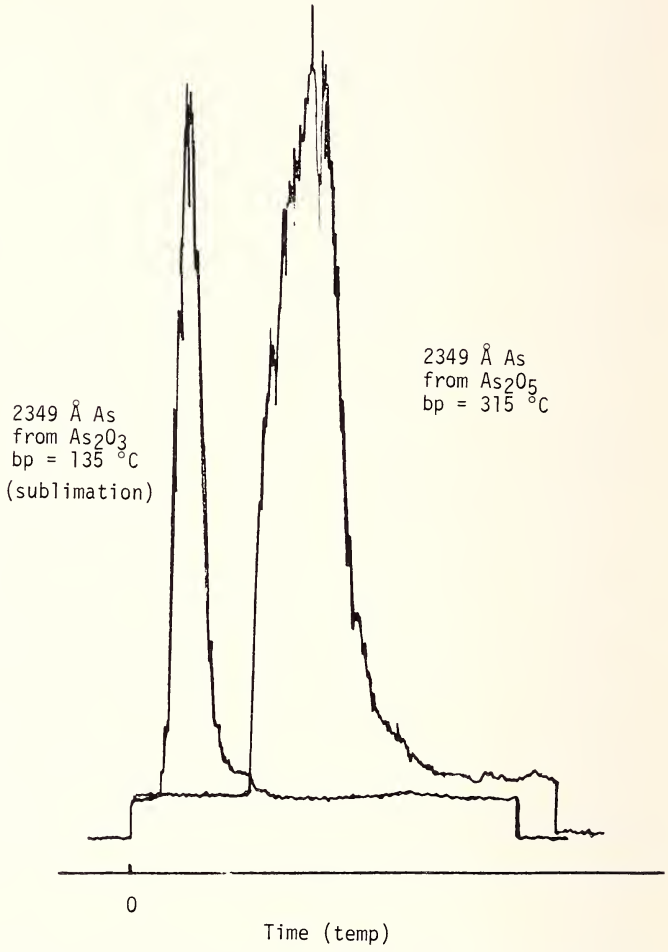

Figure 3. Fractional vaporization of As from As_2O_3 and As_2O_5 and excitation of As.

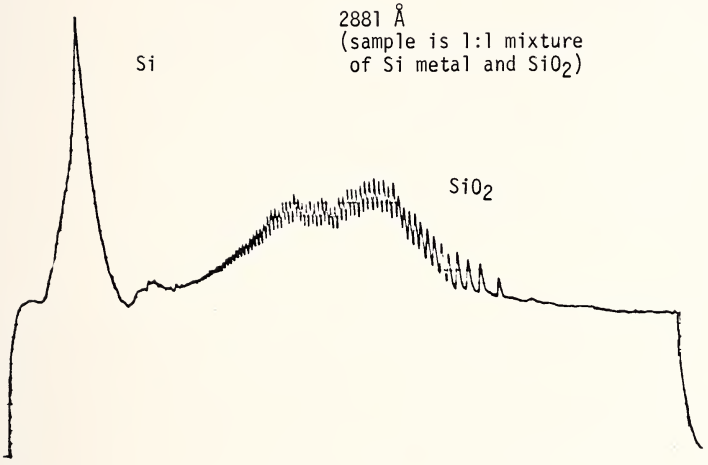

Figure 4. Fractional vaporization of Si and SiO_2 and excitation of Si.

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PREPARATION AND ANALYSIS OF AQUATIC-RELATED SAMPLES

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The use of a special room in which to prepare samples and high-purity reagents has become standard now. However, without adopting and judiciously following special operating procedures to cope with those factors which affect the accuracy of trace analyses, the analyst may encounter serious problems.

Special operating procedures have been followed within the sample preparation laboratory to provide representative aliquots of aquatic-related samples (fish, aquatic insects, water, and water sediments) to other analysts for multiple element determinations. Procedures or preparation techniques employed for aquatic-related samples have been directed toward the final use of NAA, SSMS and AA for the desired determinations. Criteria have been established to aid in the selection of applicable analytical methods. Consideration is given to determining what degree of accuracy is adequate and to the expense necessary to achieve this accuracy. By following sound sample preparation procedures in the sample preparation laboratory and by taking great care in making the final determination or measurement, significant reductions in analytical costs can be realized without compromising the accuracy of the analysis.

Keywords: Aquatic insects; atomic absorption; clean room; fish; mini-computer; sample preparation; toxic metals; trace determination.

I. Introduction

The demand for accurate trace analyses has risen greatly over the past few decades and is not expected to subside. As industrial and research facilities manufacture and develop new products, an ongoing need for

*Oak Ridge National Laboratory is operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

more sensitive and improved trace analytical techniques confronts analytical chemists. This need is compounded, unfortunately, by the rush to fulfill the nation's insatiable needs for energy. Unless effective environmental controls are used, an increase in atmospheric, terrestrial, and aquatic pollution will occur. With the delay of using approved controls for effluents and the natural belated effectiveness of others, efforts must be expanded to understand and quantify the resultant environmental impact from energy production. Such efforts are being undertaken.

Baseline studies have been and are now being made at quasi-pristine watersheds in order to characterize the speciation of trace elements and nutrients. The selection of such areas is always tainted with reservations. Nevertheless, it is thought by making such studies, the insults to receiving waters and forest lands from urban and industrial complexes can be realistically evaluated. Accurate trace analytical techniques must be employed in support of both baseline and man-made pollution studies.

II. Sample Preparation

In order to minimize the effect of those factors which have an influence on the accuracy of trace analytical methods [1], it is now common practice to use special sample preparation laboratories [2,3]. A variety of such laboratories are in existence, many of which were designed to fulfill certain needs. An example of a laboratory used for processing aquatic samples for the analyses of mercury, selenium, cadmium, lead, and zinc is shown in figure 1. The masonry walls within the room have been coated, exposed metallic surfaces on work benches and hood ledges covered, and the floor and ceiling joints sealed to reduce sources of dust particles, thus augmenting the air filtration system. All of the input air is passed through a roughing filter and a high efficiency particulate filter, thus reducing the concentration of many airborne contaminants. This sample preparation laboratory is maintained under a positive pressure at all times with the use of air locks as entry ways. One of these entry ways is shown in figure 2. Entry into the laboratory is controlled and special clothing is required.

Special operating procedures are employed to provide representative aliquots of samples for multiple element analyses since some of these analyses are performed by neutron activation techniques coupled with gamma-ray spectrometry, atomic absorption spectrometry, spark-source mass spectrometry, and cold vapor atomic absorption. An example of one successful procedure (due perhaps to its simplicity) that has been employed with numerous fish samples taken from both baseline study areas

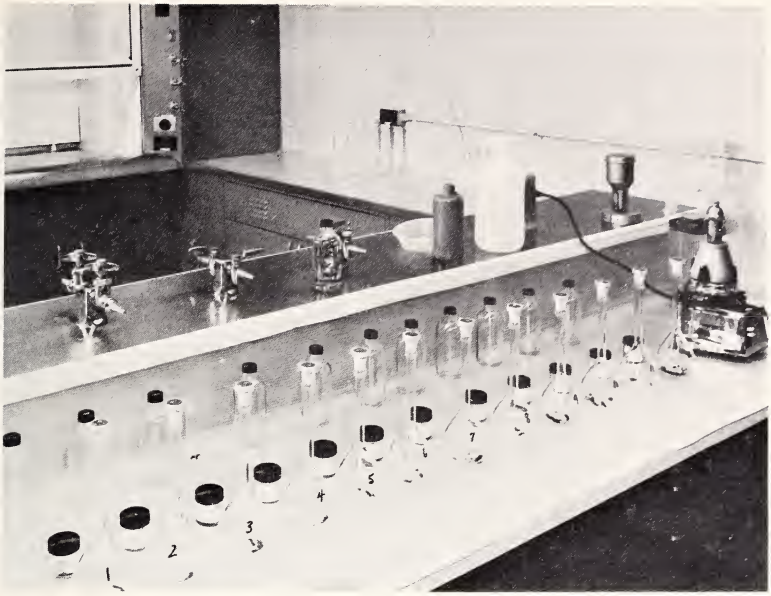

Figure 1. Sample preparation laboratory.

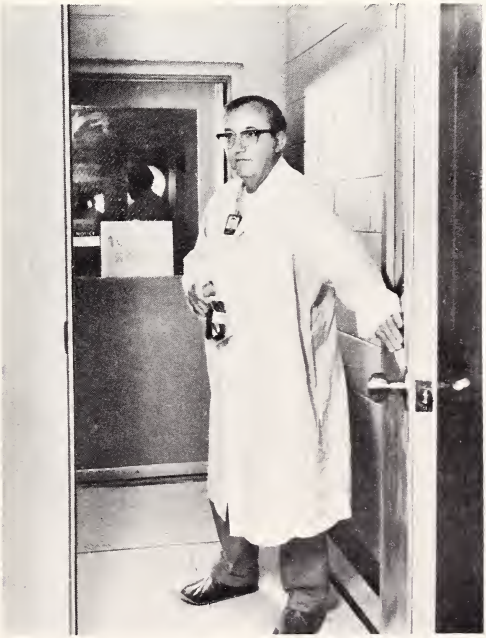

Figure 2. Air lock entrance to sample preparation laboratory.

and impacted areas involves the blending of fish muscle, skin, and organs with measured amounts of double distilled – predeionized – distilled water. Cross contamination of samples is reduced by judiciously following a prescribed cleaning procedure for the nondisposable blending equipment. Experience has shown that the water to tissue ratio will vary between 2 and 5 depending upon the consistency of homogenates desired. Aquatic insects present a more difficult processing and distribution problem. Liquid nitrogen is used to harden the insects to facilitate crushing. Though this processing procedure can withstand improvement, the analytical results obtained from homogenates of insects have been satisfactory.

Representative homogenates are analyzed directly by neutron activation analysis, or they are dissolved and analyzed by other multielement techniques such as atomic absorption spectrometry or isotope dilution spark-source mass spectrometry. Dissolutions are performed by a wet-pressure method [4] or with the special flasks shown in figure 3. Special mercury analyses are performed by the classical cold-vapor atomic absorption method, using the instrumentation shown in figure 4.

Figure 3. Dissolution flasks for aquatic samples.

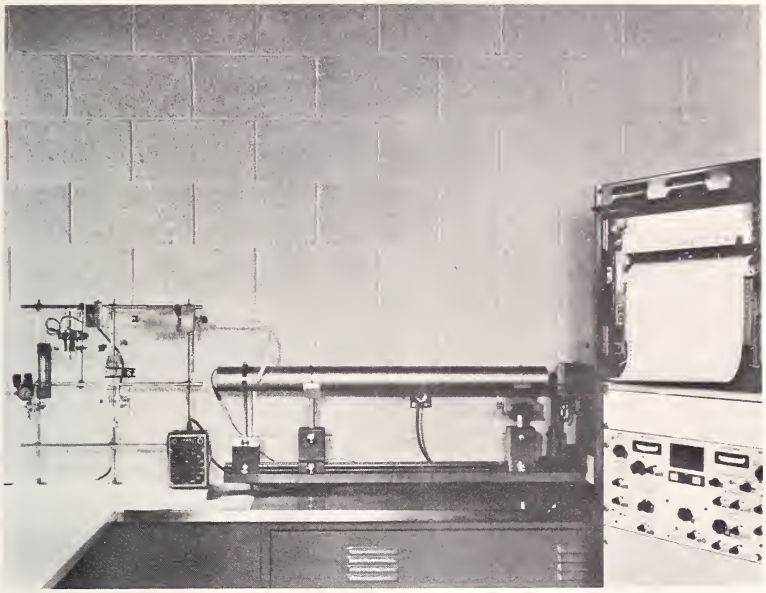

Figure 4. Cold vapor atomic absorption instrumentation for mercury analysis.

III. Improving Reproducibility

To improve the accuracy and precision of the analyses using the multielements methods mentioned above, the acquisition and reductions of data are performed with the aid of mini-computers.

For example, a PDP-8/e computer, coupled to an atomic absorption instrument, is programmed to calculate absorbance values from sample, standard, and dark current readings. The FOCAL program may be used for the manual entry of data from other analyses dependent on standard calibration curves.

Another program, MONSTER, is used with a PDP 15/20 computer for the resolution and isotopic identification of Ge(Li) gamma-ray spectra [5]. This machine routine is used routinely to process neutron activated samples at both the Oak Ridge Research Reactor and the High Flux Isotope Reactor.

A PDP 8/e computer is used to facilitate the reading of photo plates associated with spark-source mass spectrometry. In addition, an IBM 1130 is used for other data processing. The programming used allows percent transmittance data acquired by the computer to be transferred automatically to an emulsion calibration program.

These added features to the analytical instruments not only make each method more competitive, but they simplify the reduction of data in a rapid and relatively error-free manner. The analytical chemist responsible for obtaining multiple analyses must decide which of the methods to use and when. These decisions must be made with an unbiased awareness for the capability of the methods available and should be based on established needs. Such needs include the accuracy, precision, and sensitivity required; the number and type of samples; and the analyses of interest. Other considerations involve sample size and the type of processing, if any, that will be required.

Some investigators have adopted decision-making procedures to aid in the selection of proper instrumentation [6] or to establish the optimum alternatives from a preselected group [7]. This latter procedure has been studied and was found applicable for selecting analytical methods for mercury, selenium, cadmium, lead, and zinc in aquatic samples and other trace elements in various matrices. In this method of decision-making, the methods and alternatives (desired criteria) are graded or given numerical weights. Such an exercise is not only enlightening, but it also points out the need to maintain a multi-method capability. By following good sample preparation procedures in the sample preparation laboratory and by selecting the best analytical method based on some forethought and method evaluation, significant reduction in overall costs may be realized without compromising analytical accuracy.

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STABILITY OF METAL IONS IN AQUEOUS ENVIRONMENTAL SAMPLES

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The time lapse between the collection of aqueous environmental samples and the analysis affects the original ionic concentration. Studies have proven the nonionic species in a water sample have more of an effect on the veracity of an analysis than the "container wall" effect, and that adjustment to a pH of 2 at sample collection time is a "Pyrrhic victory." Lead, for example, will commonly increase an order of magnitude when unfiltered samples are adjusted to a pH of 2 upon collection. This effect is greatest when elemental ions are present in the ng ml^{-1} range and lessens as the original ionic concentration increases.

Data is presented that behooves filtration of stream water and rainwater samples prior to any acidification step. The need to acidify the resulting filtrate is also discussed. Lithium, sodium, potassium, cesium, magnesium, calcium, strontium, manganese, iron, copper, silver, zinc, cadmium, aluminum, indium, and lead are examined. The insoluble phase retained on the filter can be digested with acid and also analyzed. The separate analysis of the filtrate and filter will give a true representation of the occurrence of these metals in nature. Flame and flameless atomic absorption and emission are used to perform the trace analyses.

Keywords: Filtration losses; metallic ions; pollution monitoring; rainwater analysis; sample stability; surface water analysis.

I. Introduction

It is generally impossible to perform an analysis on aqueous environmental samples immediately after the samples are taken in the field. Because of a time lapse between the collection and the analysis of such

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samples, the original ionic concentrations may no longer be truly represented. Such knowledge is of importance in determining the fate of metallic ions introduced into an aqueous system, *i.e.*, if the metal of concern is associated with a particle in a stream it may eventually become a component of the sediment; however, if it remains in solution it may find its way into public water supplies.

Attenuation of ionic concentrations has been reported in a number of published studies on the storage of aqueous solutions. Table 1 reviews these accounts. Only the parameters relevant to this present study are tabulated. Contradictions are evident between the various studies. For example, references [16] and [17] present data showing the instability of Ag at a pH range of 3.8 to 4.5 when stored in borosilicate containers while reference [2] found that Ag was stable at a pH of 3.8 when stored in brown borosilicate containers but that losses did occur at pH 3.8 if polyethylene containers were used. Meanwhile, reference [8] reports that Ag in seawater is stable in polyethylene containers in the pH range of 3.5 to 4.0. Reference [7] finds that Cd is stable over a pH range of 3 to 10 in polyethylene containers but reference [5] shows that loss can occur in the pH range of 7.5 to 8.0. Reference [7] discloses that Cd losses do not occur at a pH less than 7.0 in borosilicate containers but reference [15] reports losses at a pH greater than 5.0. Other discrepancies are also readily apparent for Ca, Sr, Mn, and Zn, while agreement is better for the remainder of the metals. Losses are generally attributed to an adsorption of the ions onto the container material. Precipitation out of solution was recognized as a possible cause by references [3,13-15]. Where losses have occurred there is agreement that adjusting the pH to 1 to 2 will maintain ionic concentrations. Only references [5] and [6] have considered the importance of naturally occurring suspended particulate formations, but have studied their effect on a limited number of ions. Most of the authors used a deionized or distilled water matrix over various pH ranges to draw conclusions about environmental water samples which are never in a deionized state.

This paper examines the stability of 15 cations in genuine environmental aqueous matrixes and takes into consideration the influence of suspended particulates. The results are useful not only for determining the storage conditions needed to maintain accurate sample solutions, but also the environmental effect of certain cations when they are added to existing water bodies.

TABLE 1. *Studies on the stability of metallic ions*

Element concentration $\mu g\ ml^{-1}$	Container material	Matrix, conditions	pH	Results	Investigators remarks	Ref.
Fe, Mn				Refrigeration inhibits somewhat changes in water composition		[1]
Ag^{110} 0.01–1.0	Brown BG	D	1.5, 3.8	0.10, 1.0 <5% adsorbed in 60 days		[2]
		D	1.5	.01 <10% adsorption in 60 days		
		D	3.8	.01 <20% adsorption in 60 days		
	PE	D	3.8	.02 adsorbed 50% in 40 days		
Cs^{137}	PP, BG	Simulated natural water	7.4	Adsorption on BG > PP	As hardness increases, adsorption decreases.	[3]
		D	7.0			
Sr^{90} 9^{-6}–9^{-8}	PP, BG	Simulated natural water	7.4	Adsorption on PP > BG	Lowering pH, reduced colloid formation and adsorption considerably.	
		D	7.0			
Pb, 0.40	BG	D		50% loss in 1 hour		[4]
	PE	D		31% loss in 1.5 hour		
Cd^{115}, .001	BG, PE	River water	7.5–8.0	Adsorption on BG > PE, Adsorption ~10% within 24 hour	~7% Cd adsorbed on filter media.	[5]
Cd^{115}, 0.001	BG, PE	River water and natural sediments		50–90% losses within 24 hour		
Cu^{64}	BG, PE	River water		~65% loss to containers and filters within 5 min		
Cd^{109}, Zn^{65}		Estuarine sediments		99% Zn, 79% Cd adsorbed onto suspended material within 1 hour		[6]

TABLE 1. *Studies on the stability of metallic ions*—Continued

Element concentration $\mu g\ ml^{-1}$	Container material	Matrix, conditions	pH	Results	Investigators remarks	Ref.
Cd^{109}, .025-.200	PE, PP, PVC	D	3–10	<3% loss in 2 weeks		[7]
	BG	D	3–10	~75% loss in 24 hours, pH ≥ 9.0, no loss pH <7.0	Back adjustment to pH 6.0 desorbed Cd.	
Ag^{100}, .00145	PE	Sea water	3.5–4.0	No adsorption losses		[8]
Cu	PE	Filtered sea water	Natural	30% loss in 3 days, 170 further loss	Storage at −18 °C No loss after 40 days.	[9]
Mn	PE	Filtered sea water	Natural	Little variation in 7 days	Storage at −18 °C resulted in slight increase.	
Fe	PE	Filtered sea water	Natural	Steady decrease in 7 days	Storage at −18 °C resulted in decrease in 8 days.	
Ag				NH_3 reagents most stable		[10]
In^{114}	PE	Unfiltered sea water	8.0	>90% loss after 20 days		[11]
	PE	Unfiltered sea water	1.5	No loss		
	BG	Unfiltered sea water	8.0	20% loss after 75 days	Sea water should be stored in PE and adjusted to pH 1.5 at collection time to prevent adsorption losses.	
Fe^{59}	PE	Unfiltered sea water	8.0	>90% loss after 55 days		
	PE	Unfiltered sea water	1.5	No loss		
	BG	Unfiltered sea water	9.0	70% loss after 55 days		

TABLE 1. *Studies on the stability of metallic ions*—Continued

Element concentration $\mu g\ ml^{-1}$	Container material	Matrix, conditions	pH	Results	Investigators remarks	Ref.
Ag^{110}	PE	Unfiltered sea water	8.0	20% loss after 10 days, no further loss	Sea water should be stored in PE and adjusted to pH 1.5 at collection time to prevent adsorption losses.	
	PE	Unfiltered sea water	1.5	Insignificant loss		
Zn^{65}, Cs^{134}, Sr^{85}	PE, BG	Unfiltered sea water	8.0	Negligible loss after 75 days		
Cu, Fe, Zn	BG, black PE, blue PVC	Triple Ds, storage at ambient, 4 and 37 °C		Low temperatures best for prolonged storage		[12]
Na^{22}, 2.2; Cs^{134}, .0132	PE	Ds		No storage loss	Filter media adsorbed 7–17% if Cellulose used	[13]
Ca^{45}, .004	PE	Ds	10, 12		Cellulose filtration removed 66–69%	
		Ds	7.0		Filtration through glass frit removed 27%	
		Ds	9–13		Filtration through glass frit removed 97–100%	
		Ds	0–9	No loss in storage	Removal of Ca due to colloid formation occurs at pH >9.0	
Li 0.2, 1.0	BG	0.5% NaCl		Stable pH–11	Elements stable after 24 hrs were stable 4 wks	[14,15]
Cu, Fe, Pb, 2–10	BG	D, .5% NaCl		Losses occur pH >1.5 after 24 hours		
Ca, Mg, Mn, Sr, Zn, 2–10	BC	.5% NaCl		Losses occur pH >5.0 after 24 hours	Elements unstable after 24 hrs continued to decrease	
In 1.0, 10.0	BG	.5% NaCl		Losses occur pH >3.0 after 24 hours	Losses from solution were always less in .5% Na Cl matrix	

TABLE I. *Studies on the stability of metallic ions*—Continued

Element concentration $\mu g\ ml^{-1}$	Container material	Matrix, conditions	pH	Results	Investigators remarks	Ref.
Cu, Fe, Pb, 0.5–1.0	BG	.5% NaCl		Losses occur pH >3.5 after 24 hours	Losses due to adsorption on BG, or on to the ppt. present in all solutions of pH >1.5	
Ca, Mg, Sr, 0.5–1.0	BG	.5% NaCl		Losses occur pH >8.0 after 24 hours	Samples should be adjusted to pH 1.5 when collected	
Cd, 1.0	BG	.5% NaCl		Losses occur pH >5.0 after 24 hours	All test ions in same solution	
Cd, 0.2	BG	.5% NaCl		Losses occur pH >6.5 after 24 hours		
Ag, 0.0005,	PE, BG, dark storage	D	2.0	0.005 stable 36 days	Frozen storage had 15% loss of Ag at 0.001	[16]
0.001	PE, BG, dark storage	D	4.5	~50% loss after 24 days		
Pb	PP, dark storage	D	2.0, 4.5	100% loss in 4 days		
0.01	PE, BG, PP	D	6.0	100% loss in 4 days of 0.01	Samples should be acidified to pH 2 upon collection to minimize container adsorption	
	PE, PP, BG	D	4.0	60% loss after 10 days, then remained stable		
	PE, PP	D	2.0			
Cd 0.001	BG	D	2.0	No loss after 24 days		
	PE	D	2.0, 6.0	No loss after 32 days		
Zn, 0.7	BG	D	2.0	No loss after 32 days		
	BG	D	6.0	20% loss after 20 days		
	BG	D	5.0	20% loss after 60 days		
	PE	D	2.0, 5.0	No loss		

TABLE 1. *Studies on the stability of metallic ions*—Continued

Element concentration $\mu g\ ml^{-1}$	Container material	Matrix, conditions	H	Results	Investigators remarks	Ref.
Ag 0.05, 1.0	BG, PE	D, $Na_2S_2O_3$ EDTA, NaCl, NH_4OH, ethylenediamine		−49–140% loss adsorption in 30 days in BG in D1 −40–79% adsorption in 30 days in PE in D −0.1 *M* $Na_2S_2O_3$ <1% loss Ag with BG if Cl absent −1.0 *M* $Na_2S_2O_3$ <1% loss if Cl^- in range of .01–.001 *M* in PE, BG −0.1 *M* EDTA <1% loss in 248 hours, then unstable		
	BG, PE	D	4.0, 7.0, 8.0	Adsorption losses at pH 4.0 > pH 7.0, 8.0		

Note: BG = borosilicate glass.
PE = polyethylene.
PP = polypropylene.
PVC = polyvinyl chloride.
Ds = distilled water.
D = deionized water

II. Sampling

Five types of surface waters were sampled: a river, a small urban stream, a residential pond, a city park pond, and a recreational lake. It had not rained for 8 days, so there was no current runoff into these systems.

The small urban stream was sampled again immediately after a period of heavy thunderstorms. Runoff into the stream had raised it several feet, so it was considered to be representative of a highly dynamic system with equilibrium between soluble and particulate phases not yet established. This occurred 5 days after the first sampling.

Five rainwater samples were also taken. Four were from a highly industrial area and the other from an urban area that had only minor industrial development.

All of the samples were taken during summer months.

III. Experimental Procedure

The experimental treatments on the rainwater and urban stream post-rain are given in tables 2 and 3. The handling of these samples was similar to that explained below.

Treatments on the pre-rain surface waters, given in table 4, were more extensive. After the pH was measured, half of each sample was filtered, divided into 100 ml portions, and dispensed into white translucent polyethylene bottles (Nalge). Filtration was completed within 5 to 7 hours after collection. The unfiltered half of the sample was also divided into 100 ml portions and dispensed into the same type of bottle. A set of filtered and unfiltered samples at their natural pH was also frozen to be analyzed at a later date. Both the filtered and unfiltered samples were then adjusted to various pH ranges with HNO_3. After 7 days, some of the samples were spiked with known amounts of metallic ions, and these additions did not appreciably alter the natural pH. After 24 days storage from the start of the experiment, all unfiltered samples were filtered and portions of some of the previously filtered samples were refiltered. The filtrates of previously unfiltered samples were reduced to pH 1.9 if they had not been previously adjusted. The previously filtered samples and refiltered samples that were stored under natural pH conditions were dispensed into new polyethylene bottles and reduced to pH 1.9. Before filtrations all bottles were placed in an ultrasonic bath to disperse any particles that might have become attached to the container walls. Analysis of the metallic ions was then completed in 1 to 7 days.

TABLE 2. *Stability of metallic ions occurring in surface waters after a major input, normalized values*

Storage conditions	Li	Na	Mg	Ca	Sr	Mn	Cd	Pb
Filtered, natural pH	1.04	1.00	1.09	1.05	1.03	0.345	0.375	1.00
Filtered, pH 1.9[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Unfiltered, natural pH	1.00	0.950	1.00	1.18	1.13	0.012	1.00	0.500
Unfiltered, pH 1.9	1.35	.950	1.63	1.50	1.33	2.67	6.43	150
[a] Original concentration $\mu g\ ml^{-1}$	0.0023	20	5.5	22	0.054	0.058	0.00016	0.0010

Note:
Sample pH: 7.9.
Storage length: 19 days.
Unfiltered samples—filtered prior to analysis.

TABLE 3. *Stability of metallic ions occurring in rainwater, normalized values*

Storage conditions	Li	Mn	Zn	Cd	Pb
Filtered, natural pH	0.848	0.944	0.660	0.825	0.976
Filtered, pH 1.9[a]	1.00	1.00	1.00	1.00	1.00
Unfiltered, natural pH	0.845	0.983	0.849	0.793	1.08
Unfiltered, pH 1.9	.775	1.03	.879	.870	2.00
[a] Original concentration ranges, $\mu g\ ml^{-1}$, 5 samples	< .00002 – .00015	0.0039 – .0120	.010 – .052	.00022 – .00036	.010 – .074

Note:
Sample pH ranges: 4.2–6.3.
Storage length: 10–12 days.
Unfiltered samples—filtered prior to analysis.

TABLE 4. *Stability of metallic ions occurring in surface water with no recent major input, normalized values*

Storage Conditions	Li	Na	K	Cs	Mg	Ca	Sr	Mn	Fe	Cu	Cd	In	Pb
1. Filtered, natural pH	1.03	0.998	0.975	ND	0.978	0.973	0.996	0.71	ND	0.943	1.21	ND	0.767
2. 25 ml of above refiltered	1.01	.997	.965	ND	.959	.959	.995	.341	ND	2.45[b]	0.997	ND	1.13
3. Filtered, pH 1.9	1.04	1.02	1.02	ND	1.01	1.01	.978	1.09	ND	0.899	1.00	ND	0.933
4. Filtered, pH 0.7[a]	1.00	1.00	1.00	ND	1.00	1.00	1.00	1.00	ND	1.00	2.21[b]	ND	1.00
5. Unfiltered, natural pH	1.02	1.01	1.03	ND	0.996	0.956	0.986	0.163	ND	1.86	.767	ND	0.934
6. Unfiltered, pH 1.9	1.04	0.98	1.06	ND	1.00	.999	1.01	156	>80.8	2.40	1.22	ND	3.32
7. Filtered, natural pH, spiked				0.928			1.01	0.833	0.960	0.902	0.931	0.908	0.800
8. 25 ml of above refiltered				.992			0.973	.477	.240	.891	.829	.348	.390
9. Unfiltered, natural pH, spiked				.968			.986	.0666	.260	.670	.466	.208	.117
[a] Original concentration Ranges, μg ml^{-1}; Pb, $n = 3$; all others $n = 5$	.0008–.0057	12–133	.68–2.62	ND	17.5–27.0	31–62	.062–.238	.0008–.0720	ND	.0008–.005	.00005–.00012	ND	.003–.005
Spiked concentration μg ml^{-1}				.025			.050	.010	.050	.010	.001	.050	.050

[b] Values indicate contamination.
Sample pH ranges: 7.8–8.4.
Storage length: unspiked 24 days, spiked 17 days.
Unfiltered samples—filtered prior to analysis.
Note: ND = not detected.

IV. Apparatus and Techniques

The samples and blanks were filtered through 47-mm, 0.45-μm pore diameter (Millipore MF), with a holder made of borosilicate glass and a stainless steel screen filter support. The filters were leached with 100 ml of either deionized water or .016*N* HNO_3 for natural pH and reduced pH samples, respectively. This procedure also acted as a final rinse to remove any contamination to the apparatus that could have occurred during its setup. After filtration, samples were only handled inside a laminar flow clean air bench.

Instrumentation Laboratory (IL) models 151 and 353 atomic absorption/emission spectrophotometers were used. The flameless atomizers used were an IL model 355 tantalum ribbon [18] and Varian Techtron model 63 carbon tube furnace [19]. For determinations with a flame, air-acetylene was used. Specific instrumental methods and preparation techniques are given in table 5. The rationale for the particular extraction systems, apparatus, and techniques have already been explained [20]. After use, extraction flasks were rinsed with acetone, soaked in 1.6*N* HNO_3 for 15 minutes, and rinsed three times with deionized water in a clean air hood.

V. Results and Discussion

Tables 2 and 4 present the average normalized results obtained from the various treatments of the surface waters. The determinations were normalized by dividing the concentration of each storage condition of unspiked samples for a given element, by the concentration of the particular element adjusted to pH 0.7. Then the normalized results of each treatment, were averaged over all unspiked samples. The samples adjusted to 0.7 pH were assumed to be representative of the original ionic concentrations. This is justified by the complete agreement among the works cited in table 1 that samples below pH of 2 will maintain complete ionic concentrations. The exception was Cd, where some of the samples of pH 0.7 yielded results which strongly suggested that they had been contaminated with a Cd source somewhere in handling. Therefore, the sample of pH 1.9 were taken as the original concentration for Cd. This is still in agreement with the previous works and also the author's own experience with the stability of Cd solutions. Where the samples were spiked, the amount was mathematically added to the 0.7 pH concentration of the element and this was taken to be the original concentration. The results were then treated as the unspiked samples.

TABLE 5. *Instrumental methods and techniques*

Element	λ nm		Method	D.L.[a]	Precision,[b] at 10 times D.L.
Li^+	670.8	FE	Scanning, 1,000 μg ml^{-1} Na radiation buffer, yellow optical filter	0.00002 μg ml^{-1}	5%
Na^+	330.2 } D 330.3 }	FAA	1,000 μg ml^{-1} K radiation buffer	1.0 μg ml^{-1}	5%
K^+	766.5	FAA	1,000 μg ml^{-1} Na radiation buffer	0.02 μg ml^{-1}	2%
Cs^+	852.1	FE	Scanning, 1,000 μg ml^{-1} K radiation buffer, yellow optical filter	.005 μg ml^{-1}	18%
Mg^{++}	285.2	FAA	Diluted sample, 1,000 μg ml^{-1} La releasing agent	.002 μg ml^{-1}	1%
Ca^{++}	422.7	FAA	Diluted sample, 1,000 μg ml^{-1} La releasing agent	.02 μg ml^{-1}	1%
Sr^{++}	460.7	FE	Scanning, 1,000 μg ml^{-1} La releasing agent, UV optical filter	.002 μg ml^{-1}	2%
Mn^{++}	279.4	NFAA	Carbon tube furnace, direct determination, calibration by standard additions, 2.5 μl sample, background correction by H_2 continuum HCl	.0002 μg ml^{-1}	16%
Fe^{+++}	248.3	FAA	No sample pretreatment	.005 μg ml^{-1}	10%
Cu^{++}	324.8	NFAA	Tantalum ribbon atomizer, extracted sample into DZ/CCl_4 after adjustment to 0.1 *N* in HNO_3	.0001 μg ml^{-1}	4%
Ag^+	328.1	NFAA	Tantalum ribbon atomizer, $Na_2S_2O_3$ stabilizing ligand destroyed with H_2O_2 prior to adjustment to ~0.1 *N* HNO_3, extracted with DZ/CCl_4 [20]	.000001 μg ml^{-1}	5%
Zn^{++}	213.9	FAA	No sample pretreatment	.005 μg ml^{-1}	3%
Cd^{++}	228.8	NFAA	Tantalum ribbon [20] 50 μl sample, or carbon tube 2.5 μl sample direct determination, calibration by standard additions, background correction with H_2 HCl	.00003 μg ml^{-1}	5%

TABLE 5. *Instrumental methods and techniques*—Continued

Element	λ nm		Method	D.L.[a]	Precision,[b] at 10 times D.L.
In^{+++}	451.1	FE	Scanning, 1,000 μg ml^{-1} Na radiation buffer, UV optical filter	.004 μg ml^{-1}	1%
Pb^{++}	217.0	NFAA	Tantalum ribbon, DZ/$CHCl_3$ extraction system, sample adjusted to pH 11.0 + citrate [20]	.001 μg ml^{-1}[c] .0002 μg ml^{-1}[d]	10% 5%

FE = flame emission, FAA = flame atomic absorption, NFAA = nonflame atomic absorption, DZ = dithizone, HCl = hollow cathode lamp.

[a] S/N = 2.
[b] Based on calibration changes after a series of determinations.
[c] Pre-rain surface water.
[d] Post-rain surface water.
Note: FE = flame emission
FAA = flame atomic absorption
NFAA = nonflame atomic absorption
DZ = dithizone
HCL = hollow cathode lamp

Li, Na, K, Cs, Mg, Ca, and Sr generally had no significant departures from their original concentrations in the pre-rain samples, although in two of the samples K had increased about 10 percent when they were stored unfiltered. Li, Mg, Ca, and Sr increased under some of the unfiltered storage conditions in the sample of the urban creek taken after a rain.

Mn, Fe, Cu, Cd, In, and Pb displayed some changes several orders of magnitude from their original concentrations. Notably among these was the reduced recovery of these metallic ions from unfiltered spiked samples and high recovery from the unfiltered samples stored at 1.9 pH. Refiltering of the samples caused additional losses. It cannot be concluded from this study whether the loss was due to an invisible precipitate which was removed when filtered, or if ions were adsorbed onto the filter. The recovery of these ions spiked into once-filtered samples were somewhat higher than would have been predicted from the results given in table 1, the greatest loss being 20 percent for Pb.

The rainwater samples displayed better ionic stability during storage, probably because their natural pH was already acidic. The wider deviations of Li were due to concentrations near its detection limit, resulting in poor precision.

The affinity for particles of Mn, Cu, Cd, and Pb was in agreement with past works [21-26] which have established that these metals are found in association with sediments.

In a separate study it was established that Ag in rainwater and hail samples is stable for at least 30 days if $Na_2S_2O_3$ is added to the container prior to collection. Additional details can be found elsewhere [20].

VI. Conclusion

Removal of ions by adsorption onto container walls is insignificant compared to losses to the particles present in all natural aqueous systems. In nature, particles act as a storage medium for certain metallic ions which can be released back into solution under lower pH conditions, as evidenced by this study, and by certain complexing agents [27]. To discern the total metallic pollution in surface waters, the sediment must be analyzed as well as the aqueous phase.

Additional losses may result from precipitation reactions or adsorption onto the filter medium. Losses to the filter are probably more serious for lower concentrations and the higher pH.

Naturally alkaline samples must be filtered as soon as possible after taken in the field and prior to any acidification step if the distribution

between the soluble and particulate phases of metallic ions is to be determined. Samples which already exist in nature in an acid pH range tend to be more stable as illustrated by the rainwater study.

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RESIN-LOADED PAPERS—SAMPLING AND TRACE ANALYSIS USING NEUTRON ACTIVATION AND X-RAY SPECTROGRAPHY

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Resin-loaded papers, composed of approximately 50 weight percent cellulose and 50 weight percent resin, provide an ideal medium for sampling large volumes of solution, then determining the concentrated elements by either fluorescent x-ray spectrography or neutron activation. This combination of chemistry and spectrography offers a versatile quantitative approach to the determination of trace elements in the ppm-ppb range. Standards and unknowns are prepared by either multiple filtration of solutions through two or more papers to measure the collection efficiency or a radiotracer is added to the solution to serve as a collection monitor. Reagent and paper blanks are incorporated into the analytical procedure. Papers containing strong acid or base resins collect a wide range of ions. Specific ions are collected by either chemical processing of the solution prior to filtration through the resin-loaded paper or by the use of papers loaded with chelating resins having high specificity.

Advantages of the resin-loaded paper approach prior to spectrography are: Improved analytical accuracy as the standards and unknowns are present in similar matrices; sampling errors are reduced and sensitivity significantly increased by concentration of the elements from a large volume of solution; and x-ray and gamma-ray spectral interferences are greatly reduced compared to bulk analysis. Also standard papers can be reused and stored indefinitely for x-ray applications.

Examples of published applications to a wide range of industrial, environmental, and health problems are summarized together with applications by the Bureau of Mines to metallurgical and mining problems. Potential applications of new ion exchange resins and reagent-loaded papers are also considered.

Keywords: Ion exchange; sampling; x-ray fluorescence.

I. Introduction

Fluorescent x-ray spectrography, using either multichannel wavelength-dispersion or high resolution energy-dispersion techniques (EDXA), is receiving increasing interest for simultaneous multielement trace analysis. However, because of the very limited depth of direct x-ray analysis using K or L x-rays, the effective sample volume is of the order of 1 cubic centimeter or less. This small sample volume coupled with a high background of scattered radiation imposes a practical detection limit of 1 to 100 parts per million for direct x-ray analysis. Sampling errors may also be significant because of the limited quantity of sample being analyzed.

X-ray spectrography is applicable to microgram and, in favorable cases, submicrogram amounts of most elements. Therefore, one general approach for extending the capabilities of x-ray spectrography into the parts per billion range is to concentrate the elements of interest in ion exchange resin-loaded papers [1-3]. Advantages of this approach are: reduction of interelement effects because standards and unknowns have a similar low x-ray absorbing matrix; physical variables such as metallurgical history, grain size, and surface preparation are eliminated; and of the most importance, sampling errors are significantly reduced and sensitivity greatly increased by concentrating the trace elements from large samples.

Similar advantages apply to the use of resin-loaded papers in neutron activation analysis (NAA). Despite the inherent sensitivity of neutron activation, the practical concentration levels that can be determined by direct NAA procedures are seriously limited when applied to many real samples. The size of the sample, which can be used in direct NAA, is limited to a maximum of a few milliliters or grams because of the high radioactivity generated from the matrix and because of the physical limitations of sample size for controlled exposure to the neutron flux. However, large samples, several liters or hundreds of grams, are often required to reduce sampling errors characteristic of trace elements in nonhomogeneous materials, or to increase the signal from the element of interest to a statistically meaningful level.

The recent advances in high resolution detectors have greatly reduced the problems of spectral interference in multielement trace analysis [4], but there are still many unsolved problems. One approach to matrix problems is to separate trace metals from the bulk matrix after irradiation. "Hot" chemical separation requires special facilities and equipment to handle the highly radioactive samples. In the case of short-lived nuclides, the time required for chemical separation often precludes the use of this

technique. These limitations in NAA can be overcome by using separation and concentration techniques prior to activation [5]. Ion exchange resin-loaded papers offer the same advantages for NAA as for EDXA. Elements collected in these papers are in a matrix of low atomic number elements—carbon, hydrogen, oxygen, nitrogen, sulfur—that have low x-ray and gamma-ray absorption coefficients, insignificant cross sections for thermal neutrons, and/or short half-lives. The need for "hot" chemistry is eliminated by concentrating the elements of interest into resin-loaded papers prior to neutron activation.

II. Procedure

Resin-loaded papers are composed of approximately 50 percent cellulose and 50 percent powdered ion-exchange or chelating resin. Incorporation of the resin in a thin paper disk provides a convenient media for handling small quantities of resin and for supporting the resin in the analytical instrumentation. Standards and unknowns are prepared on similar resin-loaded papers, providing a match that is often impossible to achieve in direct x-ray or neutron activation analysis.

The general analytical procedure consists of the following steps:

1. Dissolution of the sample, or selective dissolution of the element or elements of interest.
2. Adjustment of pH, addition of complexing or masking agents, or other chemical treatment that may be necessary to achieve the selectivity desired in the ion-exchange process.
3. Collection of the desired elements on a resin-loaded paper disk by filtration or by suspension of the disk in the solution.
4. X-ray or neutron activation determination of the elements on the dried resin-loaded disk, using disks containing known quantities of the elements as standards.

The sample size is restricted only by the volume which may be conveniently handled during chemical treatment. For example, parts per billion and parts per million levels of gold have been determined in geological materials using samples of 200 grams [6] and, subsequently, up to 500 grams in the authors' laboratory using resin-loaded papers in combination with NAA and x-ray techniques. Large samples are essential for precise results in analyzing materials containing randomly distributed particles. For example, in analyzing a gold ore, Chow and Beamish [7] obtained values ranging from 7.2 to 50.1 parts per million gold using 50 milligram samples and direct NAA. Using 25 gram samples of the ore and the

resin-loaded paper technique, results obtained by the Bureau of Mines ranged from 9.4 to 11.5 parts per million [6].

III. Resin-Loaded Papers

The commercially available resin-loaded papers are prepared from ion-exchange resins that are strong acid or weak acid cation exchangers or strong base or weak base anion exchangers. These resins are relatively nonselective, collecting almost all cations or anions from solution according to an order to preference based on the size, charge, or other properties of the ions. If a wide range of ion collection is desired, consideration should be given to incorporation of a mixture of cation and anion resins into a paper [8]. Physical and chemical properties of commercially available resin-loaded papers are summarized in previous publications [1,2]. Any chelating or ion-exchange resin has the potential of being made into a resin-loaded paper. The particle size should be quite small—about minus 300 mesh—for good filter paper properties. Consultation with reviews of ion exchange, such as those published every 2 years by *Analytical Chemistry*, provides a source of ideas for new resin-loaded paper possibilities.

IV. Selectivity

It is often desirable to concentrate a single element or a group of elements away from the major constituents of a sample. This selectivity may be achieved in several different ways:

1. Use of selective resin-loaded paper.
2. Introduction of complexing or masking agents into the solution prior to resin-paper collection.
3. Manipulation of pH prior to resin-paper collection.
4. Preliminary chemical separation such as solvent extraction, selective precipitation, or column ion exchange, followed by resin-paper collection. Examples of each of these techniques are given in earlier publications [1-3].

The determination of different chemical forms of an element is possible using the approach employed for determining the methylmercuric and mercuric forms of mercury [9]. To achieve this separation, two specially prepared resin-loaded papers were used. Both papers were made for the

authors' laboratory by H. Reeve Angel & Co. using minus 325 Chelex 100 resin[1] [10], and Srafion NMRR resin [11]. Chelex 100 resin-loaded paper is very selective for the mercuric ion at low pH, as shown in figure 1. In contrast, methylmercuric ion is not retained by Chelex 100 resin but is collected by the Srafion NMRR resin. Passing a solution through these two resin-papers in series thus provides a means of determining the forms of mercury in the sample by either NAA or x-ray spectrography.

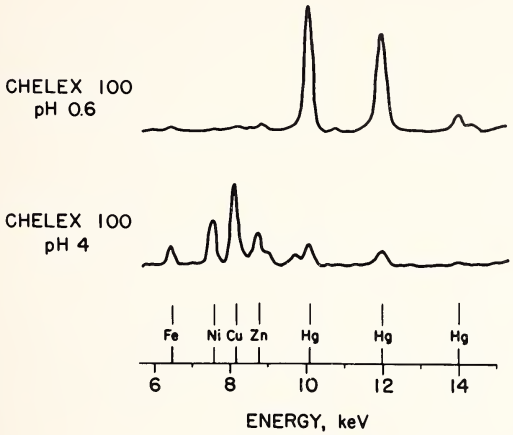

Figure 1. X-ray spectra for comparison of selectivity for Hg, Fe, Ni, Cy, and Zn by Chelex 100 resin-loaded paper at pH 4 and pH 0.6 [9].

The impregnation of ordinary filter papers with ion exchangers shows great promise. This approach has already found wide application in paper chromatography [12]. Inorganic ion exchangers are especially popular because of their selectivity, *e.g.* ceric antimonate and tungstate for mercury; zinc and cobalt ferrocyanides for silver; tin arsenate and chromium phosphate for alkali metals; titanium selenite for cadmium; titanium vanadate for strontium; and chromium molybdate for lead [12].

Stannic tungstate papers were prepared by the authors using the procedure described by Qureshi and Mathur [13]. Whatman No. 2 filter paper was cut into 3.5 cm diameter disks and dipped into a stannic chloride solution followed by 5 seconds in a hot sodium tungstate solution then washed sequentially with dilute nitric acid and distilled water. The stannic tungstate, that precipitates in the fibers of the filter paper, provides exchange sites for lead ions. The collection of lead from pH 4 solution is demonstrated by the x-ray spectra shown in figure 2. Organic ion exchangers warrant consideration for NAA and x-ray applications

[1]Reference to specific brands is made for identification only and does not imply endorsement by the Bureau of Mines.

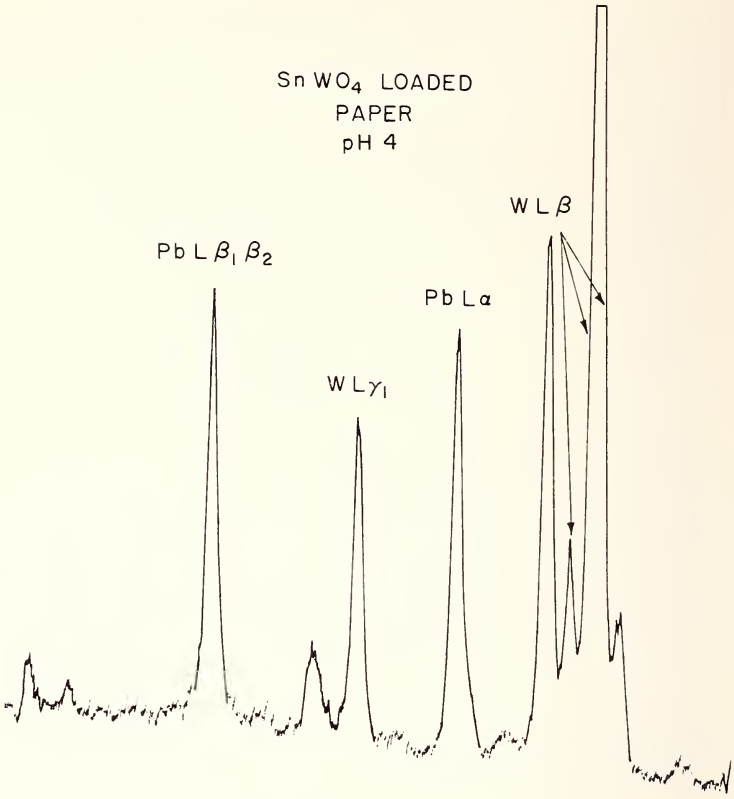

Figure 2. X-ray spectra of stannic tungstate paper after collection of lead.

because these reagents are composed of low atomic number elements. Organic exchangers having good potential include dithizone [14], 2-thenoyltrifluoroacetone [15], and bathocuproine [16].

V. Radiotracer Collection Monitors

To obtain quantitative results, the ion exchange resin-loaded paper collection process must either be quantitative for the elements of interest, or the extent of separation and collection must be adequately monitored. A resin-loaded disk is a very short ion-exchange column—the column length is equal to about the thickness of construction paper. Several filtrations, usually five to eight, are necessary to reach equilibrium between the solution and the resin particles. To check for quantitative recovery of ions

from solution, it is advisable to filter the solution through a second and third disk and determine the elements on all three disks by either x-ray or NAA. An alternate approach that the authors have found very useful is to use a radioactive tracer as a collection monitor. The chemical behavior of a radioactive isotope of an element is the same as the naturally occurring nonradioactive isotopes of that same element if the isotopes are in the same chemical form. Therefore, the ratio of radioactive to natural isotopes of the element will remain constant throughout any subsequent chemical or mechanical treatment steps, *e.g.*, solvent extraction, ion exchange, precipitation [17]. A measurement of radioactivity on standards and unknowns provides a reliable value for the quantitativeness of the collection process. Using a radiotracer monitor, it is not necessary to collect 100 percent of the ions of interest, therefore, nonstoichiometric procedures can be utilized. A good example is the "danglation" procedure employed by the authors' laboratory for the determination of gold in low grade ores [6]. A selective ion exchange resin-loaded paper was simply suspended overnight in a solution containing natural gold and a gold radiotracer (see fig. 3). Approximately 10 to 30 percent of the gold was collected in 16 hours. Even with this low and variable collection, quantitative analyses were achieved by using the collection monitor.

The authors' applications of radiotracer collection monitors with resin-loaded papers-NAA can be grouped into three categories. The first classification is a collection monitor that is not produced during neutron ac-

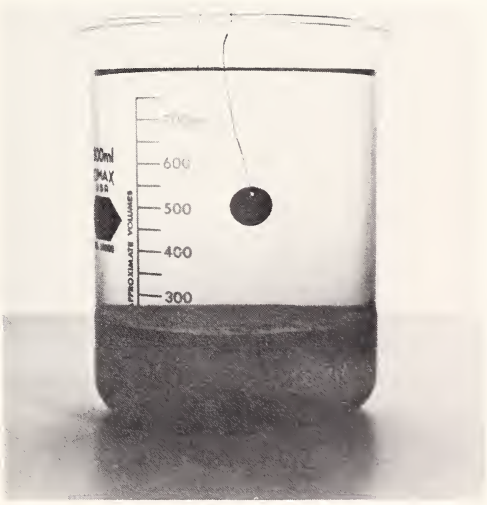

Figure 3. Collection of gold by suspended ion exchange resin-loaded paper disk.

tivation of the sample. As an example, the cyclotron-produced isotope ^{195}Au was used as the collection monitor for the determination of parts per billion concentrations of gold in geological samples [6]. The only naturally occurring isotope ^{197}Au, undergoes an (n,γ) reaction during thermal neutron irradiation yielding ^{198}Au, having a major gamma-ray peak at 412 keV. The ^{195}Au monitor emits characteristic x-rays in the 67 to 80 keV range, therefore, the spectra of ^{195}Au and ^{198}Au are easily resolved with a scintillation detector.

In the second category, the radiotracer isotope is of the same element that is being determined; however, small amounts of the radiotracer are produced during thermal neutron irradiation of the naturally occurring isotopes. In order to be a useful monitor, the radiotracer isotope must activate at a much slower rate than the isotope being determined. An example is the use of ^{203}Hg as a collection monitor in the determination of mercury in aqueous samples. Mercury has a stable isotope ^{202}Hg with a thermal neutron cross section for the reaction ^{202}Hg(n,γ)^{203}Hg that is about three orders of magnitude less than the principal reaction ^{196}Hg(n,γ)^{197}Hg. Therefore, the activity of the collection monitor ^{203}Hg is counted prior to activation, then the neutron produced ^{197}Hg is used to determine the mercury concentration in the sample. There is a thallium K x-ray peak at 72 keV from the ^{203}Hg tracer that interferes with the 68 keV Au K x-rays and 77 keV gamma-ray peaks from the ^{197}Hg when using the low resolution scintillation detector. However, this spectral interference is easily corrected as the magnitude of the interfering peak is directly proportional to the height of the principal gamma-ray peak for ^{203}Hg. The use of a chelating resin-loaded paper that is selective for the noble metals and mercury eliminates problems of spectral interferences from other elements.

In the third category, the chemical properties of the radiotracer and the elements being determined are similar; however, the radiotracer has a different atomic number. For example, the authors used cyclotron-produced ^{195}Au as a collection monitor for the determination of platinum, palladium, and gold in geological samples. Srafion NMRR chelating resin-loaded paper was used to concentrate the noble metals. Although the collection rate was different for each of the three elements, acceptable analyses were obtained by adding the ^{195}Au collection monitor to both standards and unknowns. Typical calibration data are shown graphically in figure 4.

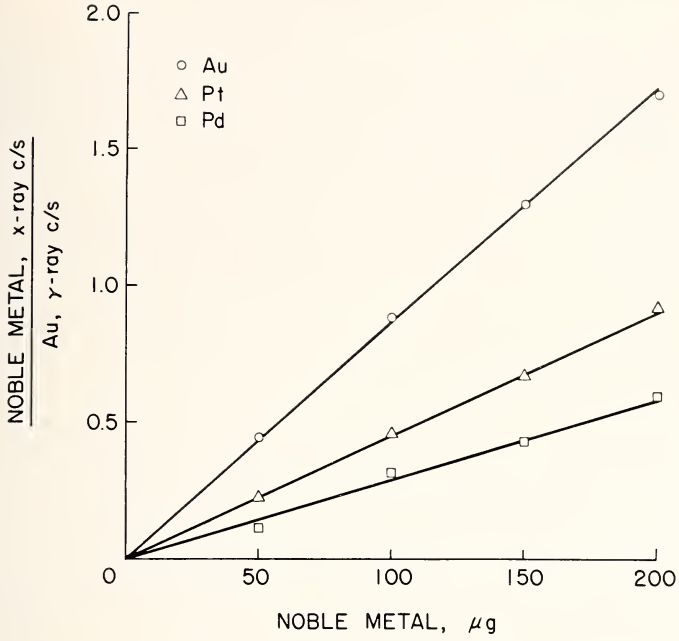

Figure 4. Calibration relationship for gold, platinum, and palladium using ^{195}Au as the collection monitor.

VI. Conclusions

Resin-loaded papers can greatly extend the range and versatility of x-ray spectrography and neutron activation for trace analyses. Chemical preconcentration into the resin-loaded papers reduces or eliminates problems of matrix correction, variations in physical properties of the sample, increases sensitivity by several orders of magnitude, and decreases sampling errors. Suitable standards are easily prepared to match the samples. Problems in nonstoichiometric collection of microgram quantities from complex solutions can be overcome by the use of radioactive collection monitors. With the commercial availability of dedicated computers coupled to neutron activation and energy dispersion x-ray systems, the use of preconcentration techniques, such as resin-loaded papers, will be of increasing importance.

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This book is the formal report of the proceedings of the 7th Materials Research Symposium: Accuracy in Trace Analysis. This volume contains the invited and contributed papers presented at the symposium, and which treat problems of sampling and sample handling as well as the usually-discussed analytical methodology. Many important techniques and methods are described, and extensive references are presented, to give deeper insight into the problems of obtaining accurate results in trace analytical chemistry. Accordingly, this volume should not only stimulate greater interest research in these areas but should provide a valuable guide for everyday analytical problems

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