National Institute of Standards & Technology

Report of Investigation

Reference Material 8017

Polyvinylpyrrolidone Coated Silver Nanoparticles

(Nominal Diameter 75 nm)

This Reference Material (RM) is intended primarily for use as a benchmark and investigative tool for the evaluation of potential environmental, health, and safety risks that may be associated with manufactured nanomaterials during their product life-cycle. A unit of RM 8017 consists of five serum vials containing a lyophilized polyvinylpyrrolidone (PVP)-coated silver (Ag) nanoparticle cake. Each vacuum sealed vial contains nominally 2 mg Ag and 20 mg of PVP (molar mass approximately 40 kDa). The RM must be reconstituted with 2 mL of deionized water before use; the reconstituted Ag concentration is nominally 1 mg/mL.

Reference Values: Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet 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 statistical agreement among multiple analytical methods [1]. Dimensional values are reported in Table 1 based on the analyte form and method listed. The mass of Ag in a vial is reported in Table 2. Values for cumulative size distributions are provided in Table 3. A synopsis for the methods used is provided in this report (see "Methods for Reference Value Measurements").

Information Values: An information value is considered to be a value that will be of interest and use to the RM user, but insufficient information is available to assess adequately the uncertainty associated with the value or only a limited number of analyses were performed [1]. Information values cannot be used to establish metrological traceability. Values and figures of interest to the RM user are provided in the "Information Values" section of this report.

Expiration of Value Assignment: The reference values for **RM 8017** are valid indefinitely, within the measurement uncertainty specified, provided the RM is handled and stored in accordance with the instructions given in this report (see "Instructions for Handling, Storage, and Use"). This report is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM: NIST will monitor this RM over the period of its validity. If substantive technical changes occur that affect the reference values, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination for the material procurement, processing, and technical measurements leading to the value assignments was conducted initially by R.I. MacCuspie and subsequently by J.M. Gorham and V.A. Hackley of the NIST Materials Measurement Science Division.

Processing of the reference material was conducted at NIST by T.J. Cho, J.M. Gorham, J. Liu, and R.I. MacCuspie of the NIST Materials Measurement Science Division.

Analytical measurements at NIST, unless stated otherwise, were conducted by A.J. Allen, V.A. Hackley, J. Liu, R.I. MacCuspie, T.M. Nguyen, and J. Pettibone of the NIST Materials Measurement Science Division; J.E. Bonevich of the NIST Materials Science and Engineering Division; and K.E. Murphy and L.L. Yu of the NIST Chemical Sciences Division.

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Endotoxin assays were conducted at the Nanotechnology Characterization Laboratory, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, MD.

Statistical consultation for this RM was provided by B. Toman of the NIST Statistical Engineering Division.

Support aspects involved with the issuance of this RM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNING TO USERS

FOR LABORATORY USE ONLY; NOT FOR CLINICAL USE.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling and Storage: Until required for use, the RM should be stored in the dark at room temperature in its original sealed vial. The reference values reported are only valid for samples analyzed within 24 h of reconstitution. Based on studies conducted at NIST, reconstituted material should be physically and chemically stable for at least 5 d (if stored as stated below and not diluted); data suggests that stability may extend to 1 month or longer under these conditions. Periodic monitoring of particle size by dynamic light scattering (DLS), atomic force microscopy (AFM), and transmission electron microscopy (TEM) is recommended.

Reconstituted material should be stored in the dark at temperatures from 4 °C to 25 °C and should not be subjected to freezing or near freezing temperatures.

Use: Remove the aluminum tear-away crimp seal, and carefully open the rubber septum capping the vial. A small rush of air into the vial may be audible. Add two milliliters of filtered deionized high purity water to reconstitute the suspension. *If intended for biological testing, biological grade (i.e., sterile and endotoxin free) deionized water should be used.* Replace the rubber septum, and shake gently by hand for 20 s to 60 s. Visible clumps should dissolve in less than 1 minute. DO NOT SONICATE. The lyophilized cake will self-disperse with gentle shaking and without the need for sonication or vortexing [2] which may cause adverse effects on the RM. Let stand for 1 h prior to use.

PREPARATION AND ANALYSIS[1]

Material Source and Processing: RM 8017 was prepared from material obtained from a commercial producer (Nanocomposix Inc., San Diego, CA) in suspension form, having been formulated to NIST specifications. Prior to material processing, 10 mL borosilicate serum vials that conform to USP Type I requirements were baked at >200 °C for >20 h, then stored under sterile conditions. During processing, the material was aliquotted into the sterilized vials using an electronic pipette and sterile, pyrogen-free pipette tips, and then loosely covered using a butyl rubber stopper. The material was then lyophilized in the vials, back-filled with ultra-high purity argon gas, and vacuum sealed. An aluminum cap was then crimped onto the vials to protect the integrity of the vacuum seal between the glass and the butyl stopper.

Heterogeneity Assessment: Five factors were identified that could introduce systematic heterogeneity during lyophilization of the reference material. They were the drying process run, tray number, tray position, vial row location, and vial column location. Vials of the material were selected according to a sampling design that balanced these factors in a way that permitted a statistical test to determine whether these factors had any systematic effect on the material. A total of 84 vials were sampled and 545 individual measurements were conducted using the dynamic light scattering method. Each individual measurement was a mean particle size in nanometers of the particular sample. ANOVA tests of the five factors and their two-way interactions did not identify any significant effect at the 95 % level of confidence, showing that the production process did not introduce any systematic differences in the material. A simple one way ANOVA test of differences among the 84 vials did show heterogeneity at the 95 % significance, indicating that a between vial random heterogeneity is present and therefore a between vial uncertainty term is included in the combined uncertainty of the reference values. For isotope dilution mass spectrometry (IDMS) measurements, the relative variability in the mass of Ag observed between vials is ± 0.85 % (relative standard deviation, n = 12), which is no greater than the variability expected for the measurement method, indicating no detectable heterogeneity in the mass of Ag beyond 0.9 %.

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[1] Certain commercial instruments, materials, or processes are identified in this report 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 instruments, materials, or processes identified are necessarily the best available for the purpose.

REFERENCE VALUES

Reference Values For Mean Silver Particle Size: Analyses to assign reference values were conducted using best practices as determined independently for each method listed in Table 1. The DLS and Ultra-Small-Angle X-ray Scattering (USAXS) measurements yield the ensemble mean equivalent spherical diameter for a population of particles, obtained for replicate measurements on a sample or subsample for each tested vial. The reference values are maximum likelihood estimations of the mean particle size based on a random effects model [3] which accounts for both within and between vial heterogeneity. The expanded uncertainty, $U = ku_c$, is calculated for the sample mean particle size of a randomly selected vial, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO/JCGM Guides [4,5]. The coverage factor, $k = 2$, represents a 95 % uncertainty interval for DLS, AFM and TEM. The coverage factor, $k = 2.1$, represents a 95 % uncertainty interval for USAXS.

For AFM and TEM, a Type B component representing the uncertainty arising from the calibration artifact is included in this calculation. For DLS a Type B component is derived from ISO 22412:2008, which stipulates an expectation of ± 2 % uncertainty relative to a monodisperse qualification standard. For USAXS, identifiable Type B components were determined to be insignificant relative to Type A. The TEM and AFM measurements yield individual sizes for large numbers of particles per sample (each sample representative of one vial), with each sample subsampled once (i.e., a subsample is a single deposition onto a substrate). Replication was achieved by sampling multiple vials. The reference values were obtained as sample means and percentiles of ordinary non-parametric two-stage bootstrap samples [6] where the first stage was a random selection of one of the vials and the second stage was a subsample, drawn with replacement, of the measurements of the vial.

Table 1. Reference Values and 95 % Uncertainty Intervals for Mean Silver Particle Size[(a,b)]

Methods	Analyte Forms	Particle Size (nm)		
Atomic Force Microscopy (AFM)	dry, deposited on substrate	70.1	$\pm$	6.0
Transmission Electron Microscopy (TEM)	dry, deposited on substrate	74.6	$\pm$	3.8
Ultra-Small-Angle X-ray Scattering (USAXS)	liquid suspension	67.9	$\pm$	0.8
Dynamic Light Scattering (DLS)[(c)]	liquid suspension, diluted	105.6	$\pm$	4.6

[(a)] The measurand is the particle size as determined by the method listed. The reference values are metrologically traceable to the SI unit of length, expressed as nanometers.
[(b)] For AFM "size" is the number-weighted maximum particle height. For TEM, DLS and USAXS size is the equivalent spherical diameter weighted by number, intensity (z-average) and volume, respectively.
[(c)] Measurements conducted at 173º scattering angle (backscatter).

Reference Mass Value for Silver (Ag): The measurand is the mass of Ag in a vial as determined by IDMS. The reference value is metrologically traceable to the SI unit of mass, expressed as milligrams. The uncertainty estimates evaluated by Type A and Type B methods, for each component of the ID equation, were combined in a Kragten spreadsheet [7] using the law of propagation of uncertainty, according to the ISO/JCGM Guide [4], to obtain the combined standard uncertainty. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO/JCGM Guide [4]. The coverage factor, $k = 1.98$, used to obtain an approximate level of confidence of 95 %, is determined from the Student's t-distribution with ν_{eff} effective degrees of freedom.

Table 2. Reference Mass Value and 95 % Uncertainty Interval for Silver[(a)]

Silver (Ag)
(mg)

2.162 $\pm$ 0.020

[(a)] To convert total Ag mass into Ag concentration at reconstituted volume of 2 mL, divide mean mass by 2 to obtain a reconstituted Ag concentration of 1.081 mg/mL; any additional uncertainty due to the volume of water added should be taken into consideration and added to the uncertainty listed above.

Reference Cumulative Percentile Size Distribution Values: The measurand is the cumulative size at the percentile indicated as determined by AFM and TEM. The reference values are metrologically traceable to the SI unit of length, expressed as nanometers. The expanded uncertainties, U, are calculated as $U = ku_c$ where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO/JCGM Guides [4,5]. A Type B component representing the uncertainty arising from the calibration artifact is included in this calculation. The coverage factor, $k = 2$, represents a 95 % uncertainty interval.

Table 3. Reference Cumulative Percentile Size Distribution Values for AFM and TEM Methods

Percentile (%)	AFM (nm)	TEM (nm)
5	53.7 ± 6.6	66.3 ± 4.2
10	58.6 ± 6.2	68.8 ± 4.0
15	62.4 ± 6.2	70.3 ± 4.0
20	65.3 ± 6.0	71.4 ± 4.0
25	67.0 ± 6.0	72.2 ± 4.0
30	68.3 ± 6.0	72.8 ± 3.8
35	69.3 ± 6.0	73.5 ± 3.8
40	70.3 ± 6.0	74.0 ± 3.8
45	71.1 ± 6.0	74.5 ± 3.8
50	71.9 ± 6.0	75.0 ± 3.8
55	72.6 ± 6.0	75.6 ± 3.8
60	73.3 ± 6.0	76.0 ± 3.8
65	74.1 ± 6.0	76.5 ± 3.8
70	74.8 ± 6.0	77.1 ± 3.8
75	75.7 ± 6.2	77.6 ± 3.8
80	76.6 ± 6.2	78.2 ± 3.8
85	77.6 ± 6.2	78.9 ± 3.8
90	79.0 ± 6.4	79.8 ± 3.8
95	81.1 ± 6.8	81.3 ± 4.0

METHODS FOR REFERENCE VALUE MEASUREMENTS

Atomic Force Microscopy (AFM): Reconstituted samples from 19 randomly selected vials were immobilized on 3-aminopropyldimethylethoxysilane functionalized silicon/silicon oxide substrates using a procedure consistent with ASTM E2859-11 [8]. Undiluted liquid samples (20 µL) were incubated on the substrate for 10 min, after which the solution was rinsed away using filtered deionized water and subsequently dried in a clean bench using compressed air. Imaging in tapping mode was initiated within 6 h of deposition using a Dimension 3100 equipped with an automated stage and a Nanoscope V controller (Bruker AXS, Santa Barbara, CA). A MikroMasch calibration grating (Model TGZ01, Serial No. G030245, NanoAndMore, Lady's Island, SC) with a mean step height of a 19.5 nm ± 0.8 nm was used to verify operation and calibration. AFM cantilevers had a nominal force constant of 42 N/m and an aluminum reflective coating on the detector side. The tips were fabricated from single crystal silicon with a radius of seven nanometers or smaller. Tips were checked at specific intervals and replaced after 100 images. Calibration was rechecked after 90 images for a given tip. Gwyddion [9], a free software package, was used on the acquired images to correct for tip defects and to determine the average background. Maximum particle heights were then exported and the background was subtracted from each image. The low-end threshold was set to 20 nm to avoid undifferentiated substrate related artifacts. A total of >22 000 individual particle measurements were included in the analysis.

Transmission Electron Microscopy (TEM): Measurements were conducted using a JEM3010 (JEOL, Peabody, MA) equipped with an Orius CCD camera (Gatan, Warrendale, PA) and a Titan (FEI, Hillsboro, OR) equipped with a Gatan UltraScan 1000 CCD camera, both operated at 300 kV. The pixel resolution was 1.4 nm/pixel and 2.8 nm/pixel for the JEM3010 and Titan, respectively. The magnification was calibrated using a gold-shadowed cross grating with 463 nm line pitch (Product 607, Ted Pella, Inc., Redding, CA) following the vendor's instructions. The magnification was not changed after calibration, and samples were focused using only stage height controls (fixed lens excitation). After reconstitution, samples from nine randomly selected vials were prepared on three millimeter amine functionalized silicon oxide TEM grids (NG01-051B, NanoPlus SiO Grid, Dune Scientific, Eugene, OR). Briefly, 5 µL of suspension previously diluted 1:10 in filtered deionized water was placed on the grid and incubated for 10 min. The grid was covered to prevent evaporation. Suspension was then wicked away by touching the grid edge using a laboratory wipe. Then 5 µL of deionized water was placed on the grid and similarly wicked away. The grid was dried with a gentle stream of compressed air and stored until needed. Image frames were acquired within 2 d of sample preparation from multiple, widely separated regions. At least 15 individual locations were used within a selected site on each grid to acquire image frames. Each site contained at least 1000 individual particles for analysis. Image frames were acquired at 2 s exposure times. Of the nine vials, eight were analyzed using the JEOL TEM and one using the FEI. ImageJ [10] was used to analyze images by thresholding, and measuring the contiguous area of pixels that fall within the threshold set for a particular micrograph. The area was then used to determine the equivalent diameter assuming a perfectly spherical particle. A particle size threshold of two nanometers was imposed to eliminate any counting of pixels arising from the speckled, amorphous background of the support grid. Following the protocol recommended in Rice et al. [11], each image was visually inspected and any touching particles (i.e., agglomerates, overlapping particles) were excluded from analysis, while particles touching the edge of image frames were also excluded. Over 16 000 individual particles were included in the reference value assignment.

Ultra-Small-Angle X-ray Scattering (USAXS): Measurements were performed at ChemMatCARS sector 15-ID of the Advanced Photon Source (Argonne National Laboratory, Argonne, IL).[(2)] This USAXS instrument has been described in detail elsewhere [12]. Data were collected during two experimental runs separated by 7 months and generating 52 individual measurements from 19 randomly selected vials analyzed within 2 h of reconstitution. In each case the sample suspension was pumped into a vertical 1.5 mm diameter quartz capillary tube and measurements were conducted at two separate positions on the capillary. The beam size was 0.4 mm x 0.4 mm, and the mean sample thickness was 1.48 mm. Thus, with an Ag volume fraction of $\approx 10^{-4}$ and a mean particle diameter of ≈ 70 nm, each USAXS measurement sampled $\approx 1.33 \times 10^8$ Ag nanoparticles in suspension. The USAXS data were reduced, the blank (empty capillary) scattering subtracted, and the data calibrated to give absolute scattering intensity, $I(Q)$, versus scattering vector, Q, where $Q = (4\pi/\lambda) \sin(\Theta)/2$, λ = the X-ray wavelength, and Θ = half the scattering angle. These reduced and calibrated data were analyzed to provide the size distribution of the features giving rise to the measured scattering intensity profile in Q. The size distribution entropy maximization algorithm, MaxEnt [13], was applied to extract the Ag nanoparticle volume fraction distribution and the mean equivalent spherical diameter.

Dynamic Light Scattering (DLS): Backscatter DLS measurements were performed using a Zetasizer-Nano ZS (Malvern Instruments Inc., Westborough, MA), following the protocol previously described [14]. NIST RM 8013 *Gold Nanoparticles, Nominal 60 nm Diameter* was used to evaluate and confirm proper performance of the instrument throughout the period of analysis. Measurement parameters were as follows: laser wavelength, 633 nm (He-Ne); scattering angle, 173º; number of sub-runs, 11 at 10 s each; 50 % of sub-runs with highest intensity were automatically rejected as a dust filter, yielding a total measurement duration for analysis of 55 s; measurement temperature, 20 ºC ± 0.1 ºC; medium viscosity, 1.0031 mPa·s; medium refractive index, 1.330. Following reconstitution according to the prescribed procedure, a 10 µL aliquot was extracted and diluted into 990 µL of filtered deionized water in a clean disposable semi-micro poly(methyl methacrylate) cuvette (1:100 dilution); this procedure was performed in a HEPA filtered clean bench. Samples were allowed to equilibrate at the measurement temperature for 180 s prior to initiating measurement. Using a combination of stratified random and targeted selection, 84 vials were analyzed for reference value assignment. All vials used for TEM were included in the DLS analysis. For most vials, a single subsample was prepared and analyzed; for a few select vials three subsamples were prepared and analyzed. At least five replicate measurements were performed on each subsample and averaged to obtain the sample means. The individual measured correlation functions were fit using the cumulants approach in compliance with ISO standards 13321:1996 and 22412:2008, to yield the z-average mean equivalent spherical hydrodynamic diameter; the measured size includes the core and the PVP corona.

Isotope Dilution Mass Spectrometry (IDMS): Silver mass was measured by IDMS using a X series II ICP-MS (ThermoFisher Scientific, Waltham, MA) equipped with matrix tolerant (Xt) cones and operated at 1400 W. A known amount of isotopically enriched ^{109}Ag (in solution) was added to each of twelve randomly selected vials containing the entire reconstituted sample, and was followed by the addition of 3.5 g of concentrated, high-purity nitric acid. The contents of each vial were gently swirled, taking care not to contact the rubber septum, at which point the reconstituted samples were observed to change from a cloudy suspension to a clear, pale yellow-colored solution. The samples were allowed to stand for at least 1 h prior to quantitatively transferring the contents of each vial to low density polyethylene bottles. Each sample was diluted to an analysis mass fraction of 3.5 ng/g ^{109}Ag in 2 % volume fraction nitric acid. The amount content of the added isotopically enriched ^{109}Ag solution was calibrated against primary Ag standards using reverse IDMS. Solution was introduced via a peristaltic pump into a low-flow (100 µL/min) perfluoroalkoxy micro-concentric nebulizer. The nebulizer was fitted to an impact-bead spray chamber cooled to 2 °C. Intensities for ^{107}Ag and ^{109}Ag were measured at dwell times of 10 ms each. Measurements were made in continuous mode using peak jump data acquisition with one point per peak. Five blocks of data, each one minute in duration, were acquired per sample. Measured intensities were corrected for dead-time and the intensity ratios were corrected for mass bias and drift. The mass of Ag was calculated *via* the isotope dilution measurement equation from the corrected ^{109}Ag/^{107}Ag intensity ratio [15].

INFORMATION VALUES

Electron Microscopy Imaging: TEM micrographs of silver particles are provided in Figure 1.
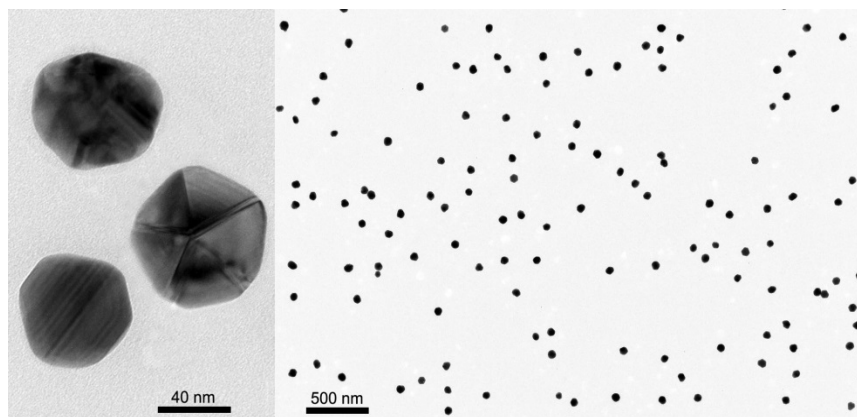

Figure 1. Electron Microscopy Imaging: TEM Micrographs. The left panel shows individual silver particles from different TEM images showing crystal habit and form, and the right panel shows density and uniformity of deposition from an image collected for particle size determination.

Elemental Impurities: Semi-quantitative determination of trace elements by ICP-MS was intended primarily to assess the impurity content of Group IB elements copper (Cu) and gold (Au) in RM 8017 and are listed in Table 4. ICP-MS was calibrated using a multi-element calibration solution that is traceable to NIST Spectrometric, Single Element Standard Solutions. SRM 1643e *Trace Elements in Water* was used for quality assurance. Three replicate measurements were performed on a single vial of RM 8017.

Endotoxin Determination: The objective of these assays was to estimate the maximum endotoxin concentration present in RM 8017 in its undiluted reconstituted form. For this purpose, five vials were randomly selected, and reconstituted following the standard procedure outlined in the "Instructions for Handling, Storage, and Use." Endotoxin contamination was assessed by the kinetic turbidity and gel-clot Limulus Amoebocyte Lysate (LAL) assays. The general protocols for these assays (STE-1.2 and STE-1.3, respectively) can be found in reference 16. Assays were conducted at a dilution factor of 1:1000, based on preliminary spike recovery results. Results are listed in Table 4.

Table 4. Information Values.

Method	Value	Detection Limit (approximately)		
Element[a]				
Copper (Cu)	ICP-MS	14 ng/g	8.1	ng/kg
Gold (Au)	ICP-MS	<1 ng/g	1	ng/g
Silicon (Si)	ICP-MS	<12 µg/g	3.2	µg/kg
Iron (Fe)	ICP-MS	<12 µg/g	3.8	µg/kg
Endotoxin level	Turbidity-LAL	≤2.0 EU/mg-Ag	2.0	EU/mg-Ag
Endotoxin level	Gel-clot LAL	<30 EU/mg-Ag	30	EU/mg-Ag

[a] All other impurity elements were below 1 µg/g or below the detection limit.

UV-Vis Absorbance: Figure 2 provides a representative UV-Vis absorbance spectrum for a reconstituted RM 8017. The average surface plasmon resonance (SPR) peak for Ag was centered at 457 nm based on 24 measurements from eight vials. Based on the Ag concentration in Table 2 and the mean SPR peak absorbance, an extinction coefficient $\varepsilon = 79.8$ mL/(mg·cm), is estimated where $A = \varepsilon CL$, where C is the Ag concentration in milligrams per milliliter and L is the path length in centimeters.
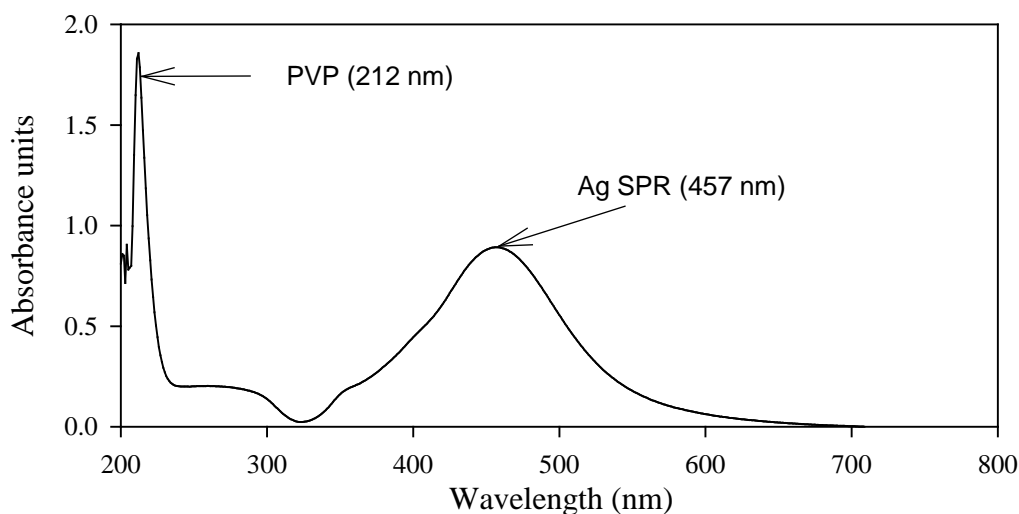

Figure 2. UV-Vis absorbance spectrum for reconstituted RM 8017 (1:100 dilution with deionized water). The spectrum was blanked against deionized water. The maximum absorbance peaks are labeled for Ag (457 nm) and PVP (212 nm).

Single Particle ICP-MS (spICP-MS): A quadrupole ICP-MS equipped with a micro-flow concentric nebulizer and an impact bead spray chamber cooled to 2 °C was utilized to quantify the [107]Ag intensity in time-resolved analysis mode to obtain spICP-MS data on reconstituted RM 8017 diluted to Ag mass fractions of 35 ng/L to 60 ng/L on the order of 10^7 particles per liter [17]. Particle size was derived by the method described in Pace et. al. [18]. The transport efficiency was measured daily via the particle size method using NIST RM 8013. Silver (Ag) standards prepared daily in water by gravimetric dilution of SRM 3151 *Silver (Ag) Standard Solution* were used to establish the intensity versus mass per event calibration curve. Working suspensions were analyzed within 1 h of dilution to minimize Ag nanoparticle oxidation. Particle pulses were differentiated from the background using a 5σ criterion. Assuming spherical shape, individual pulse intensities were converted to mass via the calibration curve and then the diameter was calculated using the bulk density of Ag. The dissolved Ag fraction was determined using an intensity vs. mass calibration curve established daily from dissolved Ag standards. Analysis of the highly diluted working sample over a 24 h period indicated a non-linear increase in the dissolved fraction and a corresponding decrease in the particle size distribution. The mean mass diameter of Ag particles obtained by averaging the means of four vials of RM 8017 was 69.2 nm with a standard deviation of 0.9 nm. A comparison of the dissolved fraction determined by spICP-MS (which includes ionic species plus particles smaller than 20 nm) and ID with spiked aqueous [109]Ag in the reconstituted vial following centrifugal ultrafiltration indicates greater variability in spICP-MS results from vial to vial, but a mean dissolved fraction (4.2 %) that is statistically in agreement with the ID results (3.0 %). Size distributions obtained by spICP-MS are compared in Figure 4 with AFM, TEM and USAXS. Data on the stability of reconstituted RM 8017 stored at 4 °C shows minimal degradation of the Ag nanoparticles over a period of 90 d.

Asymmetric-Flow Field Flow Fractionation (A4F): A single vial of RM 8017 was analyzed by A4F coupled to UV, static light scattering (LS), and dynamic light scattering detectors, with [107]Ag detection by ICP-MS and the results are shown in Figure 3. Constancy of retention time and peak shape over 1 month suggest excellent stability of RM 8017 following reconstitution; some variability between replicates at both time points (not shown) was observed and attributed to the high concentration of PVP present. Represented as a superimposed scatter plot on the UV traces of Figure 3 (left panel) is the hydrodynamic diameter, D_h, measured by on-line DLS at the FWHM of the UV peaks; the mean D_h near the center of the peak is about 90 nm and the data are relatively constant (flat) across the peak. Scattering intensity is highly dependent on particle size, whereas UV is weakly dependent on size and primarily indicative of concentration. The close overlap of UV and LS traces in the right panel of Figure 3 (with only a slight shift of the LS peak to longer retention) is suggestive of a well dispersed monomodal material. ICP-MS traces for [107]Ag (not shown) indicate good alignment with UV and LS traces, but show that a very small quantity (on the order of 1 % by mass) of Ag is eluted with free PVP at very short retention times ($t_R \approx 4.5$ min) that increased slightly at 1 month compared to the 1 h time point; this Ag was not quantified and the UV-Vis signature was not consistent with previously reported Ag species, suggesting it is ionic and/or very small reduced species, qualitatively in agreement with spICP-MS findings.
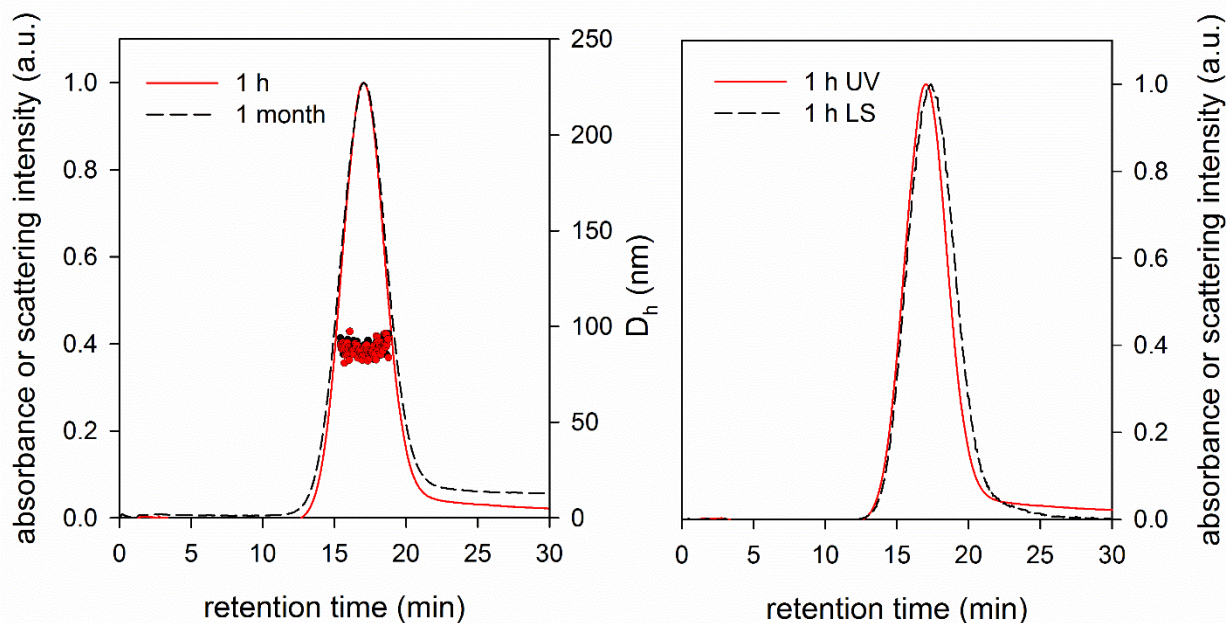

Figure 3. A4F Fractograms for reconstituted RM 8017. The left panel presents UV (400 nm) fractograms obtained at 1 h (red, solid line) and 1 month (black, dashed line) following reconstitution. The left panel also contains a superimposed scatter plot of the hydrodynamic diameter, D_h, measured by on-line DLS at the FWHM of the UV peaks; the mean D_h near the center of the peak, at about 90 nm (right axis). The fractograms were obtained using a 10 kDa polyethersulfone membrane with a 250 μm spacer in a flat channel. The mobile phase was 0.5 mmol/L NH_4NO_3 in deionized water. Both channel and cross flow rates were set to 0.5 mL/min, and 100 μL of undiluted reconstituted RM 8017 was injected during each analysis. The reconstituted sample was stored at ambient laboratory conditions between analyses. The right panel shows the UV (red, solid line) and LS (black, dashed line) traces taken 1 h after the reconstitution.

Zeta Potential and pH of Reconstituted Suspension: Within 3 h of reconstitution, electrophoretic mobility measurements were performed on RM 8017 diluted 1:100 (filtered deionized water) using phase analysis light scattering with a dip cell containing palladium-coated electrodes; two vials were analyzed. Replicate measurements were conducted on samples from both vials, and the mobility was converted to zeta potential (ZP) using the Smoluchowski equation (default setting on most instruments). The mean ZP for the 1:100 dilution was –24 mV with a standard deviation of 1 mV. A 1:10 dilution (filtered deionized water) of RM 8017 yielded a ZP of –20 mV. The pH for the as-reconstituted RM 8017 in both vials was 4.3.

Comparison of Size Distributions from Multiple Techniques: The complete set of particle size distributions (PSDs) obtained from four techniques (AFM, TEM, USAXS and spICP-MS) and the mean PSD determined by averaging all available data sets are provided in Figure 4. This comparison shows the variability for replicate and vial-to-vial measurement results and the general shape and breadth of the measured distributions.
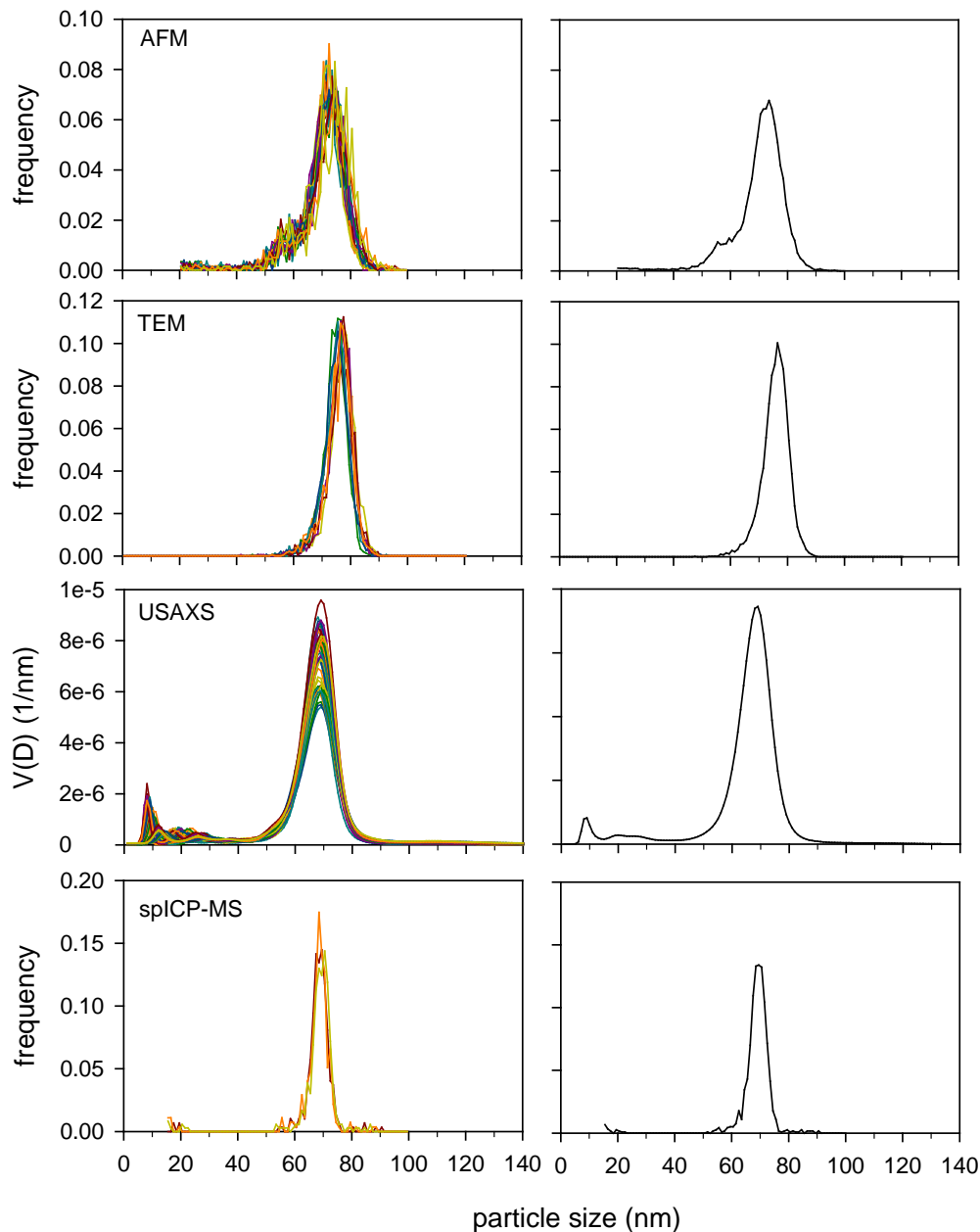

Figure 4. The left side presents the complete set of particle size distributions (PSDs) obtained from four techniques (AFM, TEM, USAXS and spICP-MS). On the right side is the mean PSD determined by averaging all available data sets. With the exception of USAXS, all PSDs have been normalized to the total counts in each data set, yielding frequency on the ordinate axis. USAXS PSDs are presented as the volume fraction. USAXS distributions are calculated using a maximum entropy fit and are plotted as the volume fraction of Ag per nanometer size increment rather than normalized frequency.

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