



Single Particle-Inductively Coupled Plasma Mass Spectrometry of Metal-Containing Nanomaterial in Surface Waters Around Las Vegas, Nevada, USA

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Background

Engineered nanomaterials (ENMs) are the building blocks of the nanotechnology revolution.

- They are defined (somewhat arbitrarily) as manufactured materials with at least one dimension between 1 nm and 100 nm.
- The predominant classes are carbon-based ENMs (fullerenes and carbon nanotubes), metal-containing ENMs (e.g., metals – Ag, Au, Pd & Fe, metal oxides – TiO₂, ZnO, CeO₂) and quantum dots (e.g., CdSe).
- Dimensions of ENMs give them unique physical, chemical, electrical, and optical properties that are dissimilar to the same material in bulk or dissolved form.
- These unique properties allow novel applications and new technologies in industry, medicine, and consumer products.
- Manufacturing and use of ENMs is ever increasing.
- Uses of metal-containing ENMs are often dispersive, resulting in significant releases to the environment.
- The unique properties of ENMs affect their environmental behavior.
 - Mobility, bioavailability, bioaccumulation and bioaccumulation, and potential adverse effects cannot be predicted.
 - ENMs are often manufactured or aggregated into complex size distributions, each size fraction potentially having different environmental behavior.
- Research into the environmental health and safety (EHS) of ENMs is expanding rapidly, in the scientific community, ENM manufacturers, and national and international regulatory agencies.
- Methods to measure ENMs in environmental media are critical for any EHS research, but they are hampered by several factors:
 - Metrics other than mass concentration are needed – at the least, particle concentration and size.
 - Expected concentrations in environmental media are very low.
 - Many natural and incidental anthropogenic nanoparticles confound analysis.
- Scanning or transmission electron microscopy (SEM, TEM) with elemental analysis by X-ray fluorescence can conclusively identify some ENMs, but quantitative analysis is precluded by non-representative sampling. Neither SEM nor TEM can be routinely applied to large numbers of samples.
- Hyphenated techniques can provide representative sampling. For metal-containing ENMs, flow-field flow fractionation (FFFF) separates sizes by hydrodynamic radii, while ICPMS is usually coupled to FFFF to provide element-selective detection.
 - FFFF-ICPMS measures the total concentration of a metal associated with nanoparticles of a certain hydrodynamic radius.
 - It cannot differentiate between the many chemical forms of a metal, so it is not a definitive analytical method.
 - It could screen samples for possible metal-containing ENMs; however, FFFF separations require at least 20-30 minutes, limiting the viability of FFFF-ICPMS for screening large numbers of samples.
- Single particle (SP)-ICPMS was investigated by Deguelle et al. for the analysis of metal colloids [1,2], and applied by others to characterizing ENMs [3-6]. This laboratory has investigated the effect of experimental conditions on the performance of SP-ICPMS [7].
- ENM characterization by SP-ICPMS is complementary to SEM, TEM, or FFFF-ICPMS.
 - SP-ICPMS measures the concentration of nanoparticles containing a metal, as well as the mass of the metal in each particle.
 - It is a single particle method like SEM and TEM, but sampling is representative.
 - It cannot directly measure nanoparticle size, as FFFF can, but it can determine the metal mass in each particle; so like FFFF-ICPMS, SP-ICPMS could potentially be used to screen for the possible presence of ENMs.
 - SP-ICPMS is fast, facilitating screening of large numbers of samples.

Rationale and Goal

- Given the lack of viable methods to selectively and quantitatively determine metal-containing ENMs in environmental media, monitoring spatial and temporal trends in concentrations of all nanoparticles containing common ENM elements may be the best approach to detecting increasing loadings of ENMs in the environment.
- This study evaluates sampling and sample-handling methods for a planned research program to evaluate such a screening approach for surface water receiving effluent from waste water treatment plants in Las Vegas, NV, USA. Preliminary results on concentrations of nanoparticles containing several metals are presented.

Experimental

Materials

- Gold and silver nanoparticles were obtained in monodisperse aqueous suspensions of known particle concentration from CorpuScular, Inc. (www.microspheres-nanospheres.com).
- Gold ENMs of nominal 30-nm, 50-nm, 80-nm, and 200-nm diameter were used. Silver ENMs were 40-nm, 60-nm, and 80-nm diameter. Sizes of the ENMs were confirmed by TEM and nanoparticle tracking analysis (NTA). TEM also confirmed spheroidal shapes (Figure 1).
- Nylon membrane syringe filters (13-mm diameter) were obtained from Whatman (Puradisk™, 0.45-µm pore size, www.whatman.com) and Steritech (5.0-µm pore size, www.steritech.com).
- Polystyrene centrifuge tubes with polypropylene caps (Falcon™, Becton, Dickinson & Co, www.bd.com) were used for working standards and samples.

Methods

- Water samples were collected at the Flamingo Wash, 36° 6.826' N, 115° 8.741' W, ca. 2 km east of Las Vegas Boulevard.
- 10 mL of water was collected just below the surface with a syringe.
- Spikes of gold and silver ENMs were added to some samples before filtering.
- Samples were diluted to 45 mL before analysis to reduce the 1.5 mg/mL total solids in the filtered samples. Samples were analyzed within 3 hours of sampling.
- Initial optimization of SP-ICPMS analysis procedures was conducted on a PerkinElmer DRCe ICPMS (www.perkinelmer.com). SP-ICPMS analyses were conducted in single-element time resolved analysis (TRA) mode with dwell times of 0.3 ms to 10 ms. Time-resolved signals were accumulated in 20 segments of 550 data-point segments (the maximum acquisition size allowed at dwell times < 10 ms).
- Sample handling procedures were evaluated and field samples were analyzed on an Agilent 7500c, with cx ion optics (www.agilent.com). Analyses on the 7500c were run in multi-element TRA mode with dwell times of 1 ms to 10 ms. SP-ICPMS signals were acquired for total acquisition times of 50 or 400 s.
- Conditions on both instruments were optimized according to factory recommendations for multi-element tuning solutions.
- ICPMS TRA data was transferred to Microsoft Excel™ spreadsheets for analysis. Initial data inspection was performed on the number of ions detected/dwell window vs. time. Data points for each element were then sorted by ions detected/dwell window.
- A cumulative distribution function (CDF) for the number of ions detected/dwell window was constructed by plotting the fractional rank of each sorted dwell window vs. the number of ions detected in that window. The cumulative distribution for a completely monodisperse suspension with an ideal detector and zero background would be a delta function from the minimum fractional rank to 1 at the expected number of ions detected for that mass of element per particle.

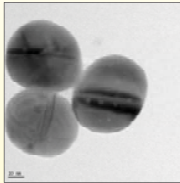


Figure 1. TEM of 60-nm Ag.

Calibration

SP-ICPMS detects the fast signal pulses produced by ion plumes of nanoparticles. It yields two metrics for metal-containing nanoparticles – the particle concentration and the metal mass in each particle. Calibration for both depends on two properties of the ICPMS signal for nanoparticles:

- ICPMS sensitivity is constant for an analyte, irrespective of whether it is dissolved or contained in nanoparticles, assuming the residence time in the plasma is sufficiently long.
- Every nanoparticle that reaches the plasma is detected. This also depends on sufficiently long residence time, so the ion plume expands enough to substantially fill the cross section of the central channel of the plasma.

The second property assures the validity of equation (1):

$$(1) \ q_i / c_p = q_i \epsilon_n$$

where q_i = flux of particles detected in plasma (s⁻¹), c_p = concentration of nanoparticles containing the detected metal in the sample (mL⁻¹), q_i = sample uptake rate (s⁻¹), and ϵ_n = nebulization efficiency.

Method performance

Suspensions of gold ENMs with nominal diameters of 30-, 50-, 80-, and 200-nm were analyzed by SP-ICPMS. Equations (1) and (2) were used to calculate the mean m_i for each ENM, and the corresponding spherical diameters were plotted in Figure 2 against the nominal values, which agreed within experimental error with TEM and NTA measurements. The accuracy of the diameters determined by SP-ICPMS demonstrates the validity of the two assumed properties above for gold nanoparticles up to 200-nm diameter.

The precision of SP-ICPMS for both nanoparticle concentration and elemental mass in single particles was evaluated by replicate analyses of a suspension of 50-nm gold ENM at a concentration of 4,000 mL⁻¹. The cumulative distribution functions obtained are shown in Figure 3, and the statistics are presented in Table 1.

- The mean number of ions detected for all the particles, which is proportional to elemental mass in the particle, has a relative standard deviation of about 2%.
- Elemental mass is proportional to the cube of the particle article diameter, so the resulting relative standard deviation in the calculated number-mean diameter is less than 0.8%.
- The relative standard deviation of the nanoparticle concentration at 4,000 mL⁻¹ is about 10%, which is what would be expected from counting statistics.

These data demonstrate that SP-ICPMS can be used to characterize the size distribution of ENMs if the elemental composition is known. Other methods for determining size distributions of suspensions, such as dynamic light scattering (DLS) and NTA, cannot be used below particle concentrations about 1,000 times greater than the concentrations analyzed here by SP-ICPMS.

SP-ICPMS can also characterize the size distribution of a known ENM in the presence of other particles, which is advantageous in characterizing experimental test media. Replicates 4 and 5 of the precision study were analyzed in the presence of a greater than 10,000-fold excess of silver nanoparticles. Alternative size characterization methods like DLS and NTS could not be used for such mixtures, while Figure 3 and the statistics in Table 1 demonstrate that SP-ICPMS yields accurate and precise measurements.

It should be noted, however, that for complex environmental matrices encountered in field studies, SP-ICPMS cannot distinguish between nanoscale materials containing the same element. In these studies, SP-ICPMS used alone is a screening-level method that can 1) indicate the possible presence of a particular ENM and 2) measure all particles containing an element, and track spatial and temporal trends in those particles.

Sample handling

Because SP-ICPMS does not directly measure nanoparticle size, fractionating a surface water sample by particle size prior to analysis would be beneficial in characterizing the particle content. Size fractionation by filtering must be carefully evaluated for the potential of altering the nanoparticle size distribution. Filtering of reagent water suspensions of 50-nm gold nanoparticles was passed through a 0.45-µm pore size syringe filter and recovery was evaluated.

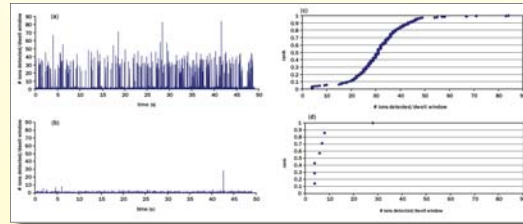


Figure 4. SP-ICPMS of 50-nm diameter gold nanoparticles (1 x 10⁴ mL⁻¹): (a) time-resolved signal of unfiltered suspension, (b) signal of filtrate suspension - 0.45 µm nylon filter, (c) CDF of unfiltered suspension, (d) CDF of filtrate suspension - 0.45 µm Nylon filter.

Results and Discussion

Because $q_i \epsilon_n$ is independent of analyte, it can be calculated from equation (1) using just one standard of known nanoparticle concentration, regardless of analyte.

Equation (1) can be rearranged to calibrate nanoparticle concentration from detected particle flux. Constant sensitivity, irrespective of analyte form, then permits the use of the analysis of a dissolved ICPMS standard to calculate the metal mass in each nanoparticle, m_{sp} , from equation (2):

$$(2) \ m_{sp} = n_i \ c_s \ q_i \ \epsilon_n / (q_i \ A_{n_i})$$

where n_i = number of ions of an analyte detected in an ion plume (number of ions detected in a dwell time containing a nanoparticle ion plume), c_s = the analyte concentration in a dissolved standard of the analyte (g mL⁻¹), q_i = ion flux measured for the dissolved standard, and A_{n_i} = relative abundance of the isotope measured.

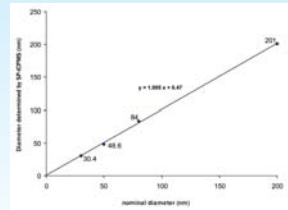


Figure 2. Calibration of gold ENM sizes. Data points are labeled with the diameters determined by SP-ICPMS and Equations (1) and (2). The ENM concentration was 1x10⁴ mL⁻¹.

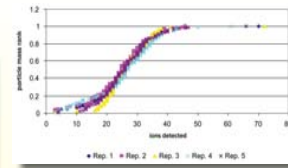


Figure 3. CDFs for 5 replicate SP-ICPMS analyses of 50-nm Au ENM, particle concentration = 4x10⁴ mL⁻¹ (5 µg L⁻¹).

- Comparison of Figure 4 (a) and (b) shows that the 0.45-µm filter retained > 98% of the 50-nm particles, even though its nominal retention size is 9 x larger.
- While the cumulative distribution function of the unfiltered suspension [Fig. 4(c)] exhibits a relatively narrow nanoparticle distribution about a median of 31 ions detected, the CDF of the filtrate [Fig. 2(d)] shows that larger particles are preferentially retained by the filter.
- These results are consistent with physical retention in the pores of the filter bed, as opposed to adsorption of gold on the nylon filters.
- Greater than 95% of either 50-nm diameter or 200-nm diameter gold nanoparticles were recovered in the filtrate when a reagent water suspension was passed through a 5.0-µm filter (data not shown), so these filters were used in field sampling. This provided a modicum of size fractionation, reduced the total solids in the analyzed samples by about 65%, and protected the concentric nebulizer used.

Recoveries of gold and silver ENMs were also evaluated in Las Vegas Wash water samples. The water was spiked to a concentration of 5.5x10³ mL⁻¹ of each ENM and then passed through the 5.0-µm nylon syringe filter.

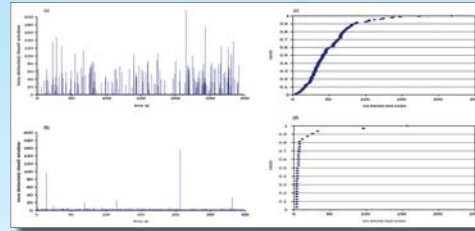


Figure 5. SP-ICPMS of 60-nm silver ENMs - (a) time resolved signal of 5x10³ mL⁻¹ spiking solution, (b) signal of Las Vegas Wash water spiked to 5.5x10³ mL⁻¹ and then passed through 5.0-µm filter, (c) CDF of 5x10³ mL⁻¹ spiking solution, (d) CDF of spiked and filtered sample.

SP-ICPMS analyses of water from Las Vegas Wash

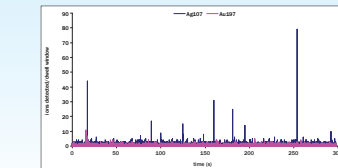


Figure 6. Ag and Au particles in Las Vegas Wash - 10 ms dwell time, 150 s acquisition/element.

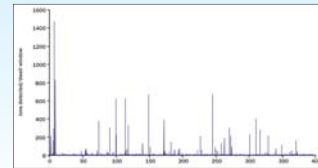


Figure 7. Ti particles in Las Vegas Wash - 3 ms dwell time, 28.5 s acquisition.

Nano-scale silver is being increasingly used as a microbicide in a number of consumer products, as well as in nutritional supplements. Because of its anti-microbial properties, and because dissolved silver can be toxic to aquatic organisms, the concentration of nano-scale Ag in surface water is of interest. Figure 6 shows the presence of silver-bearing particles with Ag mass equivalent to about 50-nm diameter metallic Ag particles. The particle concentration is approximately 200 mL⁻¹, not correcting for the low recovery. By contrast, the recovered gold-bearing particle concentration may be on the order of 10 mL⁻¹.

Titanium dioxide is a nano-scale material that is being increasingly used for its photocatalytic properties, as well as its use as a pigment. Figure 7 shows approximately 400 mL⁻¹ of titanium-bearing particles above an equivalent TiO₂ diameter of about 60 nm. The largest particle is equivalent to about 400 nm TiO₂.

Nano-scale cerium dioxide is registered in the U.S. in diesel fuel additives to increase efficiency and reduce emissions, but it is not yet widely used in this country. Cerium-bearing particles were observed in the Las Vegas Wash samples, but as can be seen in Figure 8, other lanthanoids and yttrium that usually occur in natural deposits with cerium were also present, so the source of the cerium is likely natural.

Figure 9 illustrates one effect of reducing dwell times in SP-ICPMS. The dissolved iron and molecular iron background at m/z 57 is reduced proportionally when the dwell time is reduced from 10 ms to 3 ms. Because the background signal at 10 ms is about 3 x 10³ s⁻¹, the noise is also reduced by about 2/3 when the dwell time is switched to 3 ms. Particle pulses can more clearly be seen at the shorter dwell time, improving the particle mass detection limit.

The drawback of very short dwell times can be seen in Figure 10. As the dwell time is reduced from 10 ms, the cumulative distribution function broadens, and more very small pulses are detected. This is an artifact of the discontinuous sampling of current ICPMS instrumentation, as discussed elsewhere [7].

Conclusions and Ongoing Work

- SP-ICPMS has been applied to detecting metal-bearing particles in surface water.
- In the stand-alone mode used here, SP-ICPMS can only screen for the possible presence of metal-based ENMs, because it cannot directly measure particle size.
- Simultaneous analysis of 14 elements was conducted in a single 400 s acquisition, with a particle concentration detection limit of 60 mL⁻¹ (1 pulse in a 28.5 s acquisition on each element with $q_i/c_s = 0.0006$).
- Recoveries from filtered samples, even for nanoparticles much smaller than the filter pore size, were low, and inversely related to particle size.
- Current efforts are addressing the recovery issue.
- A field study of metal-bearing particles in effluent from waste water treatment plants will begin this winter.
- Coupling SP-ICPMS to FFFF will be used as a second-tier analysis in the waste water field study.

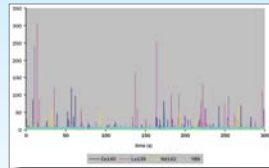


Figure 8. Lanthanoid and yttrium particles in Las Vegas Wash.

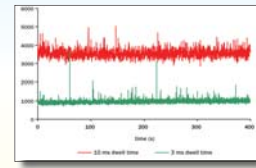


Figure 9. SP-ICPMS signals at m/z 57 at two dwell times.

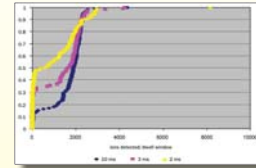


Figure 10. Effect of dwell time on cumulative distribution function.

References

- [1] C. Deguelle, P.-Y. Favarger, 2004, Talanta 62, 1054-1054.
- [2] C. Deguelle, P.-Y. Favarger, S. Weld, 2006, Analytica Chimica Acta 555, 263-268.
- [3] M. Hasselöv, 1st International Workshop on Analytical Nanoscience & Nanotechnology, Vienna, 2007.
- [4] E. K. Lesher, S. Lee, J. F. Ramville, 42nd Western Regional Meeting American Chemical Society, Las Vegas, 2008.
- [5] E. M. Heithmar, SETAC North America 30th Annual Meeting, New Orleans, LA, November 19-23, 2009.
- [6] M. Hasselöv, J. Tushnet, SETAC North America 30th Annual Meeting, New Orleans, LA, November 19-23, 2009.
- [7] E. M. Heithmar, 237th American Chemical Society National Meeting & Exposition, Salt Lake City, UT, March 22-26, 2009.

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