SINGLE-PARTICLE INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY
ANALYSIS OF SIZE AND NUMBER CONCENTRATION IN MIXTURES OF
MONOMETALLIC AND BIMETALLIC (CORE-SHELL) NANOPARTICLES

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ABSTRACT

It is challenging to separate and measure the physical and chemical properties of monometallic and bimetallic engineered nanoparticles (NPs), especially when mixtures are similar in size and at low concentration. We report that single particle inductively coupled mass spectroscopy (SP-ICP-MS), alongside field flow fractionation (FFF), has allowed for the accurate measurement of size and particle number concentrations of mixed metallic nanoparticles (NPs) containing monometallic NPs of gold (Au) and silver (Ag) and a bimetallic core-shell structured NP (Au@Ag) of equivalent size. Two sets of these NPs were measured. The first contained only 60 nm particles, where the Au@Ag NP had a 30 nm core and 15 nm shell to make a total diameter of 60 nm. The second contained only 80 nm particles (Au@Ag NP core particle of 50 nm with a 15 nm shell). FFF separation was used here as a sizing technique rather than a separation technique. It was used to confirm that suspensions containing either individual or mixtures of the Au 60 nm, Ag 60 nm and AuAg 60 nm suspensions eluted together and were of the same size. Similarly, FFF was used to show that suspensions containing individual or mixtures of the equivalent 80 nm, eluted together and were of the same size. Although the 60 nm and 80 nm suspensions did not elute at the same time they were not run together. SP-ICP-MS is then used to identify the size and concentration of the particles within the suspension. Successful separation of the NPs was effected and the limits of the instrument were obtained.

Introduction

Nanoparticles (NP) are widely used in an array of applications^{1, 2} due to their unique physical-chemical properties. These properties can be tuned by changing the size, shape and composition of the NP^{3, 4}. It has always been a challenge to fully characterize suspensions of monometallic NPs, where a multimethod approach will yield the most reliable results^{5, 6}. This process becomes increasingly difficult for more complex bimetallic NPs, or mixtures thereof, used in some applications or for those that require a mixture of different NPs. There are a variety of techniques that have been used for the determination of particle diameter, composition and particle number concentration (part. mL⁻¹) of engineered NPs; including dynamic light scattering (DLS), field flow fractionation (FFF)^{7, 8}, ultraviolet–visible spectroscopy (UV-Vis)⁹, multi-angle light scattering (MALS)¹⁰ and transmission electron microscopy (TEM)¹¹. More recently single-particle inductively coupled mass spectroscopy (SP-ICP-MS)^{12–13} has been applied although it has not been fully developed in this area. All these techniques have their own strengths and weaknesses as well as measuring size and/or composition in different ways. One of the most difficult properties to routinely measure in a NP suspension is particle number concentration.^{12, 14} This property can be measured Nanosight¹⁵, although its accuracy is questionable^{16, 17}. In fact all current methods are arduous and inaccurate with limitations in measurement; for instance, bimetallic (core-shell) NPs cannot be measured. DLS can be used to quantify size for some systems, as can FFF, which must be used with a detector, such as UV-Vis. Coupling FFF to an ICP-MS provides a lower detection limit compared with other methods¹⁸. However all these detection techniques are unable to discriminate mixtures of monometallic and/or bimetallic particles.

SP-ICP-MS is an excellent technique for the detection of metallic NPs providing data on size, size distribution, particle number concentration and other parameters¹³ and giving such data at low concentrations, which are important for many applications such as environmental measurements^{19, 20}. Advances in the measurement within shorter dwell times have led to improvements in detection by SP-ICP-MS and have also enabled for the qualitative measurement of two elements within the same NP²¹. FFF separates NPs by their hydrodynamic diameters. However FFF is unable to distinguish between mixtures of NPs with the same diameter but different compositions. Here we use FFF to establish whether mixtures of particles are nominally of the same size. This enables us to conclude that if we measure two elements with SP-ICP-MS together in that fraction that are smaller than the FFF size, and the particle number concentrations of both metals are equal then they will be from a bimetallic particles. If the sizes of the 2 elements are comparable to the FFF size and the particle number concentrations are different then the suspension will contain a mixture of monometallic NPs. We are able to show that a sizing and particle number counting method using SP-ICP-MS can be used to measure more complex suspensions of NPs within these fractions, and can discriminate between mixtures of monometallic Au and Ag NPs and bimetallic Au@Ag (core shell) NPs, yielding particle sizes and particle numbers concentrations for each constituent. The limitations to such resolution are described.

Experimental

Nanoparticles (NPs)

NP suspensions for this study were purchased from Nanocomposix (San Diego, CA, USA) and the National Institute of Standards Technology (NIST, Gaithersburg MD, USA). Particles with several sizes and compositions were used including 60 nm Ag, 60 nm Au, 60 nm Au@Ag (core size 30 nm, shell thickness 15 nm), 80 nm Ag, 80 nm Au and 80 nm Au@Ag (core size 50 nm, shell thickness 15 nm), purchased from Nanocomposix, were used either as single particle suspensions or mixtures. In addition, Au 30 nm and Au 60 nm NPs from NIST were used as certified reference materials. NP concentrations and other parameters were provided by the supplier (See supporting information S1 Table 1) and were diluted to between 10,000 and 100,000 part. mL⁻¹ for SP-ICP-MS analysis, based on manufacturers data. For FFF analysis, NPs were used at concentrations of either the nominal concentrations of the stocks (See supporting information S1 Table 1) or diluted to 1,000,000 part. mL⁻¹, depending on detector and injection volumes used. It was found that the given particle concentrations and sizes were not always as the manufacturer stated and in some cases the particle numbers were higher or lower than what was expected.

Field flow fractionation (FFF)

An eclipse DualTech hollow fiber flow field-flow fractionation separation system (Wyatt technology, Santa Barbara, CA, USA) FFF was used in combination with a Agilent 1260 Infinity LC pump and auto sampler introduction system and an online UV detector (Agilent 1260 infinity, Agilent technologies, Santa Clara, Ca, USA).

A 10 KDa PES hollow fiber was used as a separation channel with a diameter of 0.8 mm and length of 17 cm (purchased from Wyatt). The detector flow was 0.5 mL min⁻¹, focus flow of 0.2 mL min⁻¹ for 10 minutes, and a ramped cross flow of 0.15-0 mL min⁻¹ over a 10 minute period the particles eluted between 0.1 and 0.6 mL min⁻¹ cross flow) and injection volumes of 2-20 µL. The carrier solution was a sodium citrate solution at a concentration of 0.2 mM.

Inductively coupled plasma mass spectroscopy (ICP-MS)

A PerkinElmer NexION 350D ICP-MS was used with a Meinhard nebulizer, cyclonic (standard) spray chamber, a 2 mm ID injector and a glass torch. A RF power of 1600 W, auxiliary gas flow of 1.2 mL min⁻¹ and nebulizer gas flow of 1.12 mL min⁻¹ was used for measurements. The sample uptake was 0.49 mL min⁻¹. For single particle measurements (SP-ICP-MS) a dwell time of 50 µs was used with 30 to 60 s second sampling times.

Standards

1, 2, 3 and 4 ppb ionic Au and Ag standards were used as ionic calibrations. NIST Au standards of 30 nm and 60 nm were used for Au particle calibrations at concentrations of 100,000 part. mL⁻¹. The NIST 60 nm Au particles were used to determine transport efficiency.

Results and Discussion

NIST standards of nominally 30 and 60 nm certified reference materials were used to quantify the accuracy of the SP-ICP-MS system (Data in Figure S2). The diameter and size distribution measured by TEM and quoted by NIST for the nominally 30 and 60 nm standards are slightly smaller than the nominal value: 27.6 ($\pm$ 2.1) and 56.3 ($\pm$ 1.5) nm respectively. Diameters of the same NPs measured in this study were 26.9 ($\pm$ 0.3) nm and 57.3 ($\pm$ 0.1), in excellent agreement with the stated values from NIST. The 60 nm CRM were measured over the course of the experiment, and the particle number concentrations for these suspensions were consistently within 1 % of those originally measured for the TE. This along with the consistency of the size showed gave confidence in the stability of the machine throughout the experiment. Additionally, the particle number concentrations of the 30 nm certified reference materials were within 3% of the values stated by NIST, showing that the particle number counting and particle sizing by SP-ICP-MS was in good agreement with well characterized NPs.

Six test NP suspensions purchased from commercial sources (Ag, Au and Au@Ag; all at 60 and 80 nominal sizes) were analyzed by SP-ICP-MS to derive the particle size and concentration of each individual suspension for later reference. The size histograms of the NIST samples (Figure S2 A and B) very monodisperse samples, while the Nanocomposix NPs have a higher degree of polydispersity, sometimes showing bimodal distributions (examples shown in Figures S3, S4 and S5). For instance, peaks at 60.9 ($\pm$ 0.1) nm and 47.5 ($\pm$ 0.1) nm for the nominally Au-60 nm NPs (S3-B), 73.5 ($\pm$ 0.1) nm and 59.6 ($\pm$ 0.4) nm for the Ag signal of the Au@Ag-80 nm particles (S4-C) and 78.1 ($\pm$ 0.1) nm and 60.4 ($\pm$ 0.1) nm for the nominally Au-80 nm NPs (S4-B) were all observed by SP-ICP-MS. In all cases, the larger peak is closest to the manufacturer supplied data (Table S5-1). It is also evident that the secondary peak contains a substantial number of the particles: 65.8%, 65.6% and 54.2% of the particles are related to the larger diameter NP

peak in the above examples. To simplify data analysis, we fitted a Gaussian curve to the size distributions and used these fits for data interpretation (seen in Figures S3 and S4).

Figure 1A and B show the measured particle diameters (Figure 1 A) and measured particle number concentrations (Figure 1 B) for the individual NP suspensions. The total particle diameters for the separate Ag or Au NPs are 73.5 (3.9) nm, 76.2 (0.2) nm, 54.7 (0.2) and 55.5 (0.3) nm for the nominally Au-80 nm, Ag-80 nm, Au-60 nm and Ag-60 nm particles respectively. The actual stated values from the manufacturers are 81.2 (10.5), 78.9 (10.5), 60.6 (5.9) and 59.6 (5.8), respectively. In general, the data is different, but not significantly so, largely due to the poor precision in the manufacturer's data.

SP-ICP-MS measures total number of ions generated from individual NP and does not account for geometry of the NP. Thus for bimetallic (core-shell) particles, two sizes are measured that assume a solid Au and a solid Ag NP (Discussion in supporting information S6). Accounting for the geometry (calculations can be found in Merrifield and Lead 2016²²), the number of ions for the Au and Ag solid NPs translate into a larger core-shell structured NP of 57.8 (± 0.3) and 74.3 (± 0.5) for the Au@Ag-60 nm and Au@Ag-80 nm respectively. These calculated sizes can be directly compared to the manufactures (TEM) measurements of 60.8 (± 6.3) and 78.2 (± 8.8), showing good agreement. In this case we knew the composition of the NP to have an Au core and Ag shell, however calculations can be made for the total size if the reverse were true (an Ag core and Au shell), or for an alloy NP of the same composition and minimal exploration with another technique such as STEM/EDS would be required to verify this.

Figure 1B shows the particle number concentrations for each of the particle suspensions. All of the suspensions were diluted such that their concentration was expected to be 100,000 particles

mL⁻¹ and then back calculate to the original concentrations. It is evident that the manufacturer's data for most suspensions is in agreement with our data, although data for the Au-80 and Ag-60 were not in agreement with the SP-ICP-MS and we corrected for this in subsequent analyses (Table S1). For the core-shell NPs, the number concentrations of both Ag and Au signals were identical (within 0.5% of each other), suggesting that the signals either come from the same bimetallic particle or equal amounts of separate monometallic particles, although the latter is unlikely. FFF data on the individual bimetallic suspensions showed only a single elution peak, indicating that both elements elute at the same time. Figure S7 shows the 80 nm AuAg NP suspension eluting in a tight peak less than a minute after the 80 nm standard polystyrene beads. There is also a clear separation between the 80 nm and 40 nm PS standards meaning that if the manufactures had not coated the Au cores correctly in Ag we would expect to see a signal eluting just after the 40 nm PS standard. Thus, confirming that the sample contains only a bimetallic (Au@Ag) NPs and not mixtures of Ag and Au NPs.

Mixtures of Au@Ag, Au and Ag NPs in SP-ICP-MS provide an extremely challenging test of the selectivity of the SP-ICP-MS. Typical SP-ICP-MS data for mixtures of the 80 nm particles and 60 nm particles are shown in Figure 2. The Au and Ag signals for the 80 nm mixture shown in Figure 2A and 2B respectively and the Au and Ag signals for the 60 nm mixtures are shown in Figure 2C and D respectively. The Gaussian fits for the single NP suspensions (taken from Figure S3 and S4) are shown. The dotted black lines represent the Gaussian fit for the monometallic NPs while the dashed lines show the bimetallic NPs. Figure 1 A and C show a good separation of the signal from the Au core particles at 46.0 nm (A dashed line) and 29.5 nm (C dashed line) and the Au monometallic particles at 76.2 nm (A dotted line) and 54.7 nm (C dotted line). This separation allows the size and concentration of the core particles (from the Au@Ag NPs) and the monometallic Au NPs to be quantified separately.

Figures 2 B and D are the Ag signals for the 80 nm mixture and 60 nm mixture respectively. In contrast to the Au signals, the Ag shell signal (dashed lines) and Ag monometallic NP (dotted lines) overlap and they cannot be distinguished on the basis of SP-ICP-MS alone. In general, to successfully separate two Gaussian distributions a resolution of 1.2 or above needs to be achieved²³, below this the distributions are difficult to distinguish. This equates to the distance between peaks needing to be at least twice the average of the full width half maxima of each peak to accurately separate the two particle distributions and be able to measure size and particle number concentration. This is achieved for the Au signal in our mixtures (figure 2A and C) but not in the Ag signals (Figure 2B and D).

Despite the lack of separation in the Ag signal it is still possible to calculate the particle number concentrations for all three NPs in the suspension using particle number counting. There is a large enough separation between the peaks in the Au signal to determine the particle number concentration for both the Au monometallic particle and the Au core from the bimetallic NP. The Ag data contains a component from both the Ag monometallic NP and Ag shell in the core-shell NPs. The particle number of the bimetallic NPs was calculated from the Au signal. By subtracting this signal this from the total Ag signal, the particle number concentration for the Ag monometallic NP can be calculated. By this process, it is possible to calculate the concentrations of all the NPs.

Subsequent to this initial analysis, five mixtures of these NPs suspensions were produced (see Methods for details). The NP diameters and number concentrations for the three mixtures containing Au 80 nm and Au@Ag 80 nm NPs are shown in Figure 3 A and B respectively. For these samples the Au signal contained information for both of the Au core and Au monometallic

NPs, while the Ag signal only contained information from the Ag shell. The total measured NP number concentration was used as a check to ensure that the particles were not being over or under-represented. In all mixtures, the total number concentration measured for the Au signal is within 0.5% of that measured for the smaller and larger peak combined, showing that all particles are counted once. In all mixture ratios of the Au and Au@Ag NPs, the particle sizes and concentrations were fully resolved to their respective components, as shown in Figure 3. The % of Au NPs that could be separated analytically from the Au@Ag NPs varied with Au:Au@Ag NP ratio. At 50:50 Au:Au@Ag 95% of both NPs were analytically separated. At 80:20 Au:Au@Ag 100% of the Au NPs were separated and only 84% of the Au@Ag NPs, while the reverse is true for the 20:80 Au:Au@Ag mixture. In figure 3A the particle diameters for the Au-80 nm, Au-core and Ag shell were all measured within 3% of those measured for the individual NPs. The particle number concentrations of the mixtures, after measurement and back-calculation from the mixtures were not significantly different from their expected values, based on Figures 1 and 2 and the mixture ratios used. Although mixtures in this case could be satisfactorily resolved, the challenge in back calculating to the true individual values from a mixture becomes more pronounced where the mixture ratios are high (or low) and where the NP samples are polydisperse. For these bimodal suspensions and at these mixture ratios, the actual and expected values are in good agreement, with < 20% difference in size and number concentration.

Figure 4 shows the measured sizes (Figure 4 A) and calculated particle numbers (Figure 4 B) for the 80 nm and 60 nm mixed NP suspensions i.e. a three component mixture of the Ag, Au and Au@Ag NPs. The Au core and NP can be quantitatively resolved due to their differences in size. However, the Ag signal cannot be resolved as the Ag monometallic NPs and the Ag shell of the Au@Ag NPs are too similar. Neither the Au-monometallic NPs nor the total diameter for the

Au@Ag NPs could be distinguished and calculated. However, the particle number concentrations for all three particles were calculated from the acquired data and are shown in figure 4B. The measured particle number concentrations for the 80 nm mixture are shown on the left hand side of the graphs in figure 4A and B respectively. The particle numbers concentrations for the 80 nm mixture were approximately 66,100, 53,400 and 70,000 particles mL^{-1} for the Au, Ag and Au@Ag particles respectively. These particle number concentrations are within 5% of the original added amounts (64,600, 53,900 and 73,300 part. mL^{-1}), showing excellent agreement. For the nominally 60 nm particles, concentrations were within 7% of the expected values (107,000, 65,800, 37,800 part. mL^{-1} for the Au-60 nm, Ag-60 nm and Au@Ag-60 nm particles compared to the expected values of 100,000, 66,000 and 40,000 part. mL^{-1}). The measured values for the particle sizes and the measured and corrected values for the particles number concentrations can be found in S8.

Conclusions

In summary, we have shown by FFF that commercially brought monometallic (Au and Ag) NPs and bimetallic (AuAg) NPs nominally of the same size elute together and have the same size distribution. We have also shown that FFF alone is unable to distinguish between either individual NPs or mixtures of these NPs. It was also shown that the SP-ICP-MS system is capable of collecting data on size and number concentration of complex mixtures of metal and core-shell metal NPs. The obtained data resolution shows that this method is an excellent method for NP metrology in complex systems, capable of adding substantial analytical capability to established multi-method approaches.¹⁷

ACKNOWLEDGMENT

The authors would like to acknowledge the National Science Foundation (1508931) along with PerkinElmer and the Center for Environmental Nanoscience and Risk, University of South Carolina, for financial support.

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Figure 1A and B. Particle diameters and concentrations for six nanoparticles Au@Ag-80 nm, Au-80 nm, Ag-80 nm, Au@Ag-60 nm, Au-60 nm and Ag-60 nm as measured by SP-ICP-MS are shown. All particles suspensions were diluted to 100,000 part. mL⁻¹ based on the data provided by the manufacturer. The 'total diameter' is SP-ICP-MS data collected in this study, accounting for the core-shell geometry. Manufacturer's data is from by transmission electron microscopy, although no data on sample preparation was provided. Figure 1B show the measured particle numbers from SP-ICP-MS

Figure2. Figure A and B show the Au and Ag signal for a NP mixture containing the nominally Au-80 nm, Ag-80 nm and Au@Ag-80 nm particles respectively. Figure 2 C and D show the Au and Ag signal for a NP mixture containing the nominally Au-60 nm, Ag-60 nm and Au@Ag-60 nm particles. The dotted black curve corresponds to the expected signal from the monometallic particles and the dashed black curve shows the Gaussian curve from the bimetallic (core-shell) particles.

Figure 3A and B: show particle diameters and particle number concentrations for three particle suspensions as measured from SP-ICP-MS. All three suspensions contain Au@Ag-80 nm and Au-80 nm particles with different ratios of particle number concentrations of 1:4 (20,000:80,000), 1:1 (50,000:50,000) and 4:1 (80,000:20,000) part. mL⁻¹. The total particle diameter for the Au@Ag-80nm particle was calculated from the measured Au and Ag signal and is marked with an *.

Figure 4A and B: show particle diameters and particle number concentrations for two particle suspensions as measured from SP-ICP-MS. The first suspension, 80 nm mixture, contains Au-80 nm, Ag-80 nm and Au@Ag-80 nm NPs. While the second mixture, 60 nm mixture, contains Au-60 nm, Ag-60 nm and Au@Ag-60 nm NPs. In Figure 4A the diameters of the Au monometallic particles and the Au core from the Au@Ag NPs can be measured accurately but the Ag signals from the monometallic and bimetallic particles are too close to resolve which signal belongs to which particle. However from the data collected all particle numbers can be calculated and can be seen to be close to the expected values in Figure 4B

Mixed solutions

FFF separation

SP-ICP-MS signal