National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1898

Titanium Dioxide Nanomaterial

This Standard Reference Material (SRM) is intended primarily for use as a benchmark and investigative tool for evaluation of the potential environmental, health, and safety risks that may be associated with manufactured nanomaterials during their product life-cycle. This SRM is also intended for use in the calibration and performance testing of gas sorption instruments used for determining the Brunauer-Emmet-Teller (BET) specific surface area of powders and porous solids. A unit of SRM 1898 consists of an amber glass bottle containing nominally 15 g of mixed-phase (anatase and rutile) nanocrystalline titanium dioxide (TiO_2) in the form of a dry agglomerated powder.

This SRM is certified for BET specific surface area determined by nitrogen gas sorption at liquid nitrogen temperature (77.3 K) using the discontinuous manometric measurement technique. Certified values for multi-point (MP) and single-point (SP) data analysis using the linear form of the single parameter BET equation are provided in Table 1. Information values for crystalline-phase content and crystallite size are listed in Table 2 and Table 3, respectively. Information values for elemental composition and purity are given in Table 4. Additional information values include particle characterization. Validated dispersion protocols for use in toxicity assays are summarized in Appendix B. Value assignment categories are based on the definition of terms and modes used at NIST for chemical reference materials [1], and uncertainties are assessed according to the ISO Guides [2,3], unless noted otherwise.

Certified Values: A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. Certified values are based on measurements performed at NIST and validated by qualified collaborating laboratories [4]. The uncertainty listed with each value is an expanded uncertainty based on a 95 % confidence interval and is calculated according to the methods in the ISO Guides [2,3].

Table 1. Certified Values for BET Specific Surface Area

Measurement Technique	Specific Surface Area Value[(a)] (m^2/g)
MP	55.55 ± 0.70
SP	53.85 ± 0.78

[(a)] The assigned value is a weighted mean of the results from measurements of ten bottles of the material using a Gaussian, linear mixed effects model [4]. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor $k = 2$, corresponding to a 95 % confidence interval, calculated by Monte–Carlo simulation of uncertainty components using methods from the ISO Guide or its Supplement [2,3]. It includes between-bottles and within-bottle heterogeneity components, plus an additional type B evaluated component.

Expiration of Certification: The certification of **SRM 1898** is valid indefinitely, within the measurement uncertainty specified, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). Periodic recertification of this SRM is not required. The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of Certification: NIST will monitor the certified value of this SRM. If substantive technical changes occur that affect the certification, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Debra L. Kaiser, Chief
Ceramics Division

Gaithersburg, MD 20899
Certificate Issue Date: 14 June 2012

Robert. L Watters, Jr., Chief
Measurement Services Division

Coordination of the technical measurements for certification was accomplished under the direction of V.A. Hackley of the NIST Ceramics Division. Certification measurements were performed at NIST by V.A. Hackley and J.F. Kelly of the NIST Ceramics Division. Physical measurements for information values were provided by I. Levin and J.S. Taurozzi of the NIST Ceramics Division. Chemical measurements for information values were provided by T.A. Butler, J.L. Molloy, J.R. Sieber, M.R. Winchester, and L.J. Wood of the NIST Analytical Chemistry Division.

B. Toman (Statistical Engineering Division) provided statistical consultation supporting the assignment of certified values and evaluation of associated uncertainty reported in Table 1.

Validation measurements were conducted by P. Klobes from Bundesanstalt für Materialforschung und -prüfung (Berlin, Germany), R. Ahmad of Quantachrome Corporation (Boynton Beach, FL, USA), and J. Kenvin of Micromeritics Instrument Corporation (Norcross, GA, USA).

A. Stefaniak, of the Division of Respiratory Disease Studies at the National Institute for Occupational Safety and Health, Centers for Disease Control, in Morgantown, WV, provided technical input and aided in the organization of the interlaboratory study.

Support aspects involved in the preparation and issuance of this SRM were coordinated through the NIST Measurement Services Division.

MATERIAL PREPARATION AND ANALYSIS[(1)]

Material Source and Processing: Aeroxide TiO_2 P25 titanium dioxide powder (Evonik North America, Parsippany, NJ) was procured through a commercial distributor. The product is commonly referred to in the technical literature as "P25." Approximately 11 kg of powder was collected by sampling in roughly equal quantities from each of five original containers, homogenized in a cone blender, and finally placed into amber glass bottles with screw tops.

Heterogeneity Assessment: Ten units were selected using a stratified random sampling procedure and used for certification and heterogeneity testing at NIST based on BET specific surface area analysis. Analysis of these ten units was randomized, and two samples were tested from each of the ten units. Heterogeneity testing was performed by comparison of multipoint BET specific surface area results. Microscale elemental heterogeneity was evaluated for aluminum, calcium, chromium, iron, silicon, titanium, and zinc using microbeam X-ray fluorescence analysis of pressed briquettes; results suggest that a sample size of 1 mg or greater is necessary to represent the bulk composition of SRM 1898 for these elements.

Interlaboratory Study: An intercomparison study using SRM 1898 was conducted with 20 participating laboratories. In this study, at least three subsamples from a single randomly assigned unit of SRM 1898 were analyzed by each participant using nitrogen gas sorption; 14 bottles were randomly selected, such that some laboratories received samples from the same bottle. A summary of the study results is provided for informational purposes.

Value Assignment and Uncertainty Analysis: Measurements to establish certified values were conducted using commercial gas sorption instruments at NIST and at three expert-qualified collaborating laboratories using a defined method based on the static volumetric technique. For certification measurements, ten units were selected using a stratified random sampling procedure, and two subsamples were measured from each unit. Analysis of the test samples was randomized and measurements performed over several weeks. Three additional units were randomly selected and used for measurements at collaborating laboratories. Information values are derived from analyses conducted principally at NIST, with contributions from collaborating laboratories.

[(1)]Certain commercial equipment, instruments, or materials are identified in this certificate in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

NOTICE AND WARNING TO USERS

The health risks associated with this material are not fully established. This material should be handled as recommended by the National Institute for Occupational Safety and Health (NIOSH). NIOSH has determined that ultrafine titanium oxide (including engineered nanoscale) is a potential occupational carcinogen [5].

INSTRUCTIONS FOR HANDLING, STORAGE AND USE

Handling: SRM 1898 is a powdered material and as such can be subject to air dispersal. For this reason, sample transfer operations should be performed where strong drafts are absent and by working with small quantities of powder. Static electricity can cause powder to adhere to glass surfaces; gentle tapping can be used to dislodge this material during transfer, or an anti-static device may be employed. Before removing sample for test applications, the bottle containing SRM 1898 should be gently and alternately rotated and inverted several times to ensure mixing.

Storage: SRM 1898 should be stored in the original amber bottle with screw cap tightly sealed. Bottles can be stored at normal laboratory ambient temperature and humidity. In order to avoid possible contamination, powder removed for testing should not be returned to the original bottle.

Use (Procedures for Determination of BET Specific Surface Area, see also "Dispersion Protocols"):

Sampling Procedure: The amber bottle with screw cap in place should be inverted and rolled gently several times prior to removing a sample. Using a clean stainless steel spatula or similar device, remove sufficient powder for analysis and transfer to an appropriate size pre-weighed glass sample tube; the mass to be analyzed should be between 0.2 g and 0.6 g. A funnel can be utilized to deliver powder more efficiently. Gentle tapping is applied to the funnel and/or sample tube in order to ensure that the powder is delivered to the bulb area. If powder persists on the inner surface of the tube stem (above the bulb), a pipe cleaner can be used to remove this material.

Gravimetric Procedure: Sample mass for gas sorption analysis should be determined using a properly calibrated analytical balance that reads to ±0.1 mg or better. The mass of the dry empty tube plus fill rod (if used) and sealing device (e.g., stopper, seal frit) should then be recorded (mass = M1). The fill rod and sealing device should then be removed and the balance tared with the empty tube to facilitate sample transfer. After transferring an appropriate mass of sample (see above), the mass of the tube + sample + fill rod + sealer should be recorded (mass = M2). After weighing operations are complete, the instrument manufacturer's instructions or accepted practice should be followed to seal and install the sample tube for outgassing (see below). Once outgassing is complete and the sample is cooled to room temperature, measure and record the mass of tube + outgassed sample + fill rod (if used) + sealing device (mass = M3). Subtract M1 from M3 to obtain the mass of the outgassed sample to ±0.1 mg; use this value for calculation of BET specific surface area. Subtract M3 from M2 to obtain the mass loss (principally water) resulting from outgassing; this value should be of order 1 % unless the sample has been subjected to high humidity levels.

Outgassing Procedure: The powder sample should be outgassed under vacuum. Heat the sample to 110 °C at a rate not to exceed 10 °C per minute. If automated evacuation control is available, the sample tube should be evacuated at a maximum rate of 0.667 kPa/s (5 Torr per second); if the rate exceeds this value then the heating ramp should be suspended until the pressure drops into the safe range. This procedure prevents sample powder uptake into the evacuation system. Once the pressure drops below 0.667 kPa (5 Torr), unrestricted evacuation is permitted. In the absence of automated evacuation control, a slower temperature ramp (e.g., 1 °C per minute) is recommended. Hold the sample temperature at the first set point (110 °C) for approximately ten minutes. Then raise the sample temperature to 200 °C at a rate not to exceed 10 °C per minute, and hold at the maximum set point temperature *for at least 2 hours and no more than 4 hours*. After completion of outgassing, turn off the heating mantle, backfill with nitrogen gas, and allow the sample to cool to room temperature. If helium is used for backfill instead of nitrogen, a gravimetric error of order 3 % to 5 % can be expected due to the density difference between helium and air; this error can be reduced by using a fill rod or increasing the sample volume (i.e., reducing the dead space), but it is preferable to backfill with nitrogen.

Analysis Procedure: Sample analysis should be initiated as soon as possible following completion of outgassing and weighing operations. Follow the instrument manufacturer's recommendations or accepted practice to measure BET specific surface area using nitrogen gas as the adsorptive at liquid nitrogen temperature. The certified values were determined using a discontinuous manometric method as described in ISO 9277 [6]; use of other methods may result in values that deviate beyond the certified uncertainty range. The following parameters should be used for measurements. For multi-point analysis, choose at least four measurement points evenly distributed over the relative pressure (p/p_0) range from 0.05 to 0.3 (where p and p_0 are the equilibrium and the saturation pressure of the adsorptive, respectively). The intercept for the linear BET plot must be positive and the correlation coefficient (r^2) for linear regression must be at least 0.999. For single-point analysis, use a p/p_0 value close to but not exceeding 0.3. The value 0.162 nm^2 must be used for the molecular cross-sectional area of adsorbed nitrogen gas. The purity of helium gas used to calibrate measurement volumes (e.g., free space determination) should be at least 99.999 %. The purity of the adsorptive nitrogen gas should be at least 99.99 %. The liquid nitrogen level should be maintained at least 50 mm above the sample and constant to within 1 mm. The analysis procedure should include a leak test.

INFORMATION VALUES

Additional measurements and data were obtained to further characterize the material and are provided as information values. An information value is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value or only a limited number of analyses were performed [1].

Interlaboratory Study: An interlaboratory study (ILS) was conducted under the auspices of the Versailles Project on Advanced Materials and Standards (VAMAS) Technical Working Area 34 on Nanoparticle Populations. The stated objective of the ILS was to assess the "real world" between-laboratory precision for determination of the BET specific surface area of an industrially-relevant metal-oxide nanomaterial in powder form; the test material was SRM 1898 and a uniform protocol was provided to each participant. The study included 20 participants representing 19 organizations including academic, commercial, and government laboratories (see Appendix A), having a range of self-identified expertise in the application of gas sorption analysis and using a range of commercial and in-house built instrumentation. The VAMAS report is available upon request [7]. Figure 1 summarizes the multi-point BET results for each laboratory.

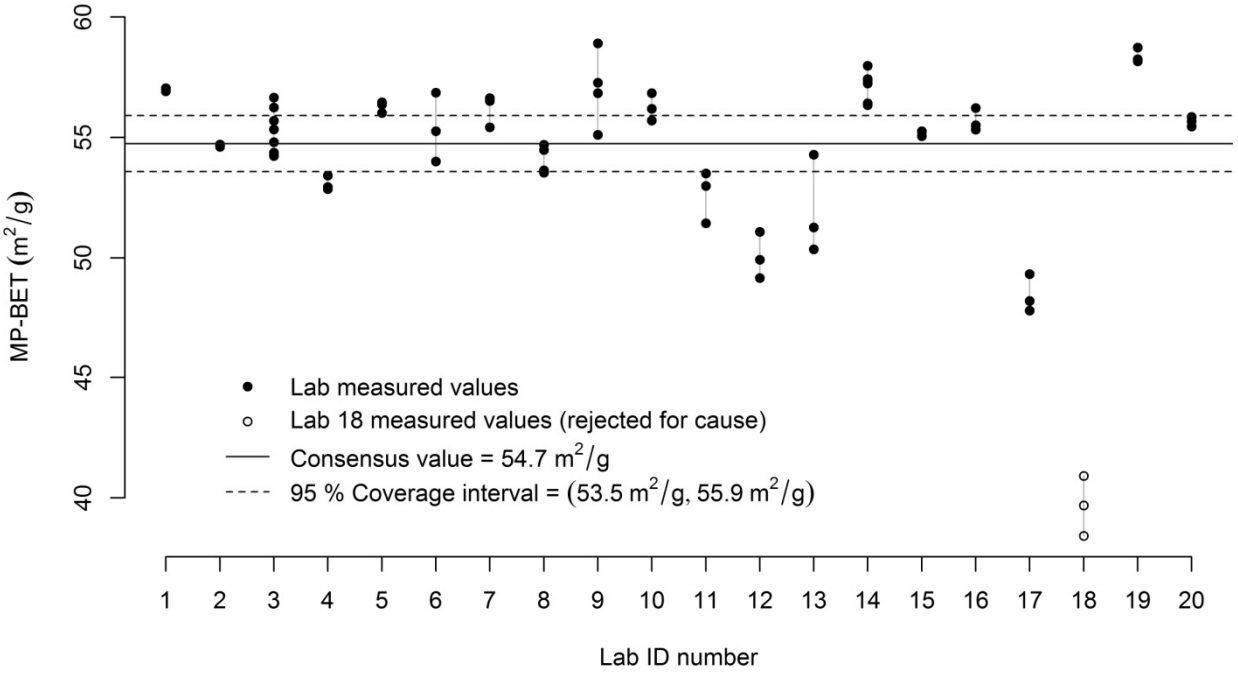

Figure 1. Values of the multipoint BET specific surface area measured by all reporting laboratories, which are designated by numbers that were randomly assigned to them. The solid line represents the consensus value (54.7 m^2/g) estimated using a linear, Gaussian mixed effects model [4] fitted to the measured values except those from laboratory 18 (open circles), which were set aside because the corresponding instrument was found to be defective. Dashed lines indicate an approximate 95 % confidence interval for the measurand (±1.2 m^2/g): this considerably greater expanded uncertainty compared with Table 1 (associated with the corresponding certified value) reflects the community's state-of-the-art in measuring this measurand. The variability between laboratories is about 3 times larger than within laboratories.

X-Ray Diffraction (XRD) Measurements: Diffraction patterns were collected with a commercial powder diffractometer using Cu $K\alpha_1$ radiation and a position-sensitive detector. The instrument operates in Bragg-Brentano configuration. Powder samples were pressed into disc sample holders 25 mm in diameter and 4 mm deep, and were rotated at 0.83776 rad/s (8 rpm, 0.133 Hz) during measurement. Scans were performed with a step size of 0.006° over a 2θ range from 10° to 150°. The diffraction pattern from SRM 660b (LaB_6) was recorded under identical measurement conditions to determine the instrumental profile parameters. Rietveld refinement was performed using the General Structure Analysis System (GSAS) software package [8]. All observed reflections were accounted for by a two-phase mixture of the polymorphs anatase and rutile, yielding relative phase fractions as shown in Table 2. Crystallite size (see Table 3) was estimated based on line-broadening with corrections for instrumental and strain-induced broadening, using double-Voigt and simplified integral-breadth methods as implemented in the software package Breadth [9].

Table 2. Information Values for Relative Phase Fractions Determined by Rietveld Refinement of Two-Phase Mixture

Phase	Relative Fraction[(a)]
Anatase	0.76 ± 0.03
Rutile	0.24 ± 0.03

[(a)] The reported uncertainty represents an expanded uncertainty (approximating a 95 % confidence interval, with $k = 3.2$ [2]) of four analyses conducted on test samples from four randomly selected units of SRM 1898.

Table 3. Information Values for Average Volume-Weighted Crystallite Size Based on Analysis of Multiple Reflections[(a,b)]

Phase	Size (nm)
Anatase	19 ± 2
Rutile	37 ± 6

[(a)] The expanded uncertainty stated for anatase expresses the dispersion of values obtained by four different analytical methods and for rutile it expresses the variability of values between four different units of material, but both are only indicative of the range to be expected for 95 % probability intervals for these measurands.

[(b)] For the purpose of comparison, the widely reported method based on the Scherrer equation and analysis of a single characteristic reflection (200 for anatase and 111 for rutile), and using the *uncorrected* full width at half maximum (FWHM) with the Scherrer constant (K) set to unity, yields the following mean values obtained from analysis of four randomly selected SRM 1898 units: anatase (23.6 nm) and rutile (44.1 nm).

Principal Element and Impurity Analysis: Chemical composition analyses were performed to provide further characterization of the material. Inductively coupled plasma optical emission spectrometry (ICP-OES) and X-ray fluorescence (XRF) spectrometry were performed at NIST. For the ICP-OES analysis, the material was digested in a closed vessel containing nitric and hydrofluoric acids using a microwave digestion system, and the resulting solutions were analyzed with the standard addition calibration method. For the XRF analysis, the material was fused using a mixture of lithium metaborate and lithium tetraborate to form glass beads that were analyzed directly with a wavelength-dispersive instrument. Analytical data were also provided by two collaborating laboratories. Specifically, glow discharge mass spectrometry (GDMS) was performed by Shiva Technologies (Syracuse, NY), and inert gas fusion (IGF) analysis and combustion analysis with infrared absorption detection were performed by Luvak, Inc. (Boylston, MA).

Table 4. Information Values for Elemental Purity and Principal Component Fractions Obtained From Chemical Analysis (Dry-Mass Basis)

Measurand	Mass Fraction Value[a,b] (g/g)	Coverage Factor, *k*
Elemental purity[c,d]	0.994 ± 0.001	2.0
Titanium mass fraction[e]	0.60 ± 0.02	2.0
Oxygen mass fraction[f]	0.40 ± 0.02	2.0
	(mg/g)	
Chlorine mass fraction [h]	0.96 ± 0.04	2.3

[a] All values pertain to the state of the material after drying for 2 h at 105 °C. There is some evidence that a small amount of water might be retained in the material after this drying procedure. However, any biases that might result are expected to be well within the stated uncertainty intervals.

[b] All uncertainties are stated as symmetric expanded uncertainty intervals with a level of confidence of 95 %. All uncertainties were estimated in accordance with the ISO Guides [2,3], except where noted.

[c] Calculated by subtracting from unity the sum of the mass fractions of all detectable impurity elements and half the limits of detection of all undetectable impurity elements, where the material was analyzed using combustion analysis with infrared absorption detection, GDMS, ICP-OES, IGF analysis, and XRF spectrometry.

[d] The presence of carbon, hydrogen, and nitrogen account for 82.9 % of the sum of the mass fraction values of all detected elemental impurities, while the presence of chlorine accounts for 16.7 %. Of the remaining 0.4 %, the principal detected elemental impurities are silicon (5 μg/g), sodium (4 μg/g), sulfur (4 μg/g), antimony (3 μg/g), fluorine (2 μg/g), niobium (1 μg/g), iron (1 μg/g), aluminum (1 μg/g), and nickel (1 μg/g).

[e] Calculated as the weighted mean of the titanium mass fraction values obtained through XRF spectrometry and ICP-OES [10,11]. The expanded uncertainty was evaluated in accordance with Supplement 1 to the ISO Guide [3].

[f] Determined using IGF analysis.

[g] Determined using XRF spectrometry.

Particle Morphology: Transmission electron microscopy (TEM) and field-emission scanning electron microscopy (FE-SEM) were used for qualitative characterization of structural elements associated with SRM 1898. There are three principal levels of structure, beginning with nanoscale crystallites that fuse to form "hard" nanoscale aggregates, which in turn associate to form microscale agglomerates. In the dry form, the agglomerates associate to yield a powder with macroscopic consistency.

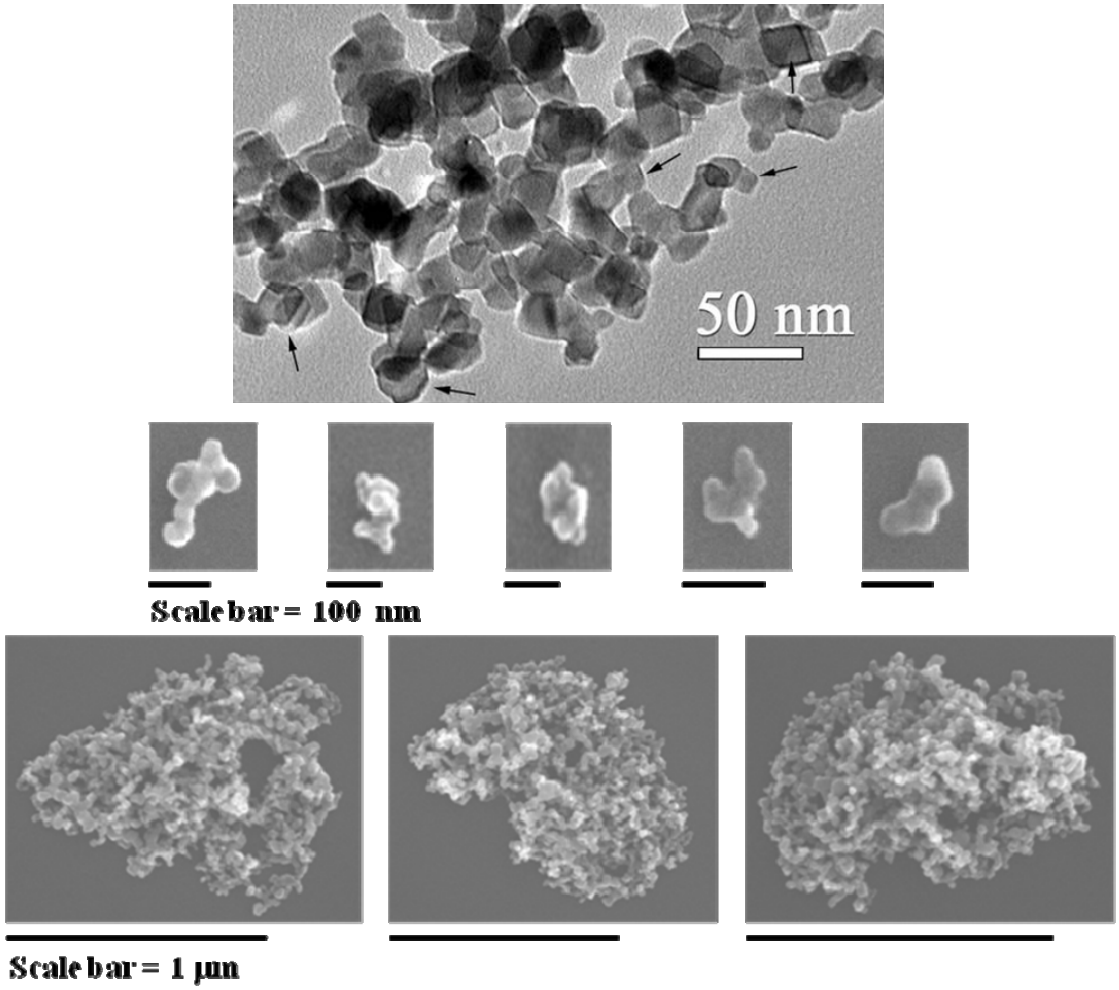

Figure 2. Three levels of structure associated with SRM 1898. Top: TEM image showing nanoscale polycrystalline structure; arrows point to individual crystallites (courtesy C. Impellitteri, National Risk Management Research Laboratory, Environmental Protection Agency). Middle: FE-SEM images of representative nanoscale aggregates of crystallites obtained following dispersion in an aqueous suspension. Bottom: FE-SEM images of representative microscale agglomerates present in poorly dispersed aqueous suspensions. Aggregates and agglomerates were electrostatically deposited from solution onto a silicon substrate with a native oxide layer.

Surface Charge Behavior: The electrophoretic mobility, zeta potential, and isoelectric point (IEP) of SRM 1898 dispersed in aqueous solution at an ionic strength of 10^{-3} mol L^{-1} was determined using phase analysis light scattering. Optimized suspensions of SRM 1898 in deionized water were diluted in a 1:40 ratio into the test media prior to titration. Zeta potential was calculated from mobility using the Smoluchowski equation, which assumes thin double-layer conditions and may not be strictly applicable to particles with a relatively thick electrical double-layer and small diameter; however, the absolute magnitude of zeta potential is not required to determine the IEP. Furthermore, the Smoluchowski value is commonly reported in the literature and is a default setting on many commercial instruments; therefore, Smoluchowski-derived values are reported for comparative purposes only.

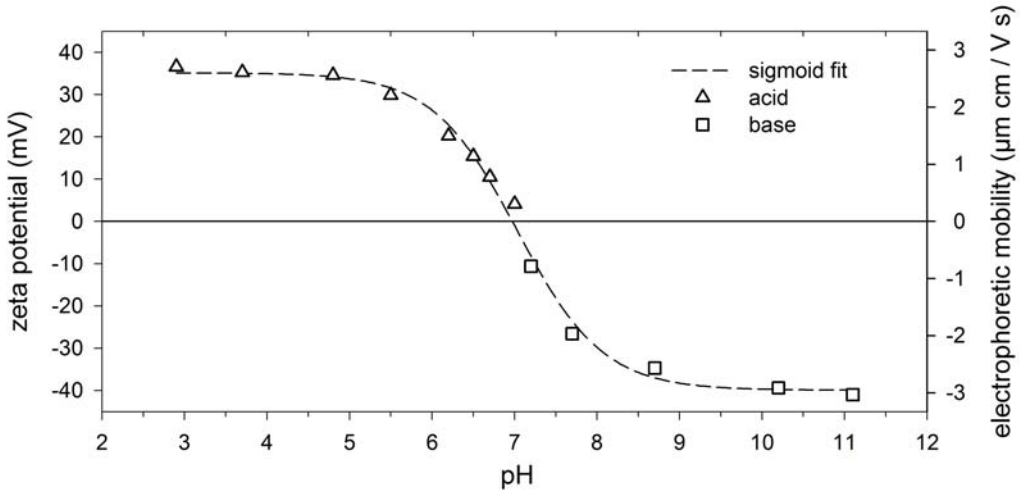

Figure 3. Acid-to-basic (Δ) and basic-to-acid (□) titrations of SRM 1898 suspensions. The five-parameter sigmoidal fit yields an isoelectric point at pH 7.0.

Dispersion Properties: For many environmental and biological testing scenarios, manufactured nanomaterials are initially dispersed into an aqueous phase. This can be facilitated by application of ultrasonic energy under controlled conditions, and by systematic optimization of the dispersion procedure [12]. Figure 4 shows the effect of sonication on the dispersal of SRM 1898 in deionized water as characterized by laser diffraction spectrometry (LDS). Without sonication most of the material exists in a highly agglomerated form. Sonication leads to fragmentation of agglomerates and the formation of a stable nanoscale population. Figure 5 shows the effect of sonication time on the volume fraction of agglomerates and the particle size of the nanoscale component. By optimizing the dispersion procedure, agglomerates can be completely eliminated, yielding a monomodal nanoscale dispersion. Table 5 shows a comparison of characteristic volume-weighted size parameters (D_m, D_{10} and D_{90})[(2)] determined using three measurement techniques, for optimized dispersion of SRM 1898 in deionized water.

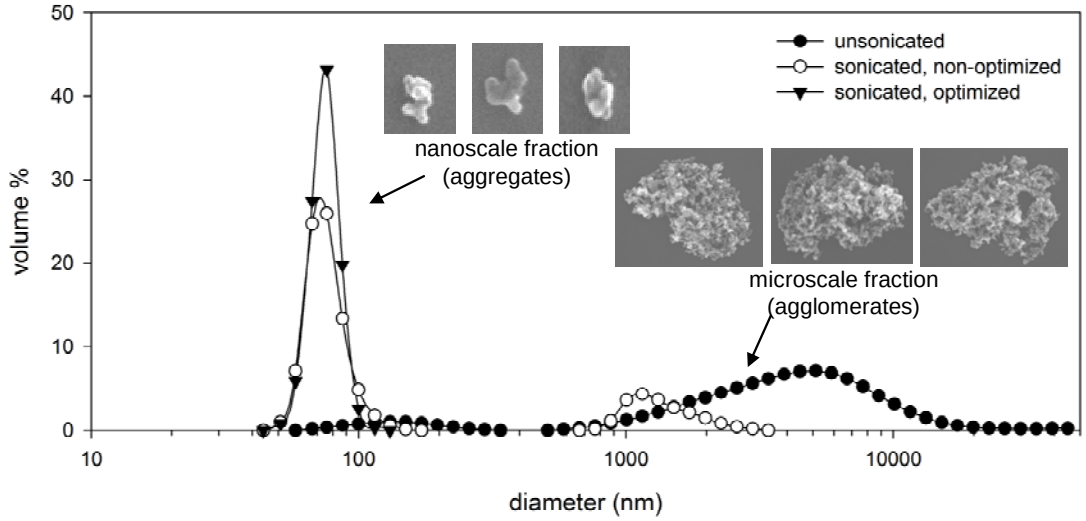

Figure 4. LDS-derived particle size distributions for SRM 1898 suspensions prepared in deionized water. FE-SEM inset images are typical for characteristic particle structures in the nanoscale and microscale fractions. The characteristic particle size parameters measured by LDS are relatively constant over the range of validated TiO_2 concentrations (see Table 6). Notably, due to residual acidity associated with the powder, the suspension pH decreases with increasing concentration. Since the IEP is at pH 7, the particles can be stabilized electrostatically in acidic media at low ionic strength.

[(2)] D_m is the peak mean diameter; D_{10} is the cumulative size distribution diameter below which 10 % of the volume lies, and D_{90} is the diameter below which 90 % of the volume lies.

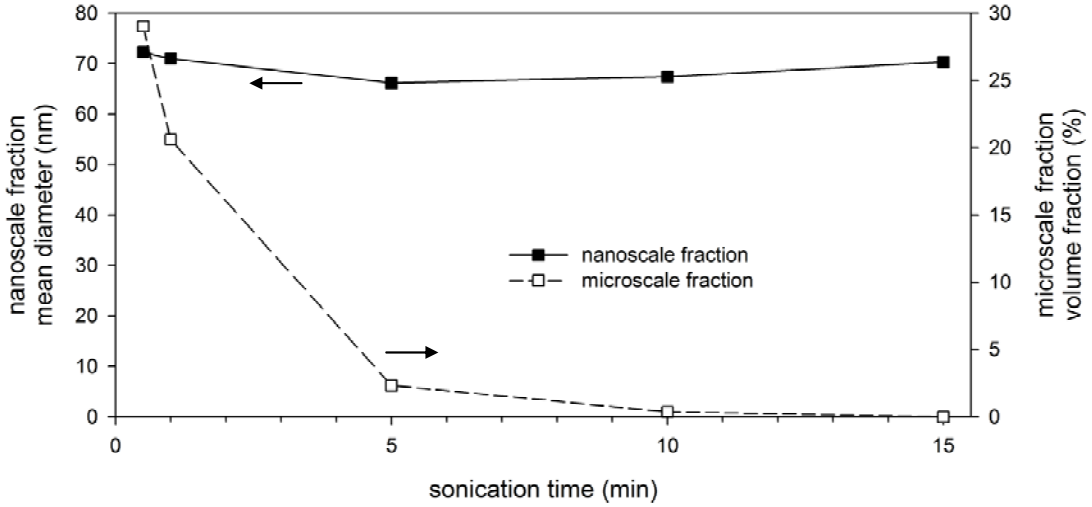

Figure 5. Nanoscale fraction mean diameter and microscale fraction relative volume fraction for SRM 1898 dispersed in deionized water as a function of sonication time at a delivered power of approximately 50 W. A combination of 50 W (calibrated delivered energy determined according to reference 12) and 15 min total sonication time (pulse mode operation) yielded optimum dispersion with elimination of microscale agglomerates.

Table 5. Informational Comparison of Particle Size Parameters by Technique for Optimized Dispersion of SRM 1898 in Deionized Water at 10 mg/mL[(a,b)]

Measurement Technique	D_m (nm)	D_{10} (nm)	D_{90} (nm)
Laser Diffraction Spectrometry	71 ± 4	59 ± 2	84 ± 5
X-Ray Disc Centrifugation	77 ± 7	32 ± 22	119 ± 23
Dynamic Light Scattering	112 ± 4	68 ± 8	151 ± 4

[(a)] Measurement of three independent dispersions obtained from a single unit of SRM 1898; uncertainty represents a 95 % confidence interval (with $k = 4.3$) derived from replicate experiments.
[(b)] Initial suspension was diluted in deionized water to achieve optimal concentration for LDS and DLS measurements.

Table 6. Informational pH Values and Particle Size Parameters (Laser Diffraction) for Optimized Dispersion of SRM 1898 in Deionized Water as a Function of Particle Concentration.[(a)] Values Represent Typical Results Obtained under Optimized Conditions.

Concentration (mg/mL)	pH	D_m (nm)	D_{10} (nm)	D_{90} (nm)
0.5	4.9	70	59	82
1	4.7	72	59	85
10	3.9	74	60	87
20	3.7	72	60	85

[(a)] Dispersion optimization followed [12,13]; see protocol for dispersion in deionized water summarized in Appendix B.

Dispersion Protocols: In order to assess the environmental and health hazards associated with nanomaterial powders during their life-cycle, the dry powder should first be dispersed into an appropriate aqueous phase to facilitate particle deagglomeration and dosing. In order to achieve maximum dispersion and sufficient temporal stability, within the context of biological assays, dispersion protocols have been developed and validated by NIST such that SRM 1898 can be used as a reproducible benchmark in these assays. Protocols are summarized in Appendix B for the generation and optimization of SRM 1898 dispersions in (1) deionized (DI) water, (2) phosphate-buffered saline (PBS), (3) Dulbecco's modified Eagle's medium containing a volume fraction of 10 % fetal bovine serum (DMEM-FBS), and (4) reconstituted hard water[(3)]. In practice, these procedures yield stabilized,

[(3)]Preparation of reconstituted hard water is summarized in Appendix B.

nanoscale dispersions with complete disruption of microscale agglomerates. The stable nanoscale species is a monomodal aggregate of fused crystallites (see Figures 2 and 4) with a modal size of order 70 nm. A transferrable, calorimetric calibration procedure for the application of ultrasonic disruption is utilized in the implementation of these protocols; full details of the calibration procedure are provided in [12]. Table 7 summarizes the mean particle size and pH values obtained from validation tests performed using the described protocols (for details, refer to Appendix B)

Table 7. Informational Mean Particle Size (D_m) and pH Values for SRM 1898 Suspensions Produced Using Dispersion Protocols for Deionized Water, PBS, DMEM-FBS, and Reconstituted Hard Water[(a)]

Medium	LDS D_m (nm)	DLS D_m (nm)	pH	TiO_2 Concentration (mg/mL)
DI Water	71 ± 4	112 ± 4	3.9	10
PBS	75 ± 4	136 ± 13	7.4	0.1
DMEM-FBS	83 ± 2	154 ± 16	7.8	0.1
Hard Water	76 ± 5	124 ± 3	7.0	0.1

[(a)] The uncertainty (approximating a 95 % confidence interval with $k = 4.3$) is derived from three replicate experiments following the optimized protocol procedures.

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at http://www.nist.gov/srm/publications.cfm (accessed June 2012).

[2] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement* (ISO GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed June 2012); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/index.cfm (accessed June 2012).

[3] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the Guide to Expression of Uncertainty in Measurement; Propagation of Distributions Using a Monte Carlo Method*; Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed June 2012).

[4] Searle, S.R.; Casella, G.; McCulloch, C.E.; *Variance Components;* John Wiley & Sons, Inc.: New York (1992).

[5] NIOSH; Approaches to Safe Nanotechnology: Managing the Health and Safety Concerns Associated with Engineered Nanomaterials; NIOSH Publication 2009-128; available http://www.cdc.gov/niosh/docs/2009-125/ (accessed June 2012); see also Occupational Exposure to Titanium Dioxide; NIOSH Current Intelligence Bulletin 63; available at http://www.cdc.gov/niosh/docs/2011-160/pdfs/2011-160.pdf (accessed June 2012).

[6] ISO 9277:2010; *Determination of the Specific Surface Area of Solids by Gas Adsorption — BET Method;* International Organization for Standardization: Geneva, Switzerland (2010).

[7] Hackley, V.A.; *Interlaboratory Study for the Measurement of BET-Specific Surface Area Using an Industrially Relevant TiO_2 Nanopowder*. VAMAS TWA-34 Project Report (2011); available upon request.

[8] Larson, A.C.; Von Dreele, R.B.; *General Structure Analysis System GSAS*, Los Alamos National Laboratory Report, LAUR 86-748 (2004).

[9] Balzar, D.; *BREADTH - A Program for Analyzing Diffraction Line Broadening*, J. Appl. Cryst., Vol. 28, pp. 244-245 (1995).

[10] Rukhin, A.L.; *Weighted Means Statistics in Interlaboratory Studies*; Metrologia, Vol. 46, pp. 323-331 (2009).

[11] DerSimonian, R.; Laird, N.; *Meta Analysis in Clinical Trials*; Control. Clin. Trials, Vol. **7,** pp. 177-188 (1986).

[12] Taurozzi, J.S.; Hackley, V.A.; Wiesner, M.R.; *Preparation of Nanoparticle Dispersions from Powdered Material Using Ultrasonic Disruption;* NIST Special Publication 1200-2 (2012); available at http://www.nist.gov/mml/np-measurement-protocols.cfm (accessed June 2012); see also Taurozzi, J.S.; Hackley, V.A.; Wiesner, M.R.; *Ultrasonic Dispersion of Nanoparticles for Environmental, Health and Safety Assessment – Issues and Recommendations*; Nanotoxicol., Vol. 5, pp. 711-729 (2010).

[13] Taurozzi, J.S.; Hackley, V.A.; Wiesner, M.R.; *Preparation of a Nanoscale TiO_2 Aqueous Dispersion for Toxicological or Environmental Testing*; NIST Special Publication 1200-3 (2012); available at http://www.nist.gov/mml/np-measurement-protocols.cfm (accessed June 2012).
[14] Taurozzi, J.S., Hackley, V.A. and Wiesner, M.R.; *Preparation of Nanoscale TiO_2 Dispersions in Biological Test Media for Toxicological Assessment*; NIST Special Publication 1200-4 (2012); available at http://www.nist.gov/mml/np-measurement-protocols.cfm (accessed June 2012).
[15] ISO 6341:1996; *Water Quality – Determination of the Inhibition of the Mobility of Daphnia magna Straus (Cladocera Crustacea) – Acute Toxicity Test;* International Organization for Standardization: Geneva, Switzerland (1996).
[16] OECD 202; *Daphnia sp., Acute Immobilisation Test and Reproduction Test*; OECD Guideline for Testing of Chemicals; Organization for Economic Cooperation and Development: Paris (adopted 1984).
[17] Taurozzi, J.S.; Hackley, V.A.; Wiesner, M.R.; *Preparation of Nanoscale TiO_2 Dispersions in an Environmental Matrix for Eco-Toxicological Assessment;* NIST Special Publication 1200-5 (2012); available at http://www.nist.gov/mml/np-measurement-protocols.cfm (accessed June 2012).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

The laboratories listed below participated in the interlaboratory study:

AAIPharma, Wilmington, NC, USA
BEL Japan Inc., Osaka, Japan
Delta Analytical Instruments, Inc., North Huntingdon, PA, USA
Dupont, Wilmington, DE, USA
Environmental Protection Agency, National Risk Management Research Laboratory, Cincinnati, OH, USA
Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany
Food and Drug Administration, National Center for Toxicological Research, Center for Phototoxicology, Jefferson, AR, USA
Hiden Isochema, Warrington, UK
Horiba Instruments Inc., Irvine, CA, USA
Particulate Technology Laboratory, TLIRI, National Taiwan University, Taiwan
Korea Research Institute of Standards and Science, Republic of Korea
Micromeritics Instrument Corporation, Norcross, GA, USA
National Institute for Occupational Safety and Health, Morgantown, WV, USA
National Institute of Standards and Technology, Engineering Laboratory, Gaithersburg, MD, USA
National Physical Laboratory, Teddington, UK
Quantachrome Corporation, Boynton Beach, FL, USA
University of Cincinnati, Cincinnati, OH, USA
University of Iowa, Department of Chemistry, Iowa City, IA, USA
University of Massachusetts, Lowell, MA, USA

APPENDIX B

Dispersion Protocols.

Table B1. Protocol for Dispersion in Deionized Water[a,b]

Step	Instructions
1	Using an analytical balance and a weighing dish, add an adequate mass of powder to achieve the desired concentration in a 50 mL suspension volume; this protocol has been validated for concentrations from 0.5 to 20 mg/mL
2	Add the weighed mass of powder to a 100 mL (approximately 5 cm diameter) cylindrical glass beaker. Add 50 mL of deionized water to the beaker containing the powder.
3	Place the beaker in a glass dish of sufficient size to accommodate the sample beaker and an ice bath solution, and secure the beaker in the center of the dish by use of clamps, etc., to ensure that the beaker remains in place during sonication[c].
4	Fill the glass dish with enough water and ice to allow the ice water bath level to encase the beaker to approximately the level of the solution contained in the beaker.
5	Immerse a standard 1.27 cm (0.5 inch) diameter titanium sonicator horn (probe) 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, as this could cause the beaker to shatter during sonication.
6	Select a sonicator setting that yields a delivered power of approximately 50 W; this requires prior calibration using the calorimetric procedure described in [12].
7	Operate the sonicator at this power level for 15 min, using an 80 % pulsed operation mode (80 % on / 20 % off during each second of operation), or similar on/off time sequence.
8	After sonication is completed, transfer the aqueous dispersion to a storage container (e.g., amber bottle) and store protected from UV light at ambient temperature until further use. Do not refrigerate.
9	The resulting suspension should have an opaque white appearance.

[a] This is a protocol summary; for complete details refer to the open-source published protocol [13].

[b] Type I biological grade deionized water with ≥18 MΩ·cm resistivity is recommended; biological grade implies sterility and absence of endotoxin contamination. Commercially available pyrogen-free water may be used.

[c] Probe-type sonicator with a standard 1.27 cm (0.5 inch) diameter titanium horn fitted with a removable flat tip (or similar ultrasonic device) is required. Bath sonicators do not provide sufficient energy.

Table B2. Protocol for Dispersion in Phosphate-Buffered Saline[a]

Step	Instructions
1	Prepare a 10 mg/mL stock SRM 1898 aqueous dispersion in biological grade deionized (DI) water according to the protocol described above or refer to reference 13.
2	Prepare 50 mL of phosphate-buffered saline (PBS) (1x) by dilution of 10x Ca/Mg-free PBS with DI water, i.e., add 5 mL of PBS (10x) to 45 mL of DI water. Measure the PBS (1x) pH and adjust if necessary to a value between 7.2 and 7.4 by addition of 0.1 mol/L HCl or NaOH as needed. Mix thoroughly after each acid or base addition step to allow for proper homogenization and attainment of equilibrium pH.
3	Weigh out 0.8 g of reagent-grade (lipid- and IgG-free) bovine serum albumin (BSA) powder and transfer to a 10 mL glass vial. Add 10 mL of DI water to the vial, seal, and gently shake to allow for complete dissolution of BSA; do not use until the solution is completely transparent (allow approximately 1 h). The final product contains a BSA concentration of 80 mg/mL.
4	Add 300 µL of the 80 mg/mL BSA stock into a clean 10 mL glass vial.
5	Add 150 µL of the 10 mg/mL stock SRM 1898 dispersion into the vial containing 300 µL of BSA stock.
6	Add 14.5 mL of the PBS (1x) prepared in step 2 and 50 µL of PBS (10x concentrate) to a 30 mL amber glass vial.
7	Using an adjustable-volume pipette, transfer 450 µL of the TiO_2/BSA mixture obtained in step 5 into the 30 mL amber vial containing 14.55 mL of PBS solution from step 6 to yield a dispersion containing 100 µg/mL TiO_2 and 1.6 mg/mL BSA in PBS (1x). The pH of the resulting dispersion should be comparable to that of the original buffer medium (nominally 7.4).
8	For toxicity assays, the user is advised to conduct a control for BSA alone in the test medium (1.6 mg/mL), without SRM 1898 present; to maintain the same dilution factor, 150 µL of DI water can be added in step 5 in place of the stock dispersion
9	The resulting dispersion, stored in the 30 mL amber vial, retains its particle size distribution for at least 48 h at room temperature; stability has been validated only in the dispersion media, without the presence of cells or other added components. The dispersion in PBS should have a white but translucent appearance.

[a] This is a protocol summary; for complete details refer to the open-source published protocol [14].

Table B3. Protocol for Dispersion in Dulbecco's Modified Eagle's Medium Containing 10 % Fetal Bovine Serum (DMEM-FBS)[(a)]

Step	Instructions
1	Prepare a 10 mg/mL stock SRM 1898 aqueous dispersion in biological grade deionized (DI) water according to the protocol described previously or refer to reference 13.
2	Prepare 50 mL of DMEM-FBS by mixing 5 mL of FBS with 45 mL of DMEM (4.5 g/L glucose and sodium pyruvate without L-glutamine and phenol red). The resulting pH should be approximately 7.8.
3	Weigh out 0.8 g of reagent grade (lipid and IgG free) bovine serum albumin (BSA) powder and transfer to a 10 mL glass vial. Add 10 mL of DI water to the vial, seal and gently shake to allow for complete dissolution of BSA; do not use until the solution is completely transparent (allow approximately 1 h). The final product contains a BSA concentration of 80 mg/mL BSA.
4	Using an adjustable pipette add 18.75 µL of the 80 mg/mL BSA stock from step 3 into a clean 10 mL glass vial.
5	Add 150 µL of the 10 mg/mL stock SRM 1898 dispersion into the vial containing 18.75 µL of BSA stock.
6	Using an adjustable pipette add 14.83 mL of the DMEM-FBS prepared in step 2 to a 30 mL amber glass vial.
7	Using an adjustable pipette, transfer 168.75 µL of the TiO_2/BSA mixture obtained in step 5 into the 30 mL amber vial containing 14.83 mL of DMEM-FBS from step 6, to yield a dispersion containing 100 µg/mL TiO_2 and 100 µg/mL BSA in DMEM-FBS. The pH of the resulting suspension should be comparable to that of the original medium prepared in step 2. This procedure results in a 1.1 % dilution of the DMEM-FBS.
8	For toxicity assays, the user is advised to conduct control tests for 100 µg/mL BSA in the diluted test medium, without SRM 1898 present; to maintain the same dilution factor, 150 µL of DI water can be added in place of the stock dispersion.
9	The resulting dispersion, stored in a 30 mL amber glass vial, retains its particle size distribution and pH (±0.1 units) for at least 48 h under relevant incubation conditions (37 ºC, 90 % humidity, 5 % CO_2 atmosphere). The suspension should have a white but translucent appearance.

[(a)] This is a protocol summary; for complete details refer to the original published protocol [14].

Table B4. Protocol for Dispersion in Reconstituted Hard Water[a]

Step	Instructions
1	Prepare stock solutions as follows [15]: Stock A: Dissolve 0.23 g KCl in 1 L of deionized (DI) water Stock B: Dissolve 2.59 g $NaHCO_3$ in 1 L of DI water Stock C: Dissolve 4.93 g $MgSO_4 \cdot 7H_2O$ in 1 L of DI water Stock D: Dissolve 11.76 g $CaCl_2 \cdot 2H_2O$ in 1 L of DI water
2	Prepare 20 mL of 100 mg/L humic acid (HA) solution, by adding 0.002 g of HA and 20 mL of DI water in a 20 mL glass vial. After adding both components, allow the solution to equilibrate for 48 h. The solution should have a pH of 4.0 ± 0.2 after equilibration. After allowing solution to equilibrate for 48 h, proceed to the next step.
3	Prepare 50 mL of 200 µg/mL SRM 1898 aqueous nanoparticle dispersion, by adding 0.01 g of SRM 1898 into 50 mL of DI water, and following the sonication procedure prescribed in reference 12.
4	In a 30 mL amber glass vial, add 3 mL of the HA solution, then 7.5 mL of SRM 1898 stock prepared in step 3, and then 3.46 mL of DI water.
5	Next, into the mixture prepared in step 4, add 0.26 mL each of Stocks A, B, C and D, in the order listed.
6	This procedure yields a dispersion containing 100 µg/mL TiO_2 and 20 mg/L HA in reconstituted hard water with a hardness of approximately 170 mg/L as $CaCO_3$; the test medium is compliant with OECD 202 [16]. The dispersion should have a white but translucent appearance, and should be stable for up to 96 h with respect to the particle size distribution.
7	The pH after preparation should be approximately 7.0. After 48 to 96 h, an increase of 0.3 to 0.7 pH units may be expected; however, dispersions should remain within the OECD 202 recommended pH range of 6 to 9.
8	If intended for toxicological assessment, the user is advised to conduct separate control tests for HA (20 mg/L) in the test medium (in the absence of SRM 1898).

[a] This is a protocol summary; for complete details refer to the open-source published protocol [17].