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Preparation of a Nanoscale TiO2 Aqueous Dispersion for Toxicological or Environmental Testing

Version 1.2

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NanoEHS Protocols

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FOREWORD

This special publication is one in a series of protocols resulting from a collaborative research agreement between the National Institute of Standards and Technology (NIST) and Duke University's Center for the Environmental Implications of Nanotechnology (CEINT). The original version of this protocol (Ver. 1.0) was first posted on the CEINT web site (http://ceint.duke.edu) and it, along with any other previous version, is superseded by this updated special publication version. Updates to this protocol may be released in the future. Visit http://nist.gov/mml/np-measurement-protocols.cfm to check for revisions of this protocol or new protocols in the series.

NIST and CEINT are interested in soliciting feedback on this method. We value user comments and suggestions to improve or further validate this protocol. Please send your name, email address and comments/suggestions to nanoprotocols@nist.gov. We also encourage users to report citations to published work in which this protocol has been applied.

1. Introduction

Toxicity and fate assessment are key elements in the evaluation of the environmental, health and safety risks of engineered nanomaterials (ENMs). While significant effort and resources have been devoted to the toxicological evaluation of many ENMs, including nanoscale TiO_2 [1-4], obtaining conclusive and reproducible results continues to be a challenge [5]. This can be traced in part to the lack of standardized dispersion protocols and the inconsistent application of dispersion procedures in relevant biological and environmental matrices [6, 7]. In order to address these issues, the National Institute of Standards and Technology (NIST), jointly with the Center for the Environmental Implications of Nanotechnology (CEINT), have developed a series of standardized and validated protocols for the dispersion of ENMs from a powdered material source for both human health and environmental testing applications. This protocol has been developed and validated using NIST Standard Reference Material (SRM) 1898[a]. SRM 1898 consists of a widely studied and industrially relevant TiO_2 nanomaterial with broad commercial penetration and a production history dating back several decades [3, 8-10].

While the procedures detailed in this protocol focus on the dispersion of SRM 1898 in aqueous media, it is believed that the adopted characterization, optimization and validation approaches can be more generally applied to the preparation of ENM dispersions in any relevant matrix. For this reason, and to allow for broader applicability, experimental details and discussions regarding the characterization, process optimization and validation steps adopted for the development of the dispersion method are detailed in a separate publication [11].

2. Principles and scope

This protocol provides a validated method for the preparation of aTiO_2 nanoparticle dispersion in high purity de-ionized water by use of ultrasound (a process referred to here as sonication), as a stock preparation compatible with biological and environmental matrices. Furthermore, this protocol can be used in combination with matrix-specific protocols for the preparation of dispersions in more complex biological or environmental media. For the preparation of TiO_2 dispersions in biologically relevant media, refer to [12]. For guidelines on reporting relevant conditions and critical parameters relating to the ultrasonic dispersion of ENMs, consult reference [13]. For additional relevant and general considerations on the application of ultrasound to prepare ENP dispersions, refer to [7, 14].

The method described herein, if applied correctly, yields aqueous monomodal nanoscale TiO_2 dispersions, characterized by a mean particle diameter of ≈ 70 nm and a pH between 3.7 and 4.9. The method is validated for the preparation of dispersions in the (0.5 to 20) mg/mL concentration range. Dispersions prepared following this protocol should be stored so as to minimize exposure to light (e.g., in amber vials), at room temperature, and used within 24 h of preparation.

[a] Information regarding SRM 1898 can be obtained at http://www.nist.gov/srm/.

3. Terminology

This protocol complies with definitions relevant to nanotechnology as set forth in the ASTM standard E2456 [15] and is consistent with the draft standard ISO TS 80004-1 [16]. Additional guidance is derived from recommendations of the International Union of Pure and Applied Chemistry [17].

nanoparticle — sub-classification of ultrafine particle that is characterized by dimensions in the nanoscale (i.e., between approximately 1 nm and 100 nm) in at least two dimensions; also referred to as "nano-object" in ISO TS 80004-1 [16].

primary particle — the smallest discrete identifiable entity associated with a particle system; in this context, larger particle structures (e.g., aggregates and agglomerates) may be composed of primary particles.

aggregate — a discrete assemblage of primary particles strongly bonded together (i.e., fused, sintered or metallically bonded).

Note—The adjective "primary", when used in conjunction with the term aggregate, is employed in the present context to indicate the smallest achievable aggregate.

agglomerate — assemblages of particles (including primary particles and/or smaller aggregates) held together by relatively weak forces (e.g., van der Waals, capillary or electrostatic), that may break apart into smaller particles upon further processing.

Note—Although we define them as distinct entities, the terms aggregate and agglomerate have often been used interchangeably to denote particle assemblies.

dispersion — used in the present context to denote a liquid (aqueous) in which particles are homogeneously suspended, or the process of creating a suspension in which discrete particles are homogeneously distributed throughout a continuous fluid phase; implies the intention to break down agglomerates into their principal components (i.e., primary particles and/or aggregates).

4. Reagents, materials and equipment

4.1. Reagents

4.1.1. AEROXIDE TiO_2 P25 (Evonik Degussa GmbH, Germany); available from distributors or obtained as a certified reference material from NIST (SRM 1898).

4.1.2. $\geq$ 18 MΩ·cm resistivity Type I biological grade de-ionized (DI) water; biological grade implies sterility and absence of endotoxin contamination.

Note—Pyrogens (also known as endotoxins) are shed from the outer membrane of Gram-negative bacteria during cell division or lysis. These toxins are relatively heat-stable and are not destroyed under typical sterilizing conditions. As a result, pyrogens are ubiquitous and can interfere with the accuracy of toxicity assays. To depyrogenize glassware, bake at 250 °C for 2 h or at 200 °C overnight.

Note—Limulus Amoebocyte Lysate (LAL) reagent grade pyrogen-free water can be obtained from commercial vendors.

Note—Sterility and absence of pyrogen contamination should be verified for all materials in contact with the dispersion. If using the LAL test for pyrogens, avoid using cellulose-based filters, as they can be a source of beta-glucan, which interferes with the LAL assay.

Note—If the dispersion is not intended for toxicological assessment, pyrogen-free conditions may not be necessary.

4.2. Materials

4.2.1. 100 mL, ≈ 5 cm diameter cylindrical glass beaker

4.2.2. 125 mm wide x 65 mm deep crystallizing glass dish

4.2.3. 50 mL borosilicate volumetric flask or pipette

4.2.4. Aluminum or polystyrene weighing dish

4.2.5. Clamps or other locking device for holding beaker in place

4.2.6. Chopped ice

4.3. Equipment

For the preparation of the aqueous dispersion:

4.3.1. Analytical balance

4.3.2. Probe-type sonicator with a standard ½ inch (1.3 cm) diameter titanium horn fitted with a removable flat tip (or similar ultrasonic device)

For verification of expected outcome:

4.3.3. pH meter and electrode

4.3.4. Laser diffraction spectrometer (LDS), ***or***

4.3.5. Dynamic light scattering (DLS) instrument, ***or***

4.3.6. X-ray disc centrifuge (XDC) particle size analyzer

5. Preparation of aqueous TiO_2 nanoparticle dispersion

Note—To avoid contamination, all glassware should be meticulously cleaned, rinsed with ethanol and dried prior to use. Glassware can be sterilized using an autoclave, by exposure to hot, dry air (130 °C-170 °C) for 2-4 h in an oven or by prolonged contact with alcohol. Avoid detergents if possible; if detergents are used, rinse with copious amounts of DI water prior to rinsing with ethanol and drying. Store and work in high-efficiency particulate air (HEPA) filtered clean bench if available; if not, containers should be capped or sealed with thermoplastic (e.g., Parafilm).

Note—Use clean, sterile pipette tips and sterile procedures.

Note—For details on the validation of the particle size distribution (PSD) of dispersions, the optimization of the TiO_2 sonication sequence, as well as stability, validation and applicability considerations, refer to [18].

5.1. Using an analytical balance and an aluminum or polystyrene weighing dish, weigh an adequate mass of dry TiO_2 powder to achieve the desired concentration in a 50 mL water volume. For guidance on mass-concentration relationships, refer to the table below.

Mass (g)	Concentration in 50 mL volume (mg/mL)
0.025	0.5
0.05	1
0.5	10
1	20

Note—For greater accuracy and reproducibility with respect to mass concentration, the TiO_2 powder should be dried overnight at an elevated temperature between (105 and 150) ºC and then allowed to cool to room temperature in a desiccator prior to use.

5.2. Add the weighed mass of powder to a 100 mL, ≈ 5 cm diameter cylindrical glass beaker. Add 50 mL of DI water to the beaker with the powder.

5.3. Place the beaker inside the 125 x 65 mm glass dish and secure the beaker in the center of the dish by use of clamps or other locking device to ensure that the beaker remains in place during sonication.

5.4. Fill the 125 x 65 mm glass dish with enough water and chopped ice to allow for the ice water bath level to encase the beaker to approximately the level of the water contained in the beaker.

5.5. Immerse the sonicator horn into the liquid in the beaker down to about 2.5 cm below the liquid level in the beaker. Center the horn in the beaker; the horn should not touch the sides or the bottom of the beaker.

5.6. Select a sonicator setting that yields a delivered power of approximately 50 W.

Note—Refer to [14] for details on the recommended calibration procedure for the determination of the sonicator's delivered power.

5.7. Operate the sonicator at this delivered power level for 15 min, using an 80 % pulsed operation mode (e.g., 80 % on / 20 % off during each second of operation time), or similar on/off time sequence.

5.8. After sonication, transfer the aqueous dispersion to an amber borosilicate glass container and store at ambient temperature until further use. Do not refrigerate.

Note—If an amber glass container is not used, store the stock dispersion in a cabinet or other container such that exposure to light is minimized. It is not established whether or not exposure to typical laboratory light levels is consequential with respect to dispersion properties or biological behavior of the TiO_2 nanoparticles.

Note—The stability of dispersions prepared according to this protocol has been validated up to 48 h; however, since this protocol is intended as a preliminary step for the preparation of dispersions in relevant media, the resulting dispersions should be used as soon as possible following preparation and in accordance with directions prescribed in medium-specific protocols.

6. Expected outcome

6.1. The obtained TiO_2 aqueous dispersion should have an opaque white appearance if prepared using P25.

Note—If source powders other than P25 are used, the appearance may vary depending on the final particle size, particle concentration and other factors.

6.2. The particle size distribution (PSD) of P25 dispersions prepared following this protocol should be monomodal, with the following volume-based mean particle (D_m), D_{10} and D_{90} values[b]:

If measured using LDS:

$D_m \approx$ (67 to 75) nm

$D_{10} \approx$ (58 to 61) nm

$D_{90} \approx$ (79 to 89) nm

If measured using DLS:

$D_m \approx$ (108 to 116) nm

$D_{10} \approx$ (60 to 76) nm

$D_{90} \approx$ (148 to 155) nm

If measured using XDC:

$D_m \approx$ (70 to 84) nm

$D_{10} \approx$ (9 to 54) nm

$D_{90} \approx$ (97 to 142) nm

The expected range for size parameters was calculated from three independent replicates of 10 mg/mL dispersions obtained following the prescribed procedure. Refer to the Appendix for details on the calculation of the expected size parameter ranges.

The volume-based mean particle diameter, as well as the D_{10} and D_{90} values for aqueous P25 dispersions prepared following the protocol should be reported by the user to allow for comparison with the values specified herein. Refer to [11] for details and discussions on PSD characterization and validation criteria, as well as illustrations of representative PSD profiles.

6.3. The pH of P25 aqueous dispersions prepared using this protocol should be between 3.7 and 4.9, with lower pH values obtained for higher P25 concentrations.

Note—The successful implementation of this protocol for ENMs other than P25, including other sources of TiO_2 or other metal oxide ENMs, is dependent on knowing the isoelectric point (IEP) or zero point of charge of the ENM; in order to obtain a stable dispersion for ENMs that are electrostatically stabilized (i.e., that do not contain a sterically stabilizing capping agent), the final pH should be at least 2 to 3 pH units below or above the IEP. This may require the addition of a compatible acid or base, and should be evaluated as part of the optimization process. Multiple test or practice runs may be required in order to determine the required quantity

[b] D_{10} and D_{90} refer to characteristic percentile size values associated with the cumulative volume or mass less than 10 % and 90 %, respectively, of the total volume or mass within the distribution. These parameters are routinely reported by LDS instruments. They may or may not be obtainable directly from commercial DLS instruments, depending on the manufacturer.

and concentration of acid or base to add; pH adjustment should, optimally, be performed after step 5.2 and prior to sonication. If dispersions are to be used for toxicological or environmental tests, additives for adjusting the pH should be selected based on their biocompatibility and relevance to the test matrix, and considering potential sonication-induced physicochemical changes [7].

7. Abbreviations

DI	de-ionized
DLS	dynamic light scattering
ENM	engineered nanomaterial
HEPA	high-efficiency particulate air
IEP	isoelectric point
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LDS	laser diffraction spectrometry
NIST	National Institute of Standards and Technology
PSD	particle size distribution
SRM	Standard Reference Material
XDC	X-ray disc centrifugation

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9. Appendix

9.1. Calculation of expected particle size parameters

The expected range for D_m, D_{10} and D_{90} values was obtained using the following equation:

$$\text{range} = \left(\boldsymbol{x} - \frac{\boldsymbol{t.s}}{\sqrt{\boldsymbol{n}}} \text{ to } \boldsymbol{x} + \frac{\boldsymbol{t.s}}{\sqrt{\boldsymbol{n}}}\right)$$

Where $\boldsymbol{x}$ and $\boldsymbol{s}$ are the average and standard deviation, respectively, of the measured size parameter from three independent replicates, $\boldsymbol{t}$ is the student test parameter for a 95% confidence interval and two degrees of freedom ($\boldsymbol{t}$ = 4.30), and $\boldsymbol{n}$ is the number of tested samples ($\boldsymbol{n}$ = 3).