

Nanomaterials in the Environment

DETECTING NANOPARTICULATE SILVER USING SINGLE-PARTICLE INDUCTIVELY COUPLED PLASMA–MASS SPECTROMETRY

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Abstract—The environmental prevalence of engineered nanomaterials, particularly nanoparticulate silver (AgNP), is expected to increase substantially. The ubiquitous use of commercial products containing AgNP may result in their release to the environment, and the potential for ecological effects is unknown. Detecting engineered nanomaterials is one of the greatest challenges in quantifying their risks. Thus, it is imperative to develop techniques capable of measuring and characterizing exposures, while dealing with the innate difficulties of nanomaterial detection in environmental samples, such as low-engineered nanomaterial concentrations, aggregation, and complex matrices. Here the authors demonstrate the use of inductively coupled plasma–mass spectrometry, operated in a single-particle counting mode (SP-ICP-MS), to detect and quantify AgNP. In the present study, two AgNP products were measured by SP-ICP-MS, including one of precisely manufactured size and shape, as well as a commercial AgNP-containing health food product. Serial dilutions, filtration, and acidification were applied to confirm that the method detected particles. Differentiation of dissolved and particulate silver (Ag) is a feature of the technique. Analysis of two wastewater samples demonstrated the applicability of SP-ICP-MS at nanograms per liter Ag concentrations. In this pilot study, AgNP was found at 100 to 200 ng/L in the presence of 50 to 500 ng/L dissolved Ag. The method provides the analytical capability to monitor Ag and other metal and metal oxide nanoparticles in fate, transport, stability, and toxicity studies using a commonly available laboratory instrument. Rapid throughput and element specificity are additional benefits of SP-ICP-MS as a measurement tool for metal and metal oxide engineered nanoparticles. *Environ. Toxicol. Chem.* 2012;31:115–121.

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Nanoparticulate silver (AgNP) is currently incorporated into myriad industrial and medical products, with more manufacturer-identified consumer products than any other nanomaterial [1]. The aim of these commodities is to take advantage of the antiseptic properties of silver (Ag), because it is a wide-spectrum biocide. Many of these products bring AgNP directly into contact with the human body [2], and all have the potential to disperse AgNP to the environment during and after their manufacture and use [3–5]. Despite the rapid progress and early acceptance of nanotechnology, adverse ecosystem effects from the inadvertent release of AgNP have not yet been established.

To provide adequate oversight and to forecast the risk that AgNP may pose, environmental exposure concentrations must be established. Mueller and Nowack [6] initially concluded from a materials flow analysis that AgNP likely would not harm aquatic ecosystems. However, an updated approach concluded that the ratio of predicted environmental concentration to predicted no-effect concentration, the risk quotient, for AgNP will be much greater than one in wastewater treatment plant effluents in Europe, the United States, and Switzerland and slightly greater than one in surface waters in Europe and

Switzerland [7]. Focusing on a different mechanism of impairment, Blaser and colleagues [8] found that ionic silver (Ag⁺) released from AgNP-bearing plastics and textiles may also be substantial enough to result in predicted environmental concentration to predicted no-effect concentration ratios greater than one. Given that AgNP is commonly used in products that are washed or disposed of in wastewater, wastewater treatment plant effluent is a logical matrix for developing methods for environmental monitoring for the emergence of AgNP.

In freshwater systems, Ag⁺ has long been recognized as a tracer of anthropogenic pollution [9] that is toxic to a wide variety of organisms [10], including bacteria [11,12]. In fact, AgNP are used because of their bactericidal properties. Less is known about the occurrence and direct toxicity of engineered AgNP. The formation of reactive oxygen species may be one mechanism by which AgNPs are also toxic [13,14]. However, several possible mechanisms exist by which AgNPs can inhibit microbial growth that may vary from those of Ag⁺, such as impairment to cell membrane architecture [11,15]. The biological impacts of nanomaterials and the biokinetics of nanoparticles [13] depend on nanoparticle size [16–18], chemical composition, surface structure [19], solubility, shape [15], and aggregation [10]. Confounding the issue is that AgNP toxicity is postulated to be linked to the release of Ag⁺ [20] and to be highly dependent on a host of environmental parameters, including concentration of ligands, interactions with organic matter, ionic strength, and pH [21]. These factors are considered important because of their influence on aggregation and settling, but it has been suggested that toxicity mechanism can be directly affected [22,23].

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All Supplemental Data may be found in the online version of this article.

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Several recent reviews [10,24–26] have identified an important issue: the lack of appropriate detection, characterization, and quantification methods for inorganic nanomaterials in environmental samples. Separation methods for particles and ions, which include dialysis, filtration, and ultracentrifugation [26,27], are often coupled with analysis methods. Filtration is the most common, although problematic, approach. Augmentation of available analytical techniques to detect and quantify AgNP and released dissolved Ag in both environmental and biological media is highly warranted. Ionic silver is highly reactive and will readily complex with sulfide ($\log K=50.1$), chloride ($\log K=9.7$), and other ligands that may mitigate toxicity [28]. For example, nanosized Ag sulfide ($\alpha\text{-Ag}_2\text{S}$) has recently been identified in final stage sewage sludge materials [29] as had been previously hypothesized because of the high binding constant for the surfaces of treatment plant particles. Impellitteri et al. [30] found that Ag^+ leaching from consumer products may be scavenged by chloride present to form the much less reactive form AgCl [30]. The formation of these nanoparticulate forms is a complicating factor if the goal is to determine the fate of engineered elemental AgNP released from products. The aggregation behavior and hydrologic transport of all forms of AgNP is likely to be similar and will depend on surface composition and particle size. However, toxicity and solubility of the various forms may be quite different and requires further investigation.

Because of its element-specific detection and high sensitivity, inductively coupled plasma–mass spectrometry (ICP-MS) is ideal to study AgNP and other inorganic nanoparticles. The operation of ICP-MS in the single-particle mode (SP-ICP-MS) provides a means of detecting individual nanoparticles. Single-particle ICP-MS relies on the extremely sensitive metal detection capability of ICP-MS, but, in contrast to traditional ICP analysis techniques, thousands of individual intensity readings are acquired, each with a very short dwell time (10–20 ms). Instead of measuring metal concentration representative of the bulk sample, the intensity readings can be collected as a function of time, where pulses above the background represent the measurement of an individual nanoparticle. Versions of the SP-ICP-MS concept have been used to measure metals in airborne particles [27] and to measure aerosols directly [31]. Degueldre and colleagues [32–34] measured laboratory-synthesized colloids in suspension and additionally developed a theoretical link between intensity and particle size. This relationship involves several poorly known parameters. Most important is the fraction of sample actually reaching the plasma. This is the topic of ongoing work, whereas the primary objective of this study was to collect data to illustrate the use of SP-ICP-MS to detect AgNP in unprocessed environmental waters. The specific aims were to ensure that nanoparticles can be analyzed without prior acid digestion by ICP-MS, to examine the deficiencies of the traditional filtration approach for AgNP characterization, to demonstrate definitively that pulses in SP-ICP-MS data are quantitatively related to nanoparticles, and to test the applicability of the method to measure the presence of AgNP in wastewater treatment plant effluent.

MATERIALS AND METHODS

Silver nanoparticles were acquired in sizes of 20, 60, and 100 nm (NanoComposix). Suspensions were supplied at a nominal concentration of 20 mg/L Ag and were stabilized in aqueous 2 mM citrate per the manufacturer. Accompanying size information (dynamic light scattering and transmission electron microscopy) verified these particles to be monodisperse, the

sizes being 20 ± 1.9 nm, 60 ± 5.3 nm, and 100 ± 6.3 nm. ASAPTM, a colloidal Ag consumer product marketed as a dietary supplement (American Biotech Labs), was also used. The product was polydisperse in size, as demonstrated by transmission electron microscopy (Supplemental Data, Fig. S1). Total Ag concentration was 10.3 mg/L and contained a variable dissolved Ag fraction dependent on lot but appeared to be very stable over time and with dilution. Nanoparticulate silver suspensions were made by diluting the stock solutions with 18.3 MOhm nanopure water to final concentrations ranging from 10 to 1,000 ng/L. Dissolved Ag standards (high-quality standards; QC-7-M), used for calibration, were diluted in 1% nitric acid (optima grade) to concentrations ranging from 0.1 to 1 $\mu\text{g/L}$. For the filtration comparisons, disposable filter membranes (25 mm) including Target[®] (National Scientific Company) nylon (0.2- μm and 0.45- μm pore sizes) and Whatman Anotop alumina (0.02- μm , 0.1- μm , and 0.2- μm pore sizes; Whatman International) were used. Samples were hand filtered by filling polypropylene 30-ml syringes (National Scientific) with the sample and pushing it into polystyrene 15-ml centrifuge tubes (BD Falcon).

Two wastewater effluents were investigated during the course of the present study. The first was collected from a municipal treatment plant in Black Hawk, CO, USA. Neither dissolved Ag nor AgNP was detected (estimated detection limit = 0.023 $\mu\text{g/L}$), and these samples were used for spiking experiments. A second treatment plant, located in Boulder, CO, USA, was also sampled. Preliminary sampling and historical effluent data spanning the last 20 years demonstrated the presence of Ag in the effluent. Both primary influent and final effluent samples were collected on March 12, 2009. Grab samples were taken and transported to the laboratory, where they were stored unfiltered at 4°C until analyzed by SP-ICP-MS.

A PerkinElmer Elan 6100 ICP-MS was used for all Ag analyses. Operating conditions are provided in the Supplemental Data. There was an online addition of 1% HNO_3 prior to nebulization into the spray chamber and into the plasma. Mass ^{107}Ag was monitored for detection, with an integration dwell time of 20 ms per reading. Consequently, a typical scan of 10,450 measurements corresponded to a run time of approximately 4 min. The dwell time per reading, readings per replicate, and total analysis time can be varied but may be limited by computing power and software capability. Longer analysis times give more data points and use more sample volume. The length of the dwell time contributes significantly to the minimum detectable particle size obtained with the method. Intensity data were recorded using PerkinElmer Elan software (version 2.4) and exported to Excel (Microsoft) for data handling and processing.

Instrument calibration was achieved by analysis of a blank and four dissolved Ag solutions ranging from 0 to 1 $\mu\text{g/L}$ in the single-particle mode. The ^{107}Ag intensity output of Ag for each solution was then averaged from the entire length of the standard analysis. No internal standard was employed, because only ^{107}Ag was detected during the run. To ensure the absence of significant instrumental drift over time, a 100 ng/L Ag dissolved standard was run in single-particle mode for every 10 AgNP samples analyzed.

In traditional ICP-MS analysis, a solution containing a dissolved metal will give a stable intensity-versus-time signal, the magnitude of which is proportional to the concentration of the metal. For dissolved metals, this is still the case when performing an analysis in SP-ICP-MS mode. In contrast, when a metal-bearing particle is ablated in the plasma during

SP-ICP-MS analysis, a pulse of ions is generated that results in a signal greater than the constant dissolved background. Thus, the signal should be steady at or near baseline until a particle passes through the plasma and creates a deviation during a single dwell time that is above the dissolved Ag background. The concept, and a comparison with traditional ICP-MS analysis, is illustrated in Figure 1. Observation of pulses is evidence of either Ag-bearing nanoparticles or Ag^+ in association with other (not primarily Ag) nanoparticles.

Dissolved Ag, regardless of concentration, will produce a stable signal with few pulses. Conversely, a range of intensities will typically occur in a sample containing AgNPs, some being only slightly greater than the background. Distinguishing between dissolved background and pulses is not a trivial task. To qualify a given intensity as a pulse, we developed an iterative algorithm. The 3σ value of all the data is calculated first and added to the average. Data points having values greater than this value are considered to be due to nanoparticles and are consequently removed. This process is repeated with the remaining data until no more pulses can be differentiated. The intensities of the pulses can be integrated to determine the concentration of AgNP. The values remaining in the data set are averaged and are considered dissolved Ag. A more detailed description and graphic depiction of this method can be found in Supplemental

Data, Figure S2. The authors note, however, that the term “dissolved Ag” is used operationally in this context to refer to both Ag^+ and any AgNP that are smaller than those that can be distinguished as AgNP by the SP-ICP-MS method.

From the previous discussion, it can be seen that determining the amount of Ag in the dissolved and nanoparticulate form is rather direct. However, quantifying nanoparticle number, concentration, and size is more difficult. Calculations rely on quantifying the efficiency of the ICP-MS sample introduction system, primarily that of the spray chamber. Although the concentration of particles is qualitatively proportional to the number of pulses observed during a run, to be more quantitative the number of pulses must be divided by the efficiency. The efficiency will depend on both the nebulizer/spray chamber design and the instrumental operating conditions, but it is generally less than 10%. Continuous signals (dissolved) are not adjusted because the efficiency is already captured by the fact that the dissolved calibration standards are presumably affected by the same sample introduction system efficiency. However, only about one in 10 particles in a sample will be detected, so the nanoparticulate data (number, total nanoparticle mass) should be multiplied by approximately 10.

By comparing pulse intensity measurements with a calibration curve derived from dissolved standards, run under the same

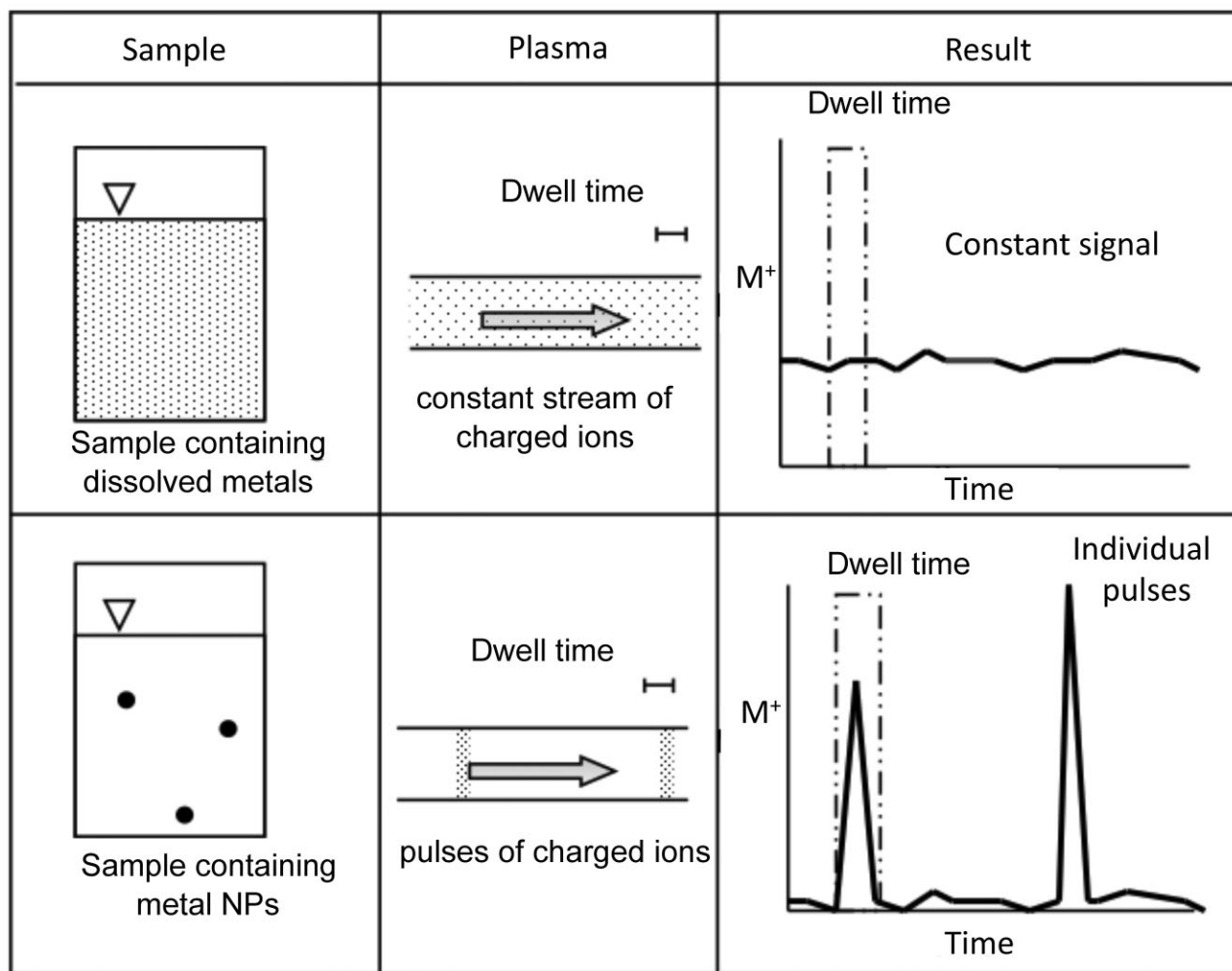


Fig. 1. Conceptual diagram for the single particle inductively coupled plasma–mass spectrometry (SP-ICP-MS) method. Samples containing dissolved metals will produce a constant stream of charged ions after passing through the plasma. The detector will then have a relatively constant intensity versus time signal for each dwell time. Conversely, a sample containing inorganic nanoparticles at a sufficiently low concentration will create a pulse of charged ions after passing through the plasma. Here, a resulting spike in intensity versus time will occur for dwell times that contained nanoparticulate metal.

conditions, intensity readings are converted to Ag concentration of the pulse. The relationship between the measured pulse concentration and the mass of metal in the nanoparticle is

$$C = \frac{m_p}{V_d} = \frac{\chi \times \rho \times V_p}{q \times t_d} \quad (1)$$

where C is measured pulse concentration ($\mu\text{g/L}$), m_p is mass of metal in the nanoparticle (μg), V_d is volume analyzed during one dwell time, χ is the fraction of analyte metal in the nanoparticle (unitless), ρ is the density of the analyte metal ($\mu\text{g/L}$), V_p is the volume of the nanoparticle (L), q is the sample flow rate (L/s), and t_d is the dwell time (s). After the concentration in each Ag pulse is determined, the background is then subtracted from the pulse signal to determine the concentration in only the pulse.

Assuming spherical geometry for AgNPs and rearranging Equation 1 to solve for d , Equation 2 allows size to be estimated from the measured concentration and other known parameters. As in the computation of particle number, the instrument efficiency must be taken into account. In this case, when preparing the dissolved Ag calibration curve, the amount of Ag actually entering the plasma is only roughly 10% of the actual concentration. Because the entire nanoparticle enters the plasma, the intensity of the pulse should be compared with the corrected dissolved Ag data (H. Pace, Colorado School of Mines, unpublished data). For natural samples, the other important parameter is χ . This will depend on the composition of the Ag-containing nanoparticle. For metallic AgNP, Ag_2S , or AgCl , the most likely forms of AgNP, χ values are 1, 0.87, and 0.75, respectively. The Ag_2S nanocrystals may have excess S on the surface depending on the environment, leading to variable Ag to S ratios ranging from 1.7 to 1.1 [29]. However, negating any associated surface S, a Ag_2S nanocrystal would be approximately 13% larger in diameter; that is, 11.49 nm compared with 10 nm. Likewise, AgCl nanocrystals would be 13.3 nm versus a 10 nm AgNP particle. The amount of Ag associated with other natural nanoparticle and colloidal phases can be determined, but the actual size of the particle will not be determinable from the Ag signal alone and will depend on additional information to characterize the other constituent.

$$d = \left(\frac{C \times q \times t_d}{\frac{4}{3}\pi \times \chi \times \rho} \right)^{\frac{1}{3}} \times 2 \quad (2)$$

ACCOMPANYING STUDIES

To understand better the concept of ablation efficiency, and to ensure that NPs can be analyzed directly by ICP-MS, we compared analysis of acid-digested versus nondigested NanoComposix NP. For digested samples, we added trace-metal-grade concentrated HNO_3 directly to AgNP, to a concentration of 67%; the digestates were further diluted prior to analysis with 1% HNO_3 . Digestion was performed at room temperature for a minimum of 30 min. Nondigested samples were prepared by diluting AgNP in both 2 mM phosphate buffer and DI water. For filtration studies, we wanted to test two main aspects of the filtering process, including the ability to recover NPs smaller than the nominal size of the filter and filter material.

RESULTS AND DISCUSSION

ICP-MS recovery

The results of the comparison of digested and nondigested AgNP are given in Figure 2. No difference was observed in the

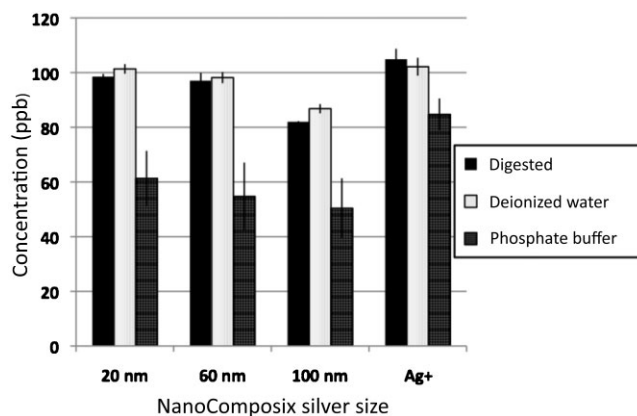


Fig. 2. Comparison of acid-digested and nondigested nanoparticulate silver (AgNP) in both deionized water and 2 mM phosphate buffer. Concentration in ppb ($\mu\text{g/L}$ Ag) is measured by ICP-MS response.

amount of Ag detected in samples dispersed in deionized water, suggesting that intact AgNP are effectively measured. Size was not a factor for the range considered, although it did appear that the 100-nm NanoComposix stock solution was less concentrated than that reported by the manufacturer. Ionization was likely inhibited when diluting with 2 mM phosphate buffer (Fig. 2), resulting in lower apparent Ag concentrations. An approximate 40% decline was observed for all AgNP sizes, and a 20% decrease was observed for dissolved Ag. Given the results of the digestion experiment, the most likely explanation for the relatively lower recovery of the AgNP in the phosphate buffer compared with the Ag^+ is that aggregation is occurring, either in the sample container or within the ICP-MS sample introduction system. This is somewhat unusual, because phosphate is the dispersing agent used in other stock solutions produced by the same manufacturer. This highlights the importance of considering not only ablation efficiency of the ICP-MS but also the need for consideration of particle stability. An initial hypothesis was that the smaller decrease in dissolved Ag recovery was due to the precipitation of an insoluble Ag phosphate ($\log K_{sp} = -17$). However, through modeling with Visual Minteq, we determined that free Ag ion was at a level several orders of magnitude lower than the predicted saturation concentration, suggesting that precipitation of Ag_3PO_4 was not responsible for the decrease in Ag analyzed.

Filtration

We examined serial filtration as a means of determining particle size of our monodisperse AgNP samples. The limitations of filtration arise from the possibility of artifacts resulting from particle-filter interactions, surface coagulation, and pore blockage and Ag^+ membrane interactions. Despite these problems, filtration is often used for natural colloids with broad size distributions [35]. Perhaps not surprisingly, filtration was found to be a problematic technique. Normalizing the amount of AgNP passing through the filter to the raw (unfiltered) samples of each size particle allowed determinations of which variables most affected the process and avoidance of possible complications such as the matrix effects as discussed previously. First, it was found that NPs smaller than the nominal filter size did not always pass through the filter (Supplemental Data, Fig. S3). The smallest of the NPs (20 nm) were able to pass through all the aluminum oxide (Anotop) filter sizes, whereas the 100-nm NPs were completely blocked by even the largest pore sizes. Second, the most important factor in filtration appeared to be the filter

membrane material. Nanoparticulate silver did not pass through the nylon membranes, regardless of NP size or filter pore sizes.

SP-ICP-MS data for ASAP

An ICP-MS output of ^{107}Ag intensity versus time for blank, dissolved (500 ng/L) Ag, and nanoparticulate (100 ng/L ASAP) Ag samples is illustrated in Figure 3. The dissolved Ag solutions, regardless of concentration, produced a stable signal with few spikes. With the AgNP suspensions, numerous spikes were observed, consistent with the mechanism of single particle detection illustrated in the Supplemental Data, Figure S2.

To confirm that the pulses were truly arising from the presence of nanoparticles, we examined the effects of concentration, filtration, and acidification. The number of pulses should be correlated directly with the number of nanoparticles in the solution. Several concentrations of ASAP ranging between 10 and 1,000 ng/L Ag were analyzed via SP-ICP-MS, and increasing numbers of pulses were observed with higher concentrations of ASAP (Fig. 4). This is mainly because at low concentrations each nanoparticle causes a unique incidence in a given dwell time. At higher concentrations, the relationship becomes nonlinear. This could be because, at higher concentrations, it is more likely that two or more nanoparticles may be analyzed simultaneously in a dwell time. An alternative explanation is that, as total Ag concentration increases, so does the background signal. This makes it increasingly difficult to detect AgNP that produces a signal near the background. In essence, at higher concentrations, small AgNPs are increasingly lost to the background. This effect might also explain the slight decrease in pulses with increasing concentration observed at low concentrations in the filtered and acidified samples.

Filtered samples were prepared with 0.02- μm Whatman Anotop alumina oxide filters. The ASAP was diluted in 1% HNO_3 and was shaken for approximately 5 h. As is evident in Figure 4, both filtration and acidification dramatically decrease the number of pulses observed in ASAP samples, strengthening the argument that pulses are indeed a result of AgNP. Incomplete digestion of acidified samples may explain the observed increase in pulse numbers with increasing concentration. The acidification approach is a common preparation step for

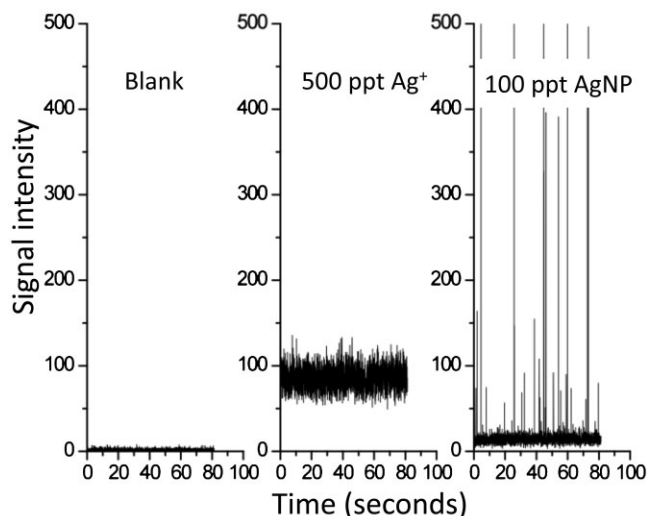


Fig. 3. Single-particle inductively coupled plasma-mass spectrometer output with different silver (Ag) solutions: blank, 500 ng/L dissolved Ag, and nanoparticulate silver (AgNP; 100 ng/L AgNP).

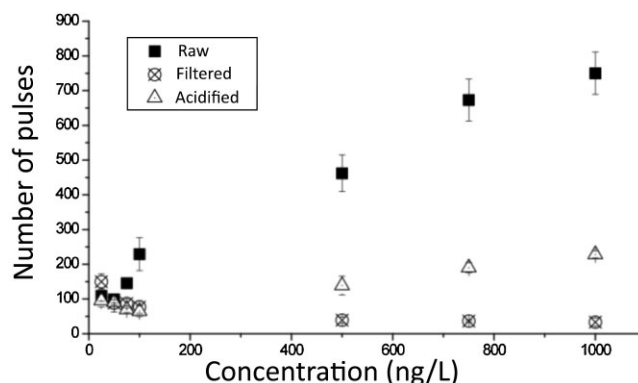


Fig. 4. Pulses positively correlate with concentration of nanoparticulate silver (AgNP). Unprocessed (raw) AgNP shows increasing number of pulses with increasing concentration. After filtering or acidifying AgNP samples, the number of pulses dropped dramatically.

ICP-MS samples. Results would likely be different if an approach designed to digest solid material completely was used.

To determine whether the results that we observed in simple matrices could be seen in environmental samples, Black Hawk wastewater effluent samples were spiked with 1 $\mu\text{g/L}$ Ag using ASAP solution. A matrix of four experiments (Fig. 5) was devised to affirm that pulses seen were truly AgNP and not an artifact of analysis such as random noise, colloid-bound Ag^+ , etc. First, both ASAP and wastewater were filtered with 0.02- and 0.45- μm filters, respectively and combined prior to analysis to ensure that no pulses would be detected (Fig. 5A). Second, only ASAP was filtered and spiked into raw wastewater to show that the dissolved (Ag^+) portion of ASAP would not bind to particulates in the wastewater and register as a pulse (Fig. 5B). Finally, when raw (unfiltered) ASAP was spiked into both filtered (Fig. 5C) and raw (Fig. 5D) wastewater, no statistical differences in the number of pulses were observed, indicating that no additional spikes were obtained when adding Ag to wastewater. The higher baseline for the samples spiked with raw ASAP compared with filtered ASAP suggests that a significant fraction of the AgNP is too small to be differentiated from the background.

Wastewater samples

The results of an analysis of samples collected from a wastewater treatment plant located in Boulder, CO, are shown in Figure 6. The influent to the plant, untreated sewage, and the final effluent were sampled. Samples were measured without filtration; however, samples were allowed to settle for several hours prior to analysis. As a proof of concept, dissolved Ag was quantified from the baseline Ag level. Nanoparticulate concentrations were determined by summing all observed pulses and applying a 10% efficiency correction. In the influent, we found 520 ng/L dissolved Ag and 200 ng/L AgNP, with the final effluent measuring 60 ng/L dissolved Ag and 100 ng/L AgNP (Fig. 6). These total Ag concentrations are consistent with historical data kept by the treatment plant for the last 20 years (D. Paterniti, Boulder, CO, Municipal Wastewater Treatment Facility, personal communication) and are in the range for wastewater predicted from material flow analysis [36]. Wastewater treatment facilities are likely places for AgNP to begin to be observed. Therefore, the use of SP-ICP-MS to monitor effluents may provide a better understanding of how AgNPs are affecting downstream aquatic environments.

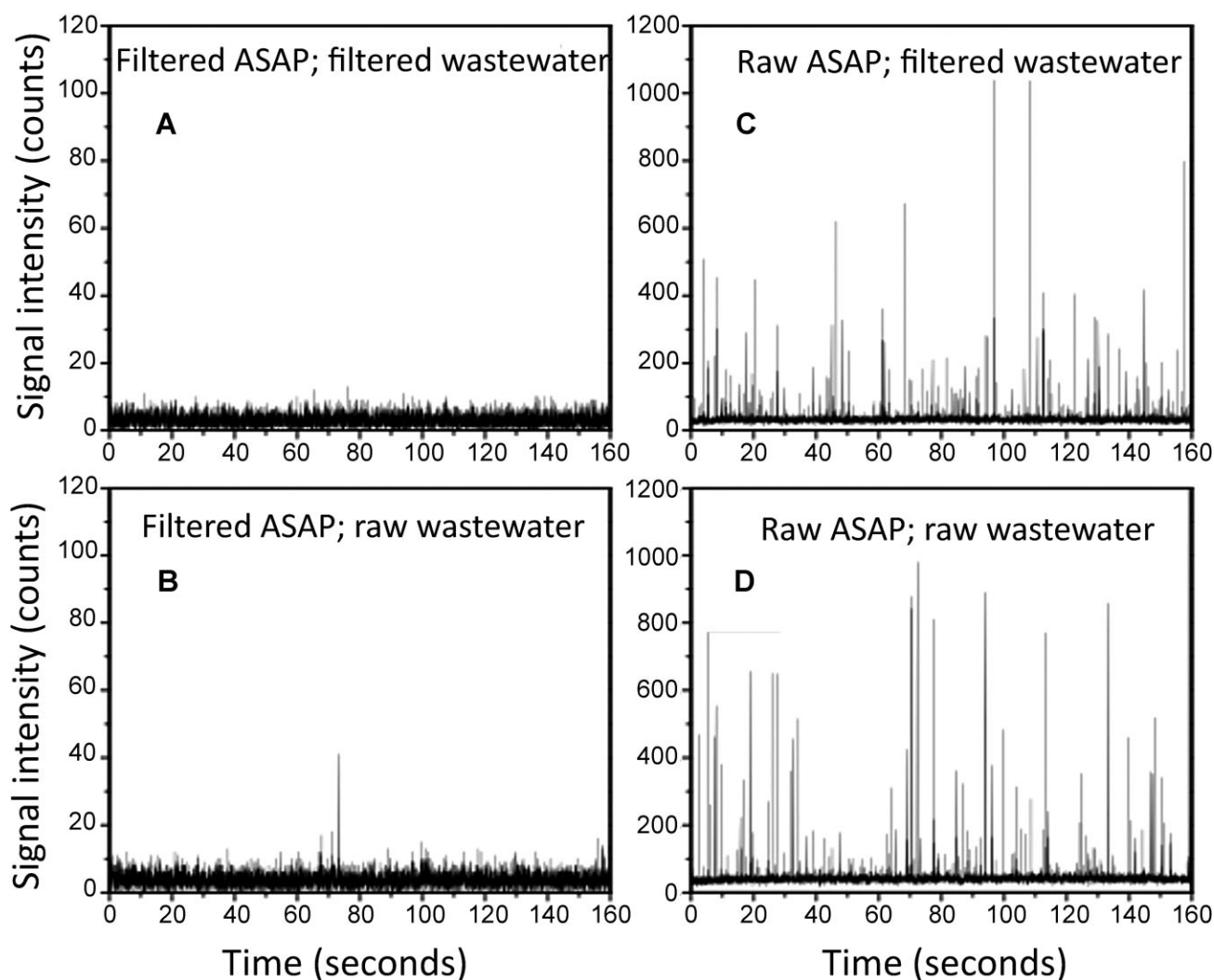


Fig. 5. Single particle inductively coupled plasma–mass spectrometry data for matrix of nanoparticulate silver (AgNP) spiked wastewater showing the effects of filtration. First, AgNP and wastewater were filtered with 0.02- and 0.45- μm filters, respectively, and combined prior to analysis to ensure that no pulses would be detected (A). Second, only nanoparticulate silver (AgNP) was filtered and spiked into raw wastewater to show that the dissolved ionic silver (Ag^+) portion of AgNP would not bind to particulates in the wastewater and register as a pulse (B). Finally, when raw (unfiltered) AgNP was spiked into both filtered (C) and raw (D) wastewater, no statistical differences in the number of pulses were observed, indicating that no additional spikes were obtained when adding silver to wastewater.

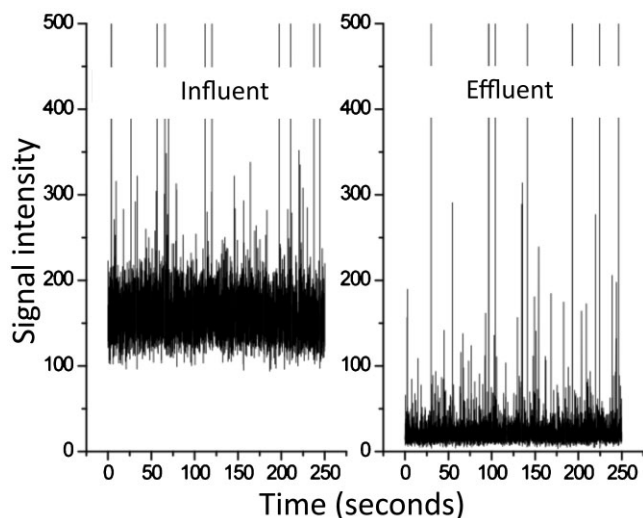


Fig. 6. Boulder, CO, USA, wastewater effluent. Evidence of dissolved silver (Ag) and nanoparticulate silver can be seen by the elevated background ionic silver (Ag^+) and pulses (nanosilver [AgNP]) in the data set.

At present, the SP-ICP-MS method cannot distinguish between engineered AgNP and other colloid-bound Ag^+ ions or precipitates (AgCl , AgS). In a previous study of five Colorado streams, filtration indicated that 60% of Ag was found to be in the colloidal form, presumably adsorbed onto natural organic matter particles [37]. The present study was performed before the rapid increase in the use of engineered AgNP. Whether colloidal natural organic matter in streams carries enough Ag^+ to register a discrete pulse in SP-ICP-MS is not known but should be considered.

It has been said that in the absence of a careful and complete description (characterization) of the nanoparticle type being evaluated, the results of nanotoxicity experiments will have limited value or significance [38]. The technique demonstrated here is the use of SP-ICP-MS to screen for nanosilver in environmental samples. The ultimate goal is to develop SP-ICP-MS to characterize the particle size distribution and particle number concentration for screening of environmental waters and the exposure solutions of ecotoxicity tests. Although highly promising, as tested, this method may be limited to analyzing particles larger than 40 nm. This may in fact exclude a

fraction of AgNPs having the most ecological interest [39]. However, SP-ICP-MS may be a useful tool in a collective quiver of methods for examining colloidal materials that could potentially be an indicator of contaminants. With further refinement of the method and our understanding of the controlling variables, such as transport in the ICP-MS sample introduction system, matrix effects, etc., we expect considerable improvement in this technique.

SUPPLEMENTAL DATA

Fig. S1. Characterization of the ASAP AgNP suspensions.

Fig. S2. Data set manipulation to differentiate between dissolved and nanoparticulate silver.

Fig. S3. Filter analysis filtration.

Table S1. Setting for a typical RTSPC analytical run (1.45 MB DOC).

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