

Use of Single Particle Inductively Coupled Plasma Mass Spectrometry to Characterize a New Silver Nanoparticle Reference Material

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ABSTRACT

In this study we use inductively coupled plasma mass spectrometry operated in single particle mode (spICP-MS) to simultaneously characterize the size distribution and dissolved silver fraction of a new silver nanoparticle (AgNP) candidate reference material, RM 8017, composed of polyvinylpyrrolidone (PVP)-stabilized 75 nm AgNPs (nominal size). We show the potential for bias in spICP-MS measurement of AgNPs if the time lapse between preparation of the dilute suspension in water and measurement is not minimized. We demonstrate that the particle size distributions measured by spICP-MS compare well with transmission electron microscopy (TEM), atomic force microscopy (AFM), and ultra-small angle X-ray scattering (USAXS) measurements. Finally, data on the stability of reconstituted RM 8017 stored at 4 °C shows minimal degradation of the AgNPs over the period of 90 days. This study shows the potential of spICP-MS as a tool to characterize the basic parameters of nanoparticle suspensions including size, size distribution, and ionic content.

Keywords: single particle ICP-MS, reference material, nanomaterial characterization, silver nanoparticles

1 INTRODUCTION

Nearly a quarter of all nanomaterials used in consumer products are purported to contain silver [1]. AgNPs are employed primarily for their antimicrobial properties. While the toxicity of silver to humans is believed to be low, silver is toxic to aquatic organisms and bacteria, leading to concerns of unintended consequences with the increased proliferation of AgNPs in the environment. The mechanism of toxicity, whether due to free Ag⁺, particulate Ag, or both, is not well understood. Assessment of the potential risks posed by AgNPs requires both well characterized AgNP suspensions to serve as test materials and analytical methodology capable of measuring the distinct silver species (Ag⁺ or particulate) at environmentally relevant concentrations. Recently, the National Institute of Standards and Technology (NIST) initiated a project to produce mono-dispersed AgNP reference materials (RMs) with well-characterized size distributions that exhibit stable, long-term shelf life [2]. The candidate RM 8017

was prepared as a lyophilized cake of PVP and nominally 75 nm AgNPs that can be reconstituted as a suspension by the addition of deionized water.

Single particle detection using ICP-MS (commonly referred to as spICP-MS) is being advanced as a method for the characterization of NPs in natural matrices at environmentally relevant concentrations [3, 4]. Single particle ICP-MS provides a means to distinguish between the dissolved metal fraction and the nanoparticle fraction of a sample without the need to physically separate the two fractions. It relies on the principal that as a nanoparticle suspended in a solution is atomized and ionized in the plasma, it will produce a flash of ions which is measured as a single pulse superimposed on the steady-state signal produced by any dissolved analyte. The intensity of the pulse generated, after subtraction of the dissolved signal intensity, is proportional to the number of atoms in the particle and thus its size, assuming a defined particle shape. The number of events counted is directly proportional to the nanoparticle number concentration. The intensity of the continuum signal provides a measure of the dissolved metal content. Besides the assumption of particle shape, it is assumed that only one particle is ionized and detected in a given acquisition period (dwell time). To accomplish this, acquisition periods sufficiently small to capture a single event, while long enough to minimize the occurrence of split events are used [5], along with very dilute suspensions (e.g., particle number concentration on the order of 10⁷ particles·L⁻¹ [3]). This latter requirement means that spICP-MS is capable of analyzing NP suspensions at environmentally relevant mass concentrations (ng·L⁻¹), although the stability of the suspensions must be closely monitored, particularly for a reactive element, such as silver. In this study we assess the reproducibility of spICP-MS for the measurement of particle size and dissolved silver content of candidate RM 8017 and provide a measure of the accuracy of the spICP-MS size measurement by comparing with established sizing methods.

2 MATERIALS AND METHODS

2.1 RM 8017

Methodologies developed for use in preparation of candidate RM 8017 have been described elsewhere [2]. This

material is composed of a lyophilized cake of nominally 75 nm AgNPs packaged in a 10 mL crimp-sealed rubber septum-capped glass vial under argon. The solids contain nominally 2 mg Ag and 20 mg PVP (approximately 40 kDa molecular weight). Multiple vials were analyzed by each method. Samples were stored in the dark until use. Each vial was reconstituted as follows. The aluminum tear-away crimp seal was removed and the rubber septum carefully lifted. A small rush of air filling the bottle may be heard. If desired, as a means to verify the vial seal, the mass of the vial before and after breaking the seal can be monitored. A mass gain of nominally 16 mg should be observed. Two mL (2.00 mL) of deionized, 18.2 mΩ cm⁻¹ water were added to reconstitute the suspension. The rubber septum was replaced and the vial well mixed (manually) for one minute. This material should not be sonicated or vortexed. The samples were allowed to stand on the bench top for 1 hour. Measurements were completed within 24 h of reconstitution. For the long term stability assessment by spICP-MS, capped vials were sealed with plastic film and stored in the dark at 4 °C.

2.2 spICP-MS

A ThermoFisher X series II¹ quadrupole ICP-MS (Waltham, MA, USA) was used. Samples were introduced into the ICP torch using a micro-flow perfluoroalkoxy (PFA) concentric nebulizer (PFA-ST, Elemental Scientific, Omaha, NE, USA) and an impact bead spray chamber cooled to 2 °C. The sample flow rate was set to approximately 0.2 mL·min⁻¹ and measured daily in triplicate. Particle sizes were derived by the method of Pace et al. [6]. The transport efficiency was measured daily *via* the particle size method using NIST RM 8013 Gold Nanoparticles (nominal 60 nm diameter). Silver standards prepared daily in water by gravimetric dilution of SRM 3151 Silver (Ag) Standard Solution (Lot 992212) were used to establish the intensity *vs.* mass per event calibration curve. The ¹⁰⁷Ag intensity was recorded in time-resolved analysis (TRA) mode using a dwell time of 10 ms for total analysis times ranging from 100 s to 360 s. Working suspensions of RM 8017 were diluted to (1.5x10⁷ to 2.5x10⁷) particles·L⁻¹ (depending on the transport efficiency) with resulting Ag mass fractions of (35 to 60) ng·L⁻¹. Working suspensions were analyzed within 1 h after preparation, (except where noted in Figure 1), in order to minimize AgNP oxidation of the dilute spICP-MS suspension. Particle pulses were distinguished from the background using a five times standard deviation (5σ) criterion [7]. Assuming spherical shaped particles, individual pulse intensities were converted to mass *via* the calibration curve and diameter calculated using the following equation:

$$d_p = \sqrt[3]{(6m_p / \pi\rho)}$$

where d_p is the particle diameter, m_p is the mass of the particle and ρ is the density of the Ag nanoparticles (assumed to be equivalent to the density of bulk Ag). The mass fraction of

¹ The identification of any commercial product or trade name does not imply endorsement or recommendation by the National Institute of Standards and Technology.

Ag⁺ was derived by dividing the signal intensity attributed to the dissolved fraction by the slope of the intensity *vs* mass fraction calibration curve established daily from dissolved silver standards prepared in water.

2.3 TEM

Transmission electron microscopy (TEM) measurements were conducted using a JEM3010 (JEOL, Peabody, MA, USA) equipped with an Orius CCD camera (Gatan, Warrendale, PA, USA). Samples from nine individual vials of the reference material candidate were studied. After redispersion, AgNP samples were prepared on amine functionalized silicon oxide thin film membrane substrates (Dune Scientific, Eugene, OR, USA) consistent with previously published protocols [8, 9]. The TEM was operated at 300 kV, and the magnification was calibrated using a gold-shadowed cross grating with 463 nm line pitch. The TEM magnification was not changed after calibration, and samples were focused using only stage height controls (fixed lens excitation). Image frames were acquired from multiple, widely separated regions.

The ImageJ software package was used to analyze TEM 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. Therefore, the average particle size does not consider any pronounced faceting of particles. Over 18 000 individual NPs were analyzed to generate the sizing histograms.

2.4 AFM

Atomic force microscopy (AFM) was carried out on a Dimension 3100 equipped with an automated stage and a Nanoscope V controller (Bruker AXS, Santa Barbara, CA, USA). Nineteen individual vials of the reference material candidate were studied using AFM. After redispersion, AgNP samples were prepared on amine functionalized silicon substrates consistent with previously published protocols [8, 9]. Images were collected in tapping mode after a tip check was performed on a calibration grating. Images were processed using Gwyddion software correcting for tip defects and acquiring an average background. Maximum particle heights were exported and background subtracted from each image. Thresholds were set to 20 nm and histograms were formed for each of the vials. For all 19 vials, >22 000 individual particle measurements were included.

2.5 USAXS

Ultra-small-angle X-ray scattering (USAXS) measurements were made at ChemMatCARS sector 15-ID of the Advanced Photon Source (APS), Argonne National Laboratory, Argonne, IL, USA. The USAXS instrument has been described in detail elsewhere [2, 10]. For candidate RM 8017, three separate sets of measurements were made over a period of 8 months; however, only the largest set of

measurements are presented here, representing two independent measurements each on 18 samples. In each case the sample suspension was pumped into a vertical 1.5 mm diameter capillary tube and USAXS measurements were made 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 a 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 $Q = (4\pi/\lambda)\sin(\phi_s/2)$, λ = the X-ray wavelength, and ϕ_s = half of the scattering angle. These reduced and calibrated data were analyzed to provide the size distribution of the features (e.g., AgNPs) giving rise to the measured scattering intensity profile in Q . The size distribution entropy maximization algorithm, MaxEnt [11], was applied to extract the Ag NP volume fraction diameter distribution.

3 RESULTS AND DISCUSSION

3.1 Stability of Dilute spICP-MS Suspensions

The effect of time on the stability of the dilute spICP-MS suspensions was initially investigated and the results are presented in Figure 1. Shown are the time-resolved signal intensity profiles and particle size distributions for a single spICP-MS suspension diluted in water to 2.5×10^7 particles·L⁻¹ (60 ng·L⁻¹), stored at room temperature, and analyzed at three time points: within 1 h, 2 h, and 24 h of preparation. The intensity profiles show a non-linear increase in mass fraction of Ag⁺ content with time (shown in white font) which increased from 14 ng·L⁻¹ to 20 ng·L⁻¹ within two hours of dilution, and was 44 ng·L⁻¹ after 24 h. The corresponding size distributions show a decrease in the measured particle size with time of storage. The mean measured AgNP size decreased by nearly 4 nm for the suspension stored for 2 h compared to that measured with 1 h of dilution, and degradation of the AgNPs was significant over the 24 h storage period. The data show that strict attention to sample preparation protocols, in particular minimization of the time lapse between preparation of the dilute suspension and analysis, will improve the accuracy of spICP-MS size measurement of AgNPs.

3.2 Reproducibility and Comparability of spICP-MS Measurements

Single particle ICP-MS measurements of AgNP size and the mass fraction of Ag⁺ for four vials of RM 8017, measured within 24 hours of reconstitution (day 1) and 1 h of the preparation of the dilute spICP-MS suspension, are summarized in Table 1. Data were corrected for particle events split between adjacent measurement windows according to a published protocol [12].

Size measurements are 10 % trimmed means of N particle events. A trimmed mean was used to avoid bias from false positives (intensities above the background threshold that are erroneously counted as particle events) and coincident particle

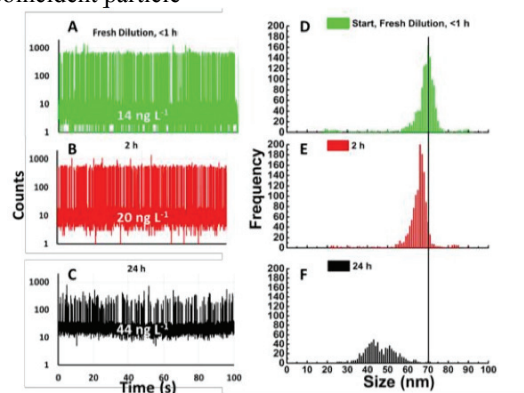


Figure 1. Time-resolved intensity profiles and corresponding particle size distributions for a spICP-MS suspension (2.5×10^7 particles·L⁻¹) of a single vial of RM 8017. The suspension was stored at room temperature and measured within 1 h (A, D), 2 h (B, E), and 24 h (C, F) of preparation. The measured mass fraction of Ag⁺ is shown in white font.

events. The mass fraction of Ag⁺ (expressed as the fraction of total Ag) measured from the dilute spICP-MS suspension is compared to the mass fraction of Ag⁺ in the reconstituted vial. The latter measurement was performed by adding a known amount of enriched ¹⁰⁹Ag⁺ in water to an aliquot from each of three vials of reconstituted RM 8017. After separating the dissolved silver fraction from the AgNP fraction by centrifugal ultrafiltration (Amicon Ultra-0.5 mL, 3k molecular weight cut-off), the mass fraction of Ag⁺ was quantified by isotope dilution analysis (IDA). The spICP-MS measurements of the Ag⁺ fraction exhibit greater variability, but are on average, in agreement with the IDA measurements.

Table 1. Reproducibility of spICP-MS Measurements of Size and Dissolved Ag Content

Vial	Size ^a , nm (N)	Ag ⁺ Fraction (%) (spICP-MS)	Ag ⁺ Fraction (%) (IDA) ^b
1	68.65 (297)	5.6	
2	68.55 (275)	7.0	2.9
3	69.18 (362)	2.9	2.8
4	70.42 (256)	1.2	3.3
Mean	69.20	4.2	3.0
SD^b	0.86	2.6	0.3

^aTrimmed Mean (10 %), ^b1 SD of the four vial means, ^cAddition of enriched ¹⁰⁹Ag⁺ followed by centrifugal ultrafiltration.

The ability of spICP-MS to accurately characterize AgNP size was investigated by comparison to measurements

performed by TEM, AFM, and USAXS described above. Figure 2 shows the size distributions measured by each method. The size distributions measured by spICP-MS show good agreement with the size distributions generated by AFM, TEM, and USAXS.

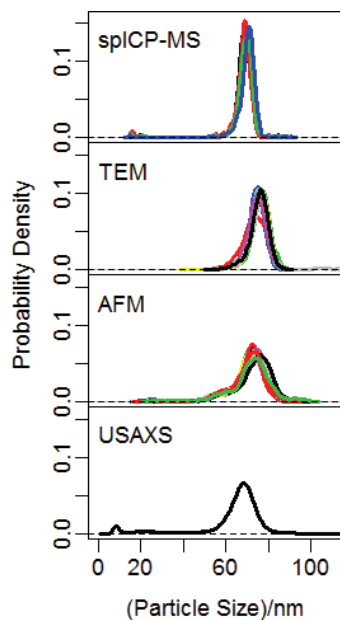


Figure 2. Size distributions of AgNPs from RM 8017 measured by spICP-MS (4 vials, particle core diameter), AFM (19 vials, particle height), TEM (9 vials, particle diameter), and USAXS (18 vials, volume-weighted size distribution per unit increment in AgNP diameter).

3.3 Stability of Reconstituted RM

Data on the stability of candidate RM 8017 are presented in Figure 3. Shown are the temporal changes in size (Fig. 3A) and fraction of Ag^+ expressed as a percentage of total Ag (Fig. 3B) for a $1000 \text{ mg}\cdot\text{L}^{-1}$ (reconstituted) suspension of RM 8017 stored at 4°C for a period of 90 days. Data for days 1, 7, and 90 are the average of measurements from multiple vials; the remaining points are data from one vial (3 replicates). Error bars are expanded uncertainties at an approximate 95 % level of confidence. No obvious trend in the data can be discerned within the uncertainty of the measurement. These results seem to indicate minimal degradation of the concentrated suspension over a 3-month period stored in the dark at 4°C , though it is recommended that this material be used within 24 h of reconstitution because value-assignment will be based on this recommended period.

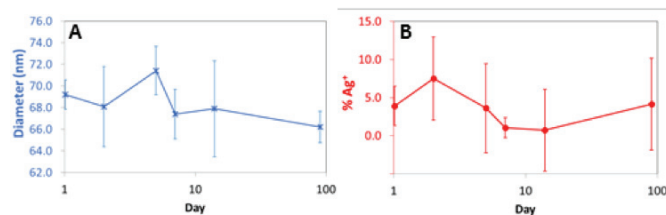


Figure 3. Stability of a concentrated aqueous suspension ($1000 \text{ mg}\cdot\text{L}^{-1}$) candidate RM 8017 stored at 4°C with size (A) and fraction of Ag^+ expressed as percentage of total Ag (B) with measurements performed on days 1, 2, 5, 7, 14, and 90. Error bars are expanded uncertainties at an approximate 95 % level of confidence.

4. CONCLUSIONS

In this study, spICP-MS was investigated as a potential new tool for use in rapidly characterizing basic parameters (size, size distribution, ionic content, and stability) of a candidate, nominally 75 nm, AgNP RM. We demonstrate that the particle size distributions measured by spICP-MS compare well with TEM, AFM, and USAXS measurements. Due to the reactivity of Ag at the low particle number concentration required for spICP-MS work, degradation of the suspension is a concern. Future work will explore different media for use in stabilizing the dilute suspensions while maintaining the integrity of the AgNP and Ag^+ species.

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