Reference Material® 8027

Silicon Nanoparticles

(Nominal Diameter 2 nm)

REFERENCE MATERIAL INFORMATION SHEET

Purpose: The non-certified values delivered by this Reference Material (RM) are intended primarily to evaluate and qualify methodology and/or instrument performance related to the physical/dimensional characterization of nanoscale particles. The RM may also be useful in the development of in vitro assays designed to evaluate the biological response of nanomaterials and for use in interlaboratory test comparisons.

Description: A unit of RM 8027 consists of five hermetically sealed pre-scored glass ampoules containing nominally 1 mL of cyclohexane-stabilized silicon (Si) nanoparticles suspended in toluene. The suspension contains particles (monomers) and a small percentage of clusters of primary particles.

Non-Certified Values: Non-certified mean size and mass fraction values derived from Dynamic Light Scattering (DLS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) measurements respectively, are provided in Table 1. Non-certified values that are the present best estimates of the true values; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient agreement among multiple analytical methods [1].

Table 1. Non-Certified Values for RM 8027[a,b]

Measurement	Value	Technique
Particle Size (nm)	2.20 ± 0.14	DLS
Si Mass Fraction (µg/g)	6.43 ± 0.31	ICP-OES

[a] Liquid suspension form.
[b] The measurand in each case is determined by the methods indicated in the text.

Additional Information: Values of potential interest to users and additional information are provided in Appendix A.

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **10 June 2025.** The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Reference Material Information Sheet and notify registered users. RM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the RM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

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Information Sheet Revision History on Page 2

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Safety: Consult the Safety Data Sheet (SDS) for hazard information. RM 8027 is NOT intended for clinical use or human consumption. NO SONICATION should be applied to this reference material. Appearance of a milky substance indicates that the RM has been compromised.

Handling and Storage: Until required for use, the RM should be stored at room temperature in its original ampoule and package, protected from intense direct light or ultraviolet radiation. Refrigeration below 0 ºC or heating above 40 ºC is discouraged. Ampoules are best stored long term in a horizontal position.

Use: Prior to opening, the glass ampoule containing the RM should be gently inverted several times to insure homogeneity. Liquid retained in the upper portion of the ampoule (the nipple), can be dislodged by gently flicking the nipple with forefinger while tilting the ampoule. The ampoule is pre-scored and should be opened by applying moderate pressure with one's thumb to snap off the nipple. It is recommended that the contents of an ampoule be used the same day as opened.

Value Assignment and Uncertainty Analysis: Analyses to establish non-certified values were conducted at NIST using best practices as determined independently for each measurement method. DLS non-certified values were calculated from the ampoule means and the expanded uncertainty was calculated as $U = k u_c$, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO/JCGM and NIST Guides [2,3]. The coverage factor $k = 2$, for 95 % expanded uncertainty intervals is based on a t multiplier with 29 associated degrees of freedom. For the Silicon mass fraction value using ICP-OES, components of uncertainty were determined according to the ISO/JCGM Guide [2]. For the uncertainty components estimated using Type A and Type B approaches, the effective degrees of freedom were calculated for each approach using the Welch-Satterthwaite formula and then combined. The expanded uncertainty (U) was obtained as follows:

$$U = k\sqrt{u_a + u_b} \text{ where } u_a = \sqrt{c_1^2 * s_{sample}^2 + c_2^2 * s_{blank}^2} \text{ and } u_b = \sqrt{c_3^2 * s_{std}^2 + c_4^2 * s_{w1}^2 + c_5^2 * s_{w2}^2}$$

where c is the sensitivity coefficient, k is the coverage factor, s_{sample}, s_{blank}, s_{std}, s_{w1} and s_{w2} are the standard uncertainties for the sample, blank, standard, standard weighing and sample weighing, respectively, and u_a and u_b are the combined uncertainties obtained using a Type A and Type B approach, respectively.

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication (NIST SP) 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Jun 2022).
[2] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at https://www.bipm.org/en/committees/jc/jcgm/publications (accessed Jun 2022).
[3] 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 https://www.nist.gov/pml/nist-technical-note-1297 (accessed Jun 2022).
[4] Rasband, W.; *ImageJ*; National Institutes of Health; available at http://rsb.info.nih.gov/ij/ (accessed Feb 2014).
[5] Nontapot, K.; Rastogi, V.; Fagan, J.A.; Reipa, V; *Size and Density Measurement of Core-Shell Si Nanoparticles by Analytical Ultracentrifugation*; Nanotechnology, Vol 24, p. 155701 (2013).
[6] ISO 13321 1996; *Particle Size Analysis - Photon Correlation Spectroscopy*; International Organization for Standardization: Geneva, Switzerland (1996).
[7] SRM 3150; *Silicon (Si) Standard Solution*; NIST; U.S. Department of Commerce: Gaithersburg, MD (19 November 2012).
[8] SRM 3139a; *Phosphorus (P) Standard Solution*; NIST; U.S. Department of Commerce: Gaithersburg, MD (29 August 2011).

Certain commercial equipment, instruments, or materials may be identified in this Reference Material Information Sheet 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.

Users of this RM should ensure that the Reference Material Information Sheet in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Values of potential interest to users: Particle mean size values using Transmission Electron Microscopy (TEM) and Analytical Ultracentrifugation (AUC) are provided in Table A1.

Table A1. Particle Mean Size for RM 8027[a]

Measurement	Particle Size (nm)	Analyte Form
TEM[a]	1.9	Dry, deposited on substrate
AUC[b]	1.7	Liquid suspension

[a] TEM images were analyzed using ImageJ [4] software package. The value is a simple mean of 560 Si particles imaged on the carbon grid.

[b] AUC measurements were performed in three solvents (toluene, chloroform, and deuterated toluene) and the simple mean hydrodynamic diameter value is provided. Particle effective density was estimated from the sedimentation coefficients in three above solvents [5].

Additional User Information: Figures A1 through A3 provide additional images and graphs that may be of interest to the RM user.

Optical Absorbance: The optical absorbance of RM 8027, diluted ten times in toluene, was recorded using a double-beam spectrophotometer and shown in Figure A1. Measurements were performed using matched quartz cuvettes (10 mm pathlength) against a filtered pure toluene. Scan conditions were bandwidth of 1.0 nm, an integration time of 0.08 s and a photomultiplier gain of 51, giving a source maximum at 510 nm that is 80 % of the maximum detection efficiency.
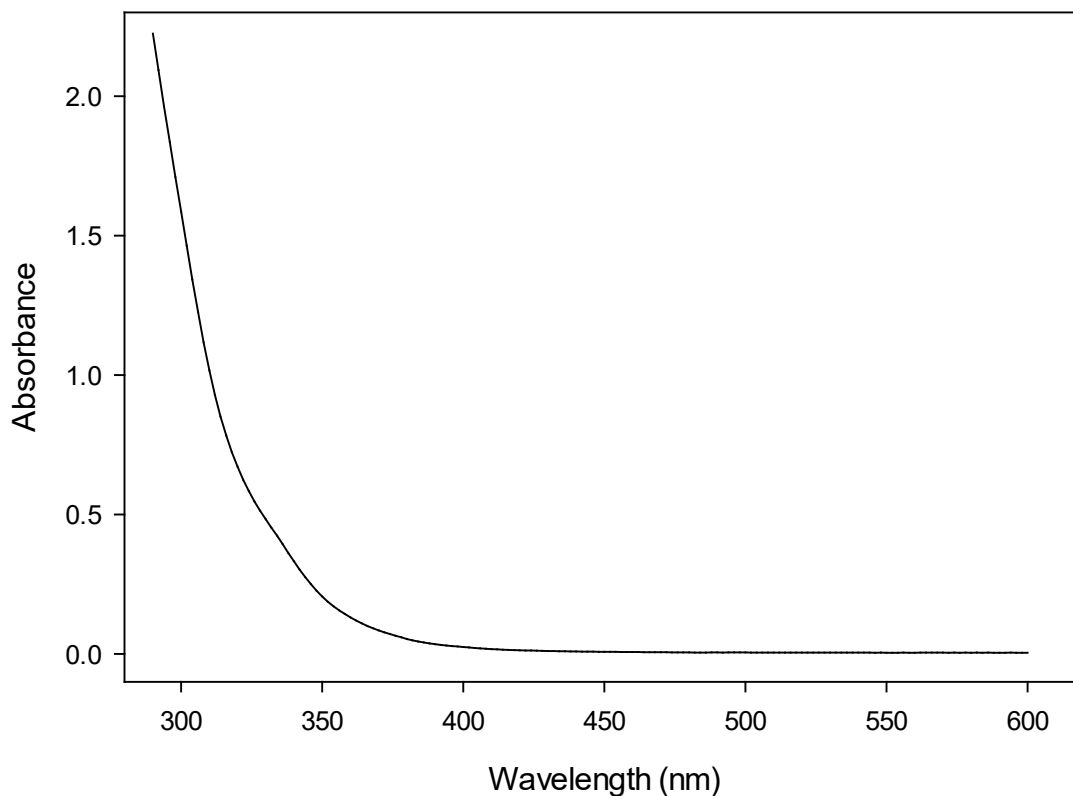

Figure A1. Representative optical absorbance spectrum for a diluted RM 8027 suspension.

TEM Imaging: TEM measures the projected image of particles deposited onto an electron-transparent substrate. Internal structure, as well as surface morphology, may contribute to the image appearance. Representative images are presented in Figure A2.
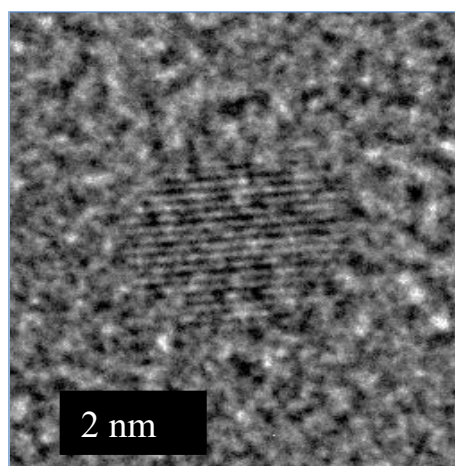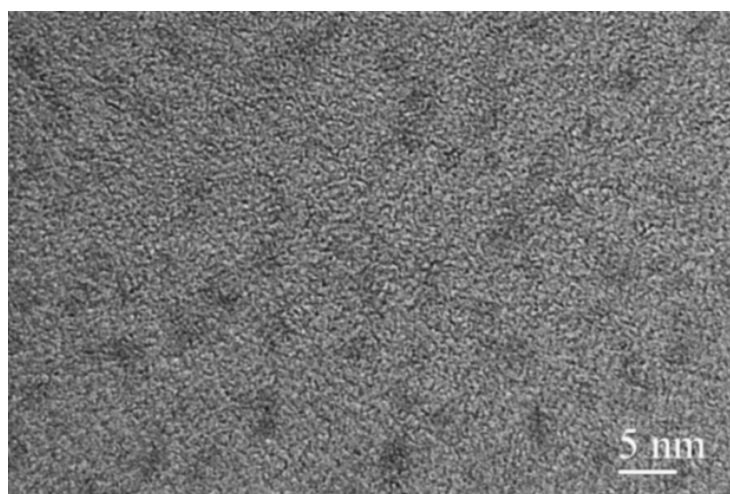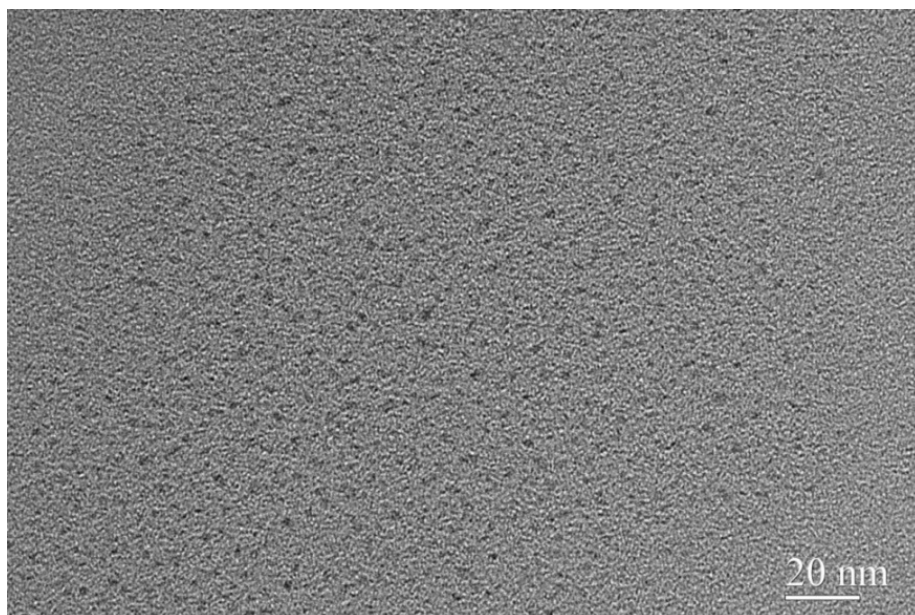

Figure A2. Si nanoparticles sampled from a single representative TEM image.
Bottom image shows a high resolution TEM image of a single Si nanoparticle.

Size Distribution Histogram: Particle size distribution generated by TEM analysis of the two carbon grid image areas is shown in Figure A3.
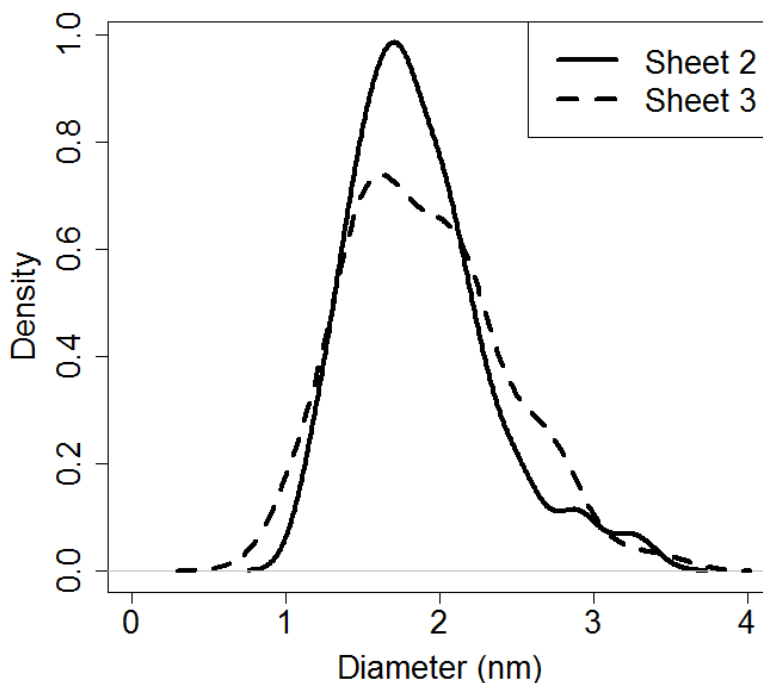

Figure A3. RM 8027 particle size distributions, obtained from the two areas of the TEM grid.

Material Source and Processing: RM 8027 was prepared at the NIST Biosystems and Biomaterials Division. Si wafers (<111>, Boron doped), were purchased from a commercial supplier. The wafers were etched using hydrofluoric acid and placed in a beaker containing toluene. The wafers were then sonicated to produce a suspension. Particle surface passivation with cyclohexane was done by hydrosilylation under ultraviolet excitation [5]. A two-stage filtering was used to remove larger particulates from the Si nanoparticle colloidal suspension in toluene. A 100 nm pore size 90 mm diameter polytetrafluoroethylene (PTFE) based filter membrane was used in the first stage and 20 nm pore size 47 mm diameter Anodisc 47 circular membrane filters in the second stage. The suspension was transferred to a clean 4 L capacity dark glass bottle and hermetically sealed. This RM was subsequently flame-sealed into 2 mL pre-scored amber glass ampoules using an automated process. Prior to use, the ampoules were cleaned with high pressure deionized water and autoclaved, then flushed with argon gas prior and during filling.

Analysis with DLS: DLS measurements were performed at the NIST Biosystems and Biomaterials Division. Si nanoparticle hydrodynamic radius measurements were performed using a Nicomp 380 ZLS DLS system (Particle Sizing Systems Inc., Santa Barbara, CA). Samples were contained in glass disposable culture tubes. Prior to sample loading, the tubes were blown with nitrogen gas and filled with 0.3 ml of the RM sample from the ampoule. The tubes were sealed using parafilm and were then centrifuged at 14 000 rpm for 5 min to separate larger particles and dust. Samples were placed in the instrument and allowed to reach thermal equilibrium. A nanoparticle DLS measurement protocol as described by the ISO 13321 [6] standard was followed. A Laplace inversion based Nicomp routine was used to find the distribution of decay rates. The decay rates are linked to the effective-sphere hydrodynamic diameters by the appropriate equations. The size distribution is reported as a discrete set of diameter bins and corresponding intensity-weighted fractions as probabilities assigned to diameter bins. The numbers provided from the instrument were normalized such that the largest probability (rather than the sum of all probabilities) was one for each ampoule. Before further analysis, probabilities were renormalized so that the sum of all probabilities was one for each ampoule. To extract an estimated mean from each ampoule, least squares optimization was used to fit a three-parameter gamma distribution to the normalized probabilities. Non-certified values were determined based on intensity-based distribution as measured by DLS.

DLS Instrument Variability Assessment: Additional measurements were performed at NIST on four randomly selected samples using two other DLS instruments from different manufacturers to assess instrument effects. These machines differed in scattering angle, laser wavelength and data analysis software. On each instrument, four ampoules of RM 8027 (nominal 2 nm), one sample of a 90 nm latex particle standard, and three samples of a 10 nm particle standard were measured. Means were estimated in the same manner as previously described. A 95 % posterior

credible interval for the consensus mean of measurements was taken with a 90 degree scattering angle based on a Markov chain Monte Carlo fit for a linear model that included random machine effects. Analysis of Variance (ANOVA) of the nominal 10 nm particle data, the fixed machine effect factor had an F-statistic of 1.10 with 2 and 6 degrees of freedom and a corresponding p-value of 0.39. ANOVA of the nominal 2 nm particle data, the fixed machine effect factor had an F-statistic of 0.87 with 2 and 9 degrees of freedom and a corresponding p-value of 0.39. This data indicated there is no statistically significant evidence of machine-to-machine variability.

Analysis with ICP-OES: Six ampoules of RM 8027 were used for the analysis. A solution of known Si concentration was used as a control [7]. Sample sizes of 0.5 g to 0.7 g were chosen for RM 8027 and the control. Two aliquots were removed from each ampoule of RM 8027. Four samples from the control RM and six blanks were prepared for the analysis. All samples were placed into 30 ml polyethylene bottles along with 2 mL of concentrated tetramethylammonium hydroxide (TMAH). For the controls and blanks, 0.5 g of toluene was also added to mimic the matrix of RM 8027. The samples were capped and left undisturbed for 3 days to 4 days to allow digestion of the Si nanoparticles. All solutions were transferred to PTFE beakers, evaporated and reconstituted with 25 g of pure water. The final solutions contained 1 µg/g of phosphorus [8] as an internal standard in 2 % TMAH. To determine the Si mass fraction in RM 8027, the calibration was performed using the method of standard additions to compensate for any matrix effects. A Si spike solution was prepared from a silicon standard [7]. Each sample was split into two subsamples and one of them was spiked with 0.5 g of the Si spike stock solution (1.43 µg/g Si). A PerkinElmer Optima 5300 DV ICP-OES instrument was used for the analysis. The Si mass fraction in the samples was measured using 251.611 nm wavelength; while the P mass fraction in the samples was measured using 213.617 nm wavelength; both Si and P used axial plasma view, an integration time of 0.512 s and a read time of 8.192 s for the measurement. Each sample measurement comprised five replicates, and each solution was measured two different times for the analysis.

Heterogeneity Assignment: During the filling process, ampoules were stored in boxes numbered 1 through 10, with box number corresponding to fill order. Homogeneity testing was performed using measurements of hydrodynamic size, and Si mass fraction. For hydrodynamic radius measurements using DLS, one sample was removed from each of 30 randomly selected ampoules (three from each box), for a total of 30 samples. To extract an estimated mean from each ampoule, least squares optimization was used to fit a three-parameter gamma distribution to the normalized probabilities. A model including random day effects showed the estimated standard deviation for day, 1.9 E–06 nm, was dominated by that for error, 0.209 nm. A model allowing for fixed day effects had a p-value of 0.702. Thus, a single mean model was fit to the entire collection of data from all 30 ampoules. The variability attributed to measurement error was estimated from four replicate measurements from each of three ampoules. An ANOVA with a fixed effect for an ampoule provided an estimated variance for repeated measurements on a single sample of 0.051668 with 9 degrees of freedom. The F-statistic for an ampoule was 0.685 with 2 and 9 degrees of freedom and a corresponding p-value of 0.529, indicating there is no statistically significant evidence of sample heterogeneity between ampoules.

Si content was evaluated using ICP-OES performed on two samples extracted gravimetrically from each of six randomly selected ampoules. Sample sizes of 0.5 g to 0.7 g were chosen for RM 8027 and a control [7]. Two aliquots were removed from each ampoule of RM 8027. Four samples from the control, and six blanks were prepared for the analysis. Analysis followed addition of an internal standard [8], TMAH digestion, and dilution with high-purity water. The relative expanded uncertainty value for the experimental Si mass fraction for RM 8027 was 4.8 %. Based on a single factor ANOVA, the observed differences are not significant among the ampoules for RM 8027 and therefore, heterogeneity was considered to be minimal.

Overall technical coordination for material preparation, processing and measurement activities was conducted by V. Reipa of the NIST Biosystems and Biomaterials Division and K. Nontapot of the Thai National Metrology Institute.

Statistical consultation on measurement design and analysis of the non-certified value data were performed by S.P. Lund of the NIST Statistical Engineering Division.

Measurements were performed by V. Reipa, P. DeRose, and A. Urbas of the NIST Biosystems and Biomaterials Division; S.A. Rabb, M.R. Winchester, and G.C. Turk of the NIST Chemical Sciences Division; J.A. Fagan, V. Rastogi, V. Prabhu of the NIST Polymers Division; A.F. Myers of the NIST Center for Nanoscale Science and Technology and K. Nontapot of the Thai National Metrology Institute.

Additional technical and coordination aspects were provided by the following: S. Choquette of the Office of Reference Materials, and J.T. Elliott and A.L. Plant of the NIST Biosystems and Biomaterials Division.

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