The persistence and transformation of silver nanoparticles in littoral lake mesocosms monitored using various analytical techniques

Lindsay M. Furtado, Md Ehsanul Hoque, Denise M. Mitran, James F. Ranville, Beth Cheever, Paul C. Frost, Marguerite A. Xenopoulos, Holger Hintelmann and Chris D. Metcalfe

A Trent University, Water Quality Center, 1600 Westbank Drive, Peterborough, ON, K9J 7B8, Canada.
B Colorado School of Mines, Department of Chemistry and Geochemistry, 1500 Illinois Street, Golden, CO 80401, USA.
C Trent University, Department of Biology, 1600 Westbank Drive, Peterborough, ON, K9J 7B8, Canada.
D Present address: Empa – Swiss Federal Laboratories for Materials Science and Technology, Technology and Society Laboratory, Lerchenfeldstrasse 5, CH-9014 St Gallen, Switzerland.
E Present address: Michigan State University, Department of Microbiology and Molecular Genetics, 220 Trowbridge Road, East Lansing, MI 48824, USA.
F Corresponding author. Email: cmetcalfe@trentu.ca

Environmental context. Silver nanoparticles discharged with municipal wastewater may contaminate surface waters and harm aquatic ecosystems. We applied several analytical techniques to investigate the persistence and transformation of silver nanoparticles in a natural lake environment, and show, through multiple lines of evidence, that they persisted in lake water for several weeks after addition. The nanoparticles were releasing silver ions through dissolution, but these toxic ions were likely binding with natural organic matter in the lake water.

Abstract. Silver nanoparticles (AgNPs) may be released into surface waters, where they can affect aquatic organisms. However, agglomeration, dissolution, surface modifications and chemical speciation are important processes that control the toxicity of AgNPs. The purpose of the study was to apply various methods for monitoring the persistence and transformation of AgNPs added to littoral lake mesocosms. Analysis of total Ag showed that the levels in the mesocosms declined rapidly in the first 12 h after addition, followed by a slower rate of dissipation with a half-life ($t_{1/2}$) of $\sim$20 days. Analysis using single particle ICP-MS (spICP-MS) showed no evidence of extensive homo-agglomeration of AgNPs. The stability of AgNPs was likely due to the low ionic strength and high concentrations of humic-rich dissolved organic carbon (DOC) in the lake water. Analyses by spICP-MS, cloud point extraction (CPE) and asymmetric flow field flow fractionation coupled to ICP-MS (AF4-ICP-MS) all indicated that the concentrations of AgNP decreased over time, and the nanoparticles underwent dissolution. However, the concentrations of dissolved silver, which includes Ag$^+$, were generally below detection limits when analysed by centrifugal ultrafiltration and spICP-MS. It is likely that the majority of free ions released by dissolution were complexing with natural organic material, such as DOC. An association with DOC would be expected to reduce the toxicity of Ag$^+$ in natural waters. Overall, we were able to characterise AgNP transformations in natural waters at toxicologically relevant concentrations through the use of multiple analytical techniques that compensate for the limitations of the individual methods.

Introduction

Silver nanoparticles (AgNPs) are used in various commercial products because of their antimicrobial properties.\[1\] Because of the growing use of these products, there is potential for AgNPs to be released into the environment.\[2\] For example, AgNPs can be released from clothing during washing, and subsequently enter freshwater ecosystems by discharges of municipal wastewater.\[2,3\] AgNP concentrations in surface waters are predicted to be in the nanogram per litre to low microgram per litre range.\[4\] Toxicity of AgNPs to aquatic organisms has been demonstrated at micrograms per litre levels.\[5–7\] which raises concerns about the ecological risks of exposure to AgNPs.

Agglomeration, dissolution, changes in chemical speciation and surface modifications are important processes that control the toxicity of AgNPs in the aquatic environment.
Agglomeration between AgNPs has been shown to reduce the bioavailability and toxicity of AgNPs\(^{[8]}\) and to promote sedimentation from the water column.\(^{[9,10]}\) Dissolution of the surface layer of AgNPs results in the release of Ag\(^+\)\(^{[11]}\) which is significant because the toxicity of AgNPs has been related to the release of dissolved Ag (dAg), which includes Ag\(^+\)\(^{[12,13]}\). However, the chemical speciation of the released Ag\(^+\) may influence bioavailability and toxicity.\(^{[9,10,14]}\) Furthermore, surface modifications of AgNPs from adsorption of natural ligands may alter toxicity by affecting agglomeration and dissolution rates.\(^{[15,16]}\) Bench-scale experiments have shown that ionic strength,\(^{[11,17]}\) dissolved organic carbon (DOC),\(^{[9,10]}\) \(pH\)\(^{[18]}\), dissolved oxygen (DO),\(^{[14,18]}\) temperature\(^{[18,19]}\) and light\(^{[20]}\) are factors that affect agglomeration and dissolution of AgNPs in natural waters. However, to better understand AgNP transformations, research is required under realistic environmental conditions. Thus far, the few studies on transformations of AgNPs in experimental mesocosms and microcosms have used high concentrations (i.e. milligrams per litre),\(^{[21–23]}\) perhaps because of the limited methods available for analysing AgNPs at environmentally relevant concentrations in complex matrices.\(^{[24]}\)

In recent years, several sensitive analytical techniques have been developed that show promise for analysis of AgNPs at environmentally relevant concentrations. Cloud point extraction (CPE) was developed by Liu et al.\(^{[25]}\) to pre-concentrate AgNPs using surfactants and complexing agents. Asymmetric flow field-flow fractionation (AF4) has been applied to the analysis of particle size distributions of nanoparticles, and when coupled with inductively coupled plasma mass spectrometry (AF4-ICP-MS), offers elemental specificity and sensitivity.\(^{[26,27]}\) Finally, single particle ICP-MS (spICP-MS) is an emerging analytical method that has great potential for monitoring the size and concentration of AgNPs\(^{[28–30]}\). However, few studies have applied these techniques simultaneously for the analysis of AgNPs at environmentally relevant concentrations in natural waters. Furthermore, it may be advantageous to combine multiple techniques when analysing AgNP transformations to validate results and compensate for the limitations associated with different analytical methods.

The objective of this study was to apply and evaluate various analytical techniques for monitoring the persistence and transformations of AgNPs in natural lake water. Studies were conducted in littoral mesocosms deployed in a lake in the Experimental Lakes Area (ELA), ON, Canada. We spiked duplicate mesocosms with a one-time addition of AgNPs at a nominal concentration of 60 \(\mu g\) Ag L\(^{-1}\). Samples were collected for analysis by total Ag quantification, centrifugal ultrafiltration, CPE, spICP-MS and AF4-ICP-MS to monitor changes in the concentration and size of AgNPs and other Ag species over time in order to understand AgNP persistence and to characterise AgNP transformations (Table 1). Based on the results, we also discuss the advantages and limitations of the various methods for AgNP analysis at environmental relevant concentrations.

### Experimental

#### Mesocosms

Two littoral mesocosms were deployed in Lake 239 at the ELA in north-western Ontario, Canada (49°40′N, 93°44′W) and monitored over a 5 week period from July to August 2012. The dimensions of the two mesocosms were 2 m in diameter and 1.5 to 1.7 m in depth, giving theoretical volumes of >5000 L. However, because of evaporation and collapsing of the mesocosm walls, the actual volumes of water in the mesocosms were lower and declined over time. The volumes measured in the two mesocosms at the end of the experiment using Cl\(^-\) as a tracer were 2824 and 3068 L. For the purposes of calculating a nominal dosing concentration, it was assumed that both mesocosms contained 4000 L of water. Mesocosms were open to bottom sediments, suspended from a floating ring and sealed to the sediments with sandbags (see Supplementary material, Fig. S1).

#### Silver nanoparticles

The suspensions of AgNPs used in the study were purchased from NanoComposix (San Diego, CA) as polyvinylpyrrolidone (PVP) capped material with an average particle size of 50 nm. The AgNPs in the stock were suspended in Milli-Q water at a nominal concentration of 1.0 mg Ag mL\(^{-1}\). Acid digestion and

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Table 1. Forms of Ag quantified by various methods employed in the study and their associated contribution to understanding silver nanoparticle (AgNP) persistence and transformations in the littoral lake mesocosms

<table>
<thead>
<tr>
<th>Method</th>
<th>Form(s) of Ag quantified</th>
<th>Contribution to understanding AgNP persistence and transformations</th>
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<tbody>
<tr>
<td>Total Ag analysis</td>
<td>AgNP</td>
<td>AgNP persistence</td>
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<tr>
<td></td>
<td>free Ag(^+)</td>
<td></td>
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<tr>
<td></td>
<td>Ag(^+) complexed with natural organic and inorganic ligands</td>
<td></td>
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<tr>
<td>Centrifugal ultrafiltration (3 kDa)</td>
<td>free Ag(^+)</td>
<td>AgNP dissolution</td>
</tr>
<tr>
<td></td>
<td>Ag(^+) complexed to inorganic or low molecular weight DOC ligands (&gt;3 kDa)</td>
<td>Ag(^+) complexation</td>
</tr>
<tr>
<td>CPE</td>
<td>AgNP (concentration and size)</td>
<td>AgNP persistence</td>
</tr>
<tr>
<td></td>
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<tr>
<td>spICP-MS</td>
<td>AgNP (concentration and size)</td>
<td>AgNP persistence</td>
</tr>
<tr>
<td></td>
<td>Ag(^+)</td>
<td>Homoe-agglomeration</td>
</tr>
<tr>
<td>AF4-ICP-MS</td>
<td>Early eluting peak Ag(^+) complexed with ligands (&gt;10 kDa)</td>
<td>AgNP dissolution</td>
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ICP-MS analysis verified that the concentration of the stock solution was 0.96 mg Ag mL$^{-1}$ and centrifugal ultrafiltration (3 kDa) showed that 2.5% of the total Ag mass comprised dAg, which was assumed to be primarily Ag$^+$. These measurements of the stock solution were performed immediately after receiving the stock solution from the manufacturer and one month before dosing. Size information provided by the supplier indicated that the AgNPs were mono-dispersed, with a primary particle diameter of 48.3 nm as determined by transmission electron microscopy (TEM), and a hydrodynamic diameter of 56.3 nm as determined by dynamic light scattering (DLS). The AgNPs were guaranteed by the supplier to remain stable for 6 months. A volume of 240 mL of AgNP stock suspension (equating to 240 mg of Ag) was added to each mesocosm on 11 July 2012 to achieve a nominal concentration of 60 μg L$^{-1}$, based on an estimated initial volume of 4000 L. After dosing at the water surface, the water column was mixed vertically using an ~45-cm plastic disc, with care taken to not disturb the sediments. The study ended after 33 days on 13 August 2012.

**Water sampling**

Intensive water sampling was carried out 1, 3, 6, 12, 24, 48 and 96 h after dosing to capture transformations of AgNPs across shorter time scales (i.e. hours to days). After the first 4 days, samples were collected weekly. The water column in each mesocosm was mixed with a disc for a few minutes before each sample collection. For each sampling time, a single sample was collected from a single location ~10 cm below the surface, with the assumption that the water column was homogenously mixed. A volume of 8 L of water was collected and immediately passed through a 35-μm mesh to remove net plankton (referred to hereafter as unfiltered water). The collected water was then subsampled for a variety of different analyses, including total Ag quantification by ICP-MS, centrifugal ultrafiltration, CPE, spICP-MS and AF4-ICP-MS (Table 1). For centrifugal ultrafiltration, spICP-MS and AF4-ICP-MS analysis, samples of unfiltered water (1 h to 21 days) were immediately flash frozen in liquid nitrogen in 5-mL cryogenic vials (Corning Inc., Corning, NY, USA) and stored at −80°C until analysis. Our previous trials with flash freezing showed that this preservation technique maintained the size and agglomeration state of AgNPs at the time of sampling until laboratory analysis could be performed (Fig. S2).

**Water chemistry**

Temperature, conductivity, and dissolved oxygen (DO) were measured at the surface, 1-m depth and just above the sediments in each mesocosm before each sampling event using multiparameter probes (YSI 35, YSI PRO ODO, and YSI30–25FT, YSI Inc., Yellow Springs, OH, USA). Subsamples of weekly unfiltered water samples were analysed for pH using a ROSS Ultra electrode (Thermo Scientific, Nepean, ON, Canada). Samples used for DOC quantification were filtered with a 0.2-μm (47 mm) polycarbonate membrane (Millipore, Billerica, MA, USA) and were kept at 4°C until analysis. DOC concentration was analysed using an O.I. Analytical 1030D carbon analyser (Xylem Scientific, Gradin Instruments, Oakville, ON). The concentrations of major inorganic anions and cations were also measured from subsamples of unfiltered water respectively using ion chromatography (Dionex ICS-1100, Thermo Scientific, Bannockburn, IL, USA; EPA Method 9056A) and flame atomic adsorption spectrophotometry (Varian FSA20, Varian Inc., Walnut Creek, CA, USA; EPA Method 7000B). Total Ag analysis

Total Ag in unfiltered water was analysed to monitor the persistence of all forms of Ag in the mesocosms over the monitoring period. After each sampling event, a sub-sample of unfiltered water (40 mL) was collected and immediately acidified with trace metal grade nitric acid (HNO$_3$, Caledon Laboratories, Georgetown, ON) to a final concentration of 4% (v/v), and stored at 4°C until analysis. The samples were heated for 6 h at 70°C before analysis to ensure all organic matter was digested. Ag analysis was performed by ICP-MS using an X Series 2 instrument (Thermo Scientific, Nepean, ON) with indium (PlasmaCal, SCP Science, Paie D’Urfe, QC, Canada) used as an internal standard (5 μg L$^{-1}$). Detection limits were determined from blank samples analysed throughout the run. Blank samples consisted of Milli-Q water (18.3 MΩ, Milli-Q Element system, Millipore Inc.) acidified to 4% HNO$_3$ with 5 μg L$^{-1}$ of indium. The limit of detection (LOD) calculated as 3 × the standard deviation (s.d.) of replicate analyses was 0.02 μg Ag L$^{-1}$.

**Centrifugal ultrafiltration**

In order to determine the concentration of Ag in the dissolved phase, water samples were subjected to ultrafiltration using Amicon Ultra-4 Centrifugal Filter Units with a 3-kDa regenerated cellulose membrane (Millipore Inc.). The term dAg is operationally defined as Ag$^+$ and any soluble Ag complexes that are not retained by the 3-kDa filter. Frozen unfiltered water samples were thawed and then pre-filtered through 0.45-μm (25 mm) nylon syringe filters (Canadian Life Sciences, Georgetown, ON) before being passed through the 3-kDa membranes by centrifuging at 2750g at room temperature for 90 min. The filtrate was acidified to 4% HNO$_3$ and stored at 4°C until ICP-MS analysis, as described above. The recovery of Ag$^+$ through the 0.45-μm membrane was 97% and through the 3-kDa membrane was 78%, based on tests with AgNO$_3$ (Sigma–Aldrich, St Louis, MO, USA) dissolved at a concentration of 10 μg Ag L$^{-1}$ in deionised water (DI) prepared by reverse osmosis. Recovery for filtration was determined as the ratio of Ag concentration measured after filtration relative to the Ag concentration measured before filtration.

**Cloud point extraction**

CPE was performed to extract and concentrate AgNPs from the water sample, leaving behind dAg in solution. CPE utilises the formation and separation of a non-polar surfactant rich phase and an aqueous phase to sequester AgNPs and dAg respectively. The addition of Ag ligands, such as thiosulfate, binds Ag$^+$ to form charged complexes that are not extractable. The CPE technique was based on the method described by Liu et al., with modifications to pre-concentrate larger volumes of water sample (i.e. 40 mL). The analysis was performed on water samples that had been pre-filtered first with a 1.2-μm filter, followed by filtration with a 0.2-μm (47-mm) polycarbonate membrane (Millipore Inc.), which would remove all particles larger than bacterioplankton. The recovery of stock 50-nm PVP AgNPs through the 0.2-μm filter was 92% at concentrations of 20 μg Ag L$^{-1}$. A 200-mL sample of the 0.2-μm filtrate was adjusted to pH 3 using 2% HNO$_3$. Acidification of the sample was performed to neutralise the surface coating, in which the highest extraction efficiency has been reported to be obtained at the zero point charge pH (pH$_{ZPC}$) of the NPs. A pH$_{ZPC}$ could not be found between pH 2 and 10 for the AgNPs used in this study. As a result, the pH of samples was adjusted to 3,
resulting in a surface zeta potential of $-17$ mV.\[^{[31]}\] This pH value was used for the previous CPE studies using PVP AgNPs as pH values lower than this could result in significant dissolution.\[^{[29]}\] Once the pH was adjusted, a 40-µL sub-sample was mixed with 0.5 mL of 1 M sodium thiosulfate (Na$_2$S$_2$O$_3$, Caledon Laboratories) and 2 mL of 5 % (w/v) non-ionic TX-114 surfactant (Sigma–Aldrich). The solution was then incubated in a water bath at 40 °C for 30 min. Samples were then centrifuged at ~828g at room temperature for 5 min to facilitate phase separation. The surfactant rich phase with the concentrated AgNPs was collected and digested using microwave-assisted digestion (ETHOS, Milestone, Shelton, CT, USA) in 9 mL of HNO$_3$ (70 % v/v) and 3 mL of H$_2$O$_2$ (30 % v/v, Sigma–Aldrich). The microwave digestion program consisted of a 5 min ramp-up to 120 °C, 10 min at 120 °C, 5 min ramp-up to 180 °C and 30 min at 180 °C. After digestion, the volume was reduced to 1 mL on a hot-plate in 20-mL glass vials and re-diluted to 10 mL using Milli-Q water. The resulting sample was concentrated by a factor of 4 and had an acid concentration less than 10 % so that it could be analysed directly by ICP-MS. The LOD was 0.01 µg L$^{-1}$. The extraction efficiency was determined using spiked PVP AgNP and AgNO$_3$ solutions in order to evaluate the performance of the method (see Supplementary material for further details). The extraction efficiency of AgNPs by CPE was 75 % in DI water, but decreased to 41 % in 0.2-µm pre-filtered Lake 239 water, indicating some effect of the sample matrix on method performance (Table S1). In both DI and Lake 239 water, laboratory results indicated that this method extracted minimal Ag$^+$ (i.e. 2 %) and was therefore effective at separating AgNPs from Ag$^+$. TEM was also performed on a sample to characterise the AgNPs extracted into the surfactant rich phase. To do so, we used a Philips CM-200 instrument (Phillips, Eindhoven, Netherlands) at 200 kV, coupled with Genesis 4000 Energy Dispersive X-ray Spectroscopy (EDXS) (EDAX Inc., Mahwah, NJ, USA). The sample was mounted onto a carbon coated grid for analysis. The TEM operating conditions are described in Table S2.

**spICP-MS**

Analysis by spICP-MS was performed to quantify the size, particle concentration and mass concentration of AgNPs (or their agglomerates). A NexION 300Q instrument supplied by Perkin Elmer (Waltham, MA) was used for spICP-MS analysis. The optimisation and validation of this analytical method has been described in previous studies.\[^{[30,32]}\] The instrument parameters were tuned to generate the maximum $^{107}$Ag intensity before data acquisition. An integration dwell time of 10 ms was used. The transport efficiency of AgNPs into the plasma was determined by the mass-based approach described by Pace et al.\[^{[29]}\] and was found to be 4.0 % using a 100-nm Au (Nanocomposix) NP standard. The particle concentration detection limit for spICPMS analysing 40-nm AgNPs was determined to be 2.5 ng L$^{-1}$ using the data collection method and instrumentation detailed above.\[^{[32]}\] The particle size detection limit under these analytical conditions was ~30-nm AgNP.

For analysis of samples collected from the mesocosms, samples flash frozen in liquid nitrogen were thawed in a water bath at room temperature (~5 min) and then sonicated for 5 min. Immediately before spICP-MS, the samples were diluted 1:5000 in 18.3 M ohm Nanopure water (Metrohm Inc., Riverview, FL, USA) to avoid particle coincidence. External calibration of a blank and four dAg solutions (QC-7M, High Purity Standards, Charleston, SC, USA) ranging from 0 to 1 µg Ag L$^{-1}$ was carried out in single particle mode. The intensity of $^{107}$Ag for each standard solution over the entire length of the analysis was then averaged. To monitor instrumental drift over time, a single Ag$^+$ standard of 100 ng L$^{-1}$ was analysed in spICP-MS mode after every 10 samples. If drift in the standard signal was detected, the particle sizing equation was adjusted accordingly. Each sample was analysed in duplicate.

The calculations for spICP-MS data were conducted as previously described\[^{[30,32]}\] Briefly, AgNPs were distinguished from the background–dissolved Ag by plotting the raw intensity data as pulse intensity v. number of pulses, where any values below the first minimum in the histogram were considered background–dissolved Ag. The intensity of the AgNP pulses was subsequently corrected by subtracting the background intensity. The calibration curve for dAg standards was converted into mass flux using the transport efficiency of silver mass into the plasma. The Ag mass in each AgNP pulse was extrapolated from the mass flux calibration curve and converted into particle diameter assuming spherical geometry. The mean particle diameter was then calculated from these data. The particle concentration was determined from the number of AgNP pulses measured in a sample adjusted by the dilution factor. Based on the mean particle diameter and particle concentration, the Ag concentration was calculated using the equation:

$$\text{Ag concentration} = \frac{\text{particles concentration} \times \text{particle volume} \times \text{Ag density}}{\text{transport efficiency}}$$

**AF4-ICP-MS**

AF4-ICP-MS was used to determine the hydrodynamic size of AgNPs and any AgNP agglomerates below <0.2-µm size, as well as to quantify AgNP concentrations. AF4-ICP-MS analysis was performed using an AF2000 Focus (Postnova Analytics; Salt Lake City, UT, USA) AF4 instrument that was directly interfaced to an X Series 2 ICP-MS instrument (Thermo Inc.). A 50-µg L$^{-1}$ solution of indium was introduced by a mixing-tee at a flow rate of 77 µL min$^{-1}$ to correct for changes in sensitivity during analysis. The indium solution was used for daily tuning of the instrument before data acquisition. A full description of the ICP-MS instrumentation and data acquisition parameters when used as an AF4 detector is provided in Table S3. AF4 separations were conducted as described previously,\[^{[14-15]}\] except the carrier fluid consisted of a solution containing sodium dodecyl sulfate (SDS) surfactant (0.05 % w/v, Sigma–Aldrich) in Milli-Q water and the cross-flow was set at 0.7 mL min$^{-1}$ to optimise peak resolution and obtain the greatest recovery of 50-nm PVP AgNPs (i.e. 109 ± 9 % with an injection of 100 µL L$^{-1}$). The final AF4 operating parameters chosen for analysis of mesocosm samples are summarised in Table S4. NanoXact standards supplied by Nanocomposix of PVP AgNPs (20 mg Ag L$^{-1}$) stabilised in Milli-Q water were used for size calibration. The manufacturer provided size information by TEM and DLS that verified the particles to be mono-dispersed (Table S5). The AF4-ICP-MS fractograms generated at the 0.7 mL min$^{-1}$ cross flow showed good resolution of the 40-, 60- and 80-nm standards (Fig. S3), but there was less satisfactory resolution of the 20- and 40-nm standards. The LOD (3 x.s.d. of noise) of the method was 1.0 µg Ag L$^{-1}$, as determined by comparing the noise to the peak height for a 10 µg Ag L$^{-1}$ standard of 50-nm PVP AgNPs.
Silver nanoparticles in a lake

For AF4-ICP-MS analysis, the flash frozen mesocosm samples were thawed in a water bath at room temperature and then sonicated for 5 min. Each sample was allowed to sit for ~30 min before injection to allow any micrometre-sized particulates to settle out. Filtration was avoided as a preparative method for AF4 analysis due to previous studies that indicated that filtration could modify particle size distributions.[33] Before mesocosm samples were analysed, a daily injection of a PVP AgNPs mixed standard of 40- and 80-nm size (100 μg Ag L\(^{-1}\)) was analysed for size calibration (using a three point calibration curve extended through the origin) and to account for any drift in retention time. A 50-nm PVP AgNP standard (100 μg Ag L\(^{-1}\)) was also injected daily to compare the hydrodynamic diameter of fresh particles to those collected in the mesocosm samples, as well as to measure AgNP concentration using a one-point, external calibration curve. A one-point calibration curve was used to avoid lengthy delays between instrument calibration and analysis of samples, and was considered sufficient given the excellent linear relationship (concentration v. area) observed for the analysis of 5–100 μg Ag L\(^{-1}\) concentrations of 50-nm PVP AgNP standards (Fig. S4). A Milli-Q water rinse was injected after the standards to ensure there was no sample carry-over before analysis of mesocosm samples. AF4-ICP-MS fractograms were processed and analysed using OriginPro 9 software (OriginLab, Northhampton, MA). Data were smoothed using the Savitzky–Golay method, with a polynomial order of 1 and 100 points of window. The mean hydrodynamic diameter was defined by the peak centre calculated in the OriginPro 9 software.

Results and discussion

Water chemistry

Water chemistry parameters measured throughout the study were similar between the two mesocosms, reflecting lake conditions of soft, circumneutral water (pH of 7.1–7.2) with high concentrations of DOC of largely humic acids (average of 8.3 mg L\(^{-1}\)). Water temperature, conductivity and DO concentrations ranged from 23 to 26°C, 31 to 34 μS cm\(^{-1}\) and 6 to 8 mg L\(^{-1}\) throughout the water column. The concentration of major cations and anions in Lake 239 indicate low ionic strength, with Cl\(^{-}\) concentrations of 0.27 mg L\(^{-1}\) (Table S6).

Total Ag

There was slow dissipation of total Ag from the water column over time after dosing of the mesocosms. The total Ag concentrations in the two mesocosms were 71 and 70 μg Ag L\(^{-1}\) at 1 h after addition, which declined to 15 and 18 μg Ag L\(^{-1}\) after 33 days (Fig. 1a). The concentrations of total Ag declined rapidly in the first 12 h post-dosing, with a dissipation half life (DT50) over this period of 0.8 and 0.9 days for the two mesocosms (Fig. 1b). After this initial 12-h period, the DT50 increased to 17 and 21 days over the duration of the study (Fig. 1c). The more rapid dissipation in the first 12 h may indicate instability of the PVP AgNP coating, but over the longer term the AgNPs were relatively persistent in the mesocosms. For example, the persistence of Ag in these lake mesocosms over the 33 days of the study was much longer than observed previously in estuarine mesocosms.[21] AgNPs dosed in estuarine mesocosms were completely removed from the water column within the first 24 h, perhaps because of the high ionic strength and high Cl\(^{-}\) concentrations.[21] Conversely, AgNPs dosed to wetland mesocosms had detectable Ag concentrations over 18 months.[23] AgNPs in the wetland mesocosms also demonstrated an initial rapid drop off over the first 8 days and a subsequent slower rate of decline. However, this initial settling time of 8 days was longer than expected by the authors, which they attributed to the high concentrations of organic matter (~50 mg L\(^{-1}\)) in the water column.[23] In the present lake mesocosm study, both the low ionic strength of Lake 239 and the relatively high DOC concentration likely reduced homo-agglomeration and thus increased AgNP persistence. DOC improves AgNP stability by adsorbing to the surface and inducing steric and electrostatic repulsion,[9,34] and at low ionic strengths there are fewer electrolytes available to reduce the surface energy barrier necessary to cause agglomeration.[11]

Therefore, the Ag persistence and water chemistry conditions in the Lake 239 mesocosms may signify that homo-agglomeration was minimal, which would slow the rate of sedimentation out of the water column. Given that total Ag analysis cannot discern the different Ag forms, more sophisticated analytical techniques are required to quantitatively and qualitatively analyse AgNPs and their transformation products.

Centrifugal ultrafiltration

The highest concentrations of dAg were detected in the first 24 h after dosing (0.15 ± 0.2 μg Ag L\(^{-1}\)), after which the concentrations fell to values below the limits of detection or quantification, with the exception of day 4 (Fig. 2a). The dAg that was detected accounted for less than 0.35 % of the total Ag concentrations (Fig. 2b). The presence of dAg in the first 24 h may have been from Ag\(^{+}\) ions already in the stock solution. Based on 2.5 % dAg measured in the stock, we could have initially added ~1.5 μg L\(^{-1}\) of Ag\(^{+}\). The initial dAg detected in the mesocosm water could also have been due to an initial release of chemisorbed Ag\(^{+}\) from the AgNP surface,[35] or a faster rate of oxidative dissolution in the first 24 h.[36] Subsequently, any Ag\(^{+}\) initially released in the mesocosms or generated by dissolution was probably removed from the dissolved phase by complexing with natural organic matter, such as DOC, as observed in other studies.[6,22] Based on thermodynamic constraints, Levan et al.[7] predicted that the majority of Ag\(^{+}\) will bind to cysteine (log K = 11.9), which is representative of thiol functional groups on DOC, in oxic fresh waters. Also, Bone et al.[38] found that the majority of oxidised Ag was associated with a cysteine-like complex in microcosms containing DOC. The AF4-ICP-MS results, discussed below, provide further evidence that the released Ag\(^{+}\) may have formed complexes with larger molecular weight DOC (>3 kDa) that would not pass through the ultrafiltration filter.

Cloud point extraction

For mesocosm samples (i.e. 0.2-μm filtrate) extracted by CPE, there was little decrease over time in the concentrations of AgNPs in the extracts (Fig. 3a). This was accompanied by a decrease in the percentage of AgNPs extracted, calculated as the Ag concentration after CPE relative to the Ag concentration in the 0.2-μm filtrate before CPE (Fig. 3b). The decrease in the proportion of AgNPs extracted over time does not appear to be the result of a decrease in particle concentration, which we found had little effect on extraction efficiency (Table S7). It is likely that the temporal decrease in the percentage of AgNPs extracted was due to AgNP dissolution, resulting in an increased proportion of Ag\(^{+}\) (either free or complexed), as only nanoparticles are extracted by CPE. The CPE technique is incapable of extracting metal ions
Fig. 1. Concentration of total Ag over the exposure period (a), and first order kinetics of loss determined in plots of ln (% initial total Ag concentration) v. time over the first 12 h (b) and after 24 h (c). Plots show the results from each replicate mesocosm (n = 2).

Fig. 2. Concentrations of dissolved Ag (dAg) (µg L⁻¹) (a) and the percentage of dAg (b) determined by centrifugal ultrafiltration (3 kDa) in mesocosm samples over time. At day 2 and after day 4, the concentrations of dAg were below the limits of detection or quantification. Data from one mesocosm replicate for the 12- and 24-h samples were removed because of contamination. The percentage of dAg was calculated as the ratio of the concentration of dAg and the concentration of total Ag in unfiltered water. Plots show the results from each replicate mesocosm (n = 2).
and metal-ion humic acid colloids because of the strong affinity of Ag$^{+}$ for thiosulfate ($S_2O_3$), which is added during the CPE procedure. These CPE results are consistent with AgNP dissolution occurring over time.

Subsequent laboratory experiments revealed a decrease in extraction efficiency with decreased particle size of PVP AgNPs (Table S8). Therefore, a decrease in particle size as a result of AgNP dissolution may have also contributed to a decline in the recovery of AgNPs in mesocosms by CPE over time. It is also possible that increased particle dissolution for smaller sized AgNPs during the CPE procedure may have resulted in a decrease in the apparent extraction efficiency. Dissolution is accelerated for AgNPs with smaller particle sizes, because of an increased surface area-to-volume ratio. Furthermore, we

Fig. 3. The concentration of silver nanoparticles (AgNPs) and the percentage of AgNPs determined by cloud point extraction (CPE) (a, b), single particle inductively coupled plasma mass spectrometry (spICP-MS) (c, d) and asymmetric flow field flow fractionation with on-line inductively coupled plasma mass spectrometry (AF4-ICP-MS) (e, f) in mesocosm samples over time. For CPE, the percentage of AgNPs was calculated as the ratio of the Ag concentration measured after CPE and the Ag concentration measured before CPE (i.e. concentration of Ag in the 0.2-μm filtrate). For sp-ICP-MS and AF4-ICP-MS the percentage of AgNPs was calculated as the ratio of the concentration of Ag measured in the particle size distributions and the concentration of total Ag in unfiltered water. Plots show the results from each mesocosm replicate ($n = 2$).
used previously published CPE methods and did not check if the sample preparation, specifically the acidification\cite{18} and incubation at higher temperatures,\cite{18,19} affected the dissolution state of the PVP AgNPs used in this study.

The effect of potential AgNP humic coating on our CPE results is uncertain. Previous studies have shown that DOC concentrations had no effect on the extraction efficiency of AgNPs by CPE.\cite{25,41} However, our laboratory tests demonstrated that the extraction efficiency decreased when AgNPs were spiked in Lake 239 water, as compared to DI water, indicating that matrix constituents in Lake 239 were affecting the method performance (Table S1). This may reflect an enhanced dissolution of the PVP AgNPs used in this study in Lake 239 water as compared to DI water during the CPE procedure, or it could have been due to modification of the PVP AgNP surface by the water chemistry properties in Lake 239 that reduced recovery in the surfactant rich phase. However, reduced extraction efficiency caused by surface modifications is not consistent with the clear temporal trends observed in the mesocosms. The CPE results still show the selectivity of the method for AgNPs rather than Ag\(^+\) in aqueous matrices, and are another indicator of dissolution of AgNPs over time.

It has been shown that the size and shape of AgNPs are preserved during CPE and storage in the surfactant-rich phase, offering the potential for characterisation by TEM.\cite{25,41} TEM analysis of the surfactant rich phase of a 24-h sample from the mesocosms revealed the presence of colloids in the nanometre size range, but no Ag signal was detected by EDXS (Fig. S5). The EDXS method requires elevated amounts of Ag in the microgram range to confirm the elemental composition of materials detected by TEM. With the amounts of nanoparticles in the CPE extract applied to the EM grid, it was not possible to confirm whether the particles detected by TEM were composed of silver.

**spICP-MS**

Analysis by spICP-MS confirmed the initial 50-nm size of the metal core of the PVP-coated AgNPs in samples collected 1 h after addition to mesocosms. The measured particle size then decreased over time (Fig. 4a). By 14 days post-addition, a high proportion of the particle sizes reached the 30-nm size limit for the spICP-MS method. The decrease in AgNP size over time is likely a result of AgNP dissolution, and the loss of Ag mass from the AgNP core. In a recent study, spICP-MS analysis showed that dissolution caused a reduction in AgNP particle size over time, but this effect was mitigated by high concentrations of DOC and inorganic ligands.\cite{18} Our studies of AgNP stability under varying physico-chemical conditions also showed a significant decrease in AgNP size in DI water, presumably due to AgNP dissolution.\cite{42}

In the present study, particle size distributions showed very little evidence of homo-agglomeration, because after 7 days there were few Ag particles detected at sizes greater than 60 nm (Fig. 5a, b). It is possible that the extensive dilution required for spICP-MS analysis may not have maintained agglomerates. However, dilution was performed immediately before analysis to minimise alterations to the particles. Furthermore, given the relatively low concentration of AgNPs in the mesocosms compared to other natural particles, it is expected that AgNPs would form hetero-agglomerates rather than homo-agglomerates.\cite{43} For example, Kennedy et al.\cite{9} demonstrated that at dilute AgNP concentrations agglomerate size decreased as a result of the low frequency of AgNP–AgNP interactions that cause homo-agglomeration. It is likely that the low ionic strength and high DOC in the lake also stabilised the AgNPs against homo-agglomeration, as discussed previously in the total Ag section.

There was a decrease in the particle concentration over time (Fig. S6), which corresponded to a decrease in the mass concentration. The AgNP concentration determined by spICP-MS decreased in the two mesocosms from 70 and 43 \(\mu\)g L\(^{-1}\) 1 h post-addition to 1.8 and 1.6 \(\mu\)g L\(^{-1}\) after 21 days (Fig. 3c). The majority of Ag was initially in the form of nanoparticles >30 nm in size, as the AgNP concentration determined by spICP-MS represented >60% of the total Ag concentration (Fig. 3d). However, the AgNP concentrations deviated from the total Ag concentration as the experiment progressed (Fig. 3d). This discrepancy is possibly a consequence of AgNP dissolution, resulting in smaller AgNPs that blend with the instrumental background below the 30-nm size detection limit. Theoretically, the decrease in AgNP size should correspond with an increase in dAg concentrations as a result of Ag\(^+\) ions being released from
the AgNP core. Similar to observations with the ultrafiltrate samples, no dAg was detected by the spICP-MS method. The released Ag\(^+\) was either lost to environmental sinks like biotic particles or bottom sediments, complexed to DOC or diluted below the instrumental LOD as a result of the 1 : 5000 dilution required for the method.

**AF4-ICP-MS**

Optimisation of AF4-ICP-MS showed good analytical results in terms of size quantification. Daily size calibration of mesocosm samples with injections of a mix of 40- and 80-nm PVP AgNPs extended through the origin (i.e. 3-point calibration) showed a strong linear relationship, with an \( R^2 \) of greater than 0.97 and a negligible change in retention time (Fig. S7). The average hydrodynamic diameter of a freshly prepared 50-nm PVP AgNP standard over multiple days of analysis was 60 ± 2 nm, which was close to the 56-nm hydrodynamic diameter determined by DLS. For mesocosm samples collected after 1 h, the measured hydrodynamic diameter was 65 and 66 nm for both mesocosms (Fig. 4b). Changes in mean hydrodynamic diameters varied between 56 and 92 nm over the first 7 days with no evidence of a decline in size over time (Fig. 4b), which contradicts the trends observed in spICP-MS. After 7 days, the AgNP peaks in the fractograms were below the LOD. This apparent discrepancy between the results from spICP-MS and AF4-ICP-MS is likely due to the accumulation of surface coatings that increased the hydrodynamic size determined by AF4-ICP-MS. The spICP-MS analytical technique uses Ag mass to determine the primary particle size, whereas the AF4-ICP-MS method separates particles based on their hydrodynamic diameter. Therefore, AF4-ICP-MS could have detected Ag associated with other particles or coated with molecules such as sulfides\(^{[44]}\), organic matter\(^{[34]}\), or biological molecules\(^{[27]}\) that have been shown to increase the hydrodynamic radius of AgNPs.

The Ag detected in the early eluting peak of the AF4-ICP-MS fractograms increased over time (Fig. 5c, d). After 7 days, the early eluting peak was also accompanied by tailing of an additional peak. This first peak may have consisted of Ag\(^+\) associated with ligands in the form of small sized particles

Fig. 5. Particle size distributions determined over time in the two mesocosm replicates using single particle inductively coupled plasma mass spectrometry (spICP-MS) (a, b) and asymmetric flow field flow fractionation with on-line inductively coupled plasma mass spectrometry (AF4-ICP-MS) (c, d). The y-axis in the AF4-ICP-MS fractograms displays the normalised \(^{107}\)Ag intensity. All AF4-ICP-MS fractograms and spICP-MS histograms are shown in the Supplementary material, respectively in Figs S8 and S9.
the total Ag concentration, there was great variability in the
with the concentrations determined by spICP-MS. When com-
the initial concentrations of AgNPs determined by AF4-ICP-
from the intensity of the nanoparticle peak in AF4-ICP-MS.
peak that could correspond to metals associated with particles.
with organic colloids (i.e. humic substances) and a second broad
peaks when analysing trace metal association with natural
ditions, neutral pH of the water and the high water temperatures
dissolution in the mesocosms is expected, given the oxic con-
interactions with natural particles that promote hetero-
driving homo-agglomeration were minimal, as compared to
in lake water, it is probable that AgNP–AgNP interactions
modifications. The minimal amounts of homo-agglomerates
compared to the trends in decreased primary particle size by
larger hydrodynamic diameters measured by AF4-ICP-MS, as

In the lake mesocosms, total Ag levels declined slowly with a
centrations of DOC in the lake water. Adsorption of DOC
AgNP concentrations decreased over time, as determined
from the intensity of the nanoparticle peak in AF4-ICP-MS.
The initial concentrations of AgNPs determined by AF4-ICP-
the two mesocosms were 47 and 58 Ag µg L⁻¹. After 7
days, the concentrations in the two mesocosms decreased to
and 21 Ag µg L⁻¹ (Fig. 3e), which is generally consistent with
the concentrations determined by spICP-MS. When com-
paring the AgNP concentration determined by AF4-ICP-MS to
the total Ag concentration, there was great variability in the
proportion of the total Ag represented by the AgNP peak, both
over time and between replicates (Fig. 3f). Hagendorfer et al.[47]
suggested that variability in AF4 channel recovery is a major
limitation for using this method for direct quantification of
nanoparticle concentrations. Surface alterations to AgNPs may
also change the recovery during AF4 separation. For example,
Delay et al.[34] demonstrated that coating of AgNPs with natural
organic matter improved the recovery as a result of the steric
hindrance that prevents membrane interactions. It would appear
that AF4-ICP-MS provides valuable information on the size
distribution of AgNPs in natural waters, but quantitative data on
AgNP concentrations must be interpreted with caution.

Persistence and transformations of AgNPs
In the lake mesocosms, total Ag levels declined slowly with a \( t_{1/2} \)
of ~20 days after the first 12 h post addition. The stability of
AgNPs was likely due to the low ionic strength and high concen-
trations of DOC in the lake water. Adsorption of DOC onto
AgNPs has been shown to improve stability[10] and the larger hydrodynamic diameters measured by AF4-ICP-MS, as compared to the trends in decreased primary particle size by
spICP-MS, may support the development of such surface
modifications. The minimal amounts of homo-agglomerates
detected by spICP-MS also provide evidence of AgNP stability
in the lake mesocosms. Given the low concentration of AgNPs in
the mesocosms relative to the high amount of natural particles
in lake water, it is probable that AgNP–AgNP interactions
driving homo-agglomeration were minimal, as compared to
interactions with natural particles that promote hetero-
agglomeration. CPE, spICP-MS and AF4-ICP-MS analyses all
indicated that there was dissolution of AgNPs over time. AgNP
dissolution in the mesocosms is expected, given the oxic con-
conditions, neutral pH of the water and the high water temperatures
during the study.[14,18,19] However, centrifugal ultrafiltration
and spICP-MS results showed that very little dAg, which includes Ag⁺, was present in the two mesocosms. The majority of released Ag⁺ probably formed complexes with DOC, consistent with the appearance of an early eluting peak in the
AF4-ICP-MS results. Given the strong association of Ag⁺ with
thiol-containing ligands present in DOC[37,38] it is probable that
Ag⁺ interacted with DOC in the mesocosms to form a complex
that would not pass through the membranes used in both the AF4
and ultrafiltration methods. This observation has significance for
toxicity to aquatic organisms, as one of the mechanisms of
toxicity of AgNPs is due to Ag⁺ released by dissolution of the
particles.[12,13] Complexation of Ag⁺ with ligands will reduce
bioavailability and mitigate AgNP toxicity.[9,10,14] Overall, we
were able to characterise AgNP transformations, both in natural
water matrices and at toxicologically relevant concentrations,
through the use of multiple analytical techniques.

Analytical techniques
The various analytical techniques used to analyse AgNPs and
their associated transformations each have advantages and
limitations. Total Ag analysis can provide information on AgNP
persistence and stability, but more sophisticated analytical
techniques are required to analyse AgNPs to confirm their
persistence and to evaluate transformations. Monitoring AgNP
dissolution in natural waters using ultrafiltration techniques is
challenging because it may exclude Ag⁺ complexed with high
molecular weight ligands (i.e. DOC). However, ultrafiltration
does provide information on the amount of ‘free’ and biologi-
cally available Ag⁺, which is the most toxic form.[12,13] Quant-
itive analysis of AgNPs using CPE may not be possible using
the previously published methods used in this study, as our
laboratory experiments demonstrated reduced recovery in more
complex water matrices (i.e. Lake 239 water) and variations in
extraction efficiency with AgNP size. It also may not be possible
to determine the size and agglomeration state of AgNPs by
TEM-EDXS analysis of CPE extracts at environmentally rele-
vant concentrations because of the high detection limit of
EDXS. Nevertheless, our CPE results do distinguish AgNPs
from Ag⁺ and thus can be used to monitor trends in AgNP
dissolution.

AF4-ICP-MS and spICP-MS were the most sensitive and
versatile analytical tools for understanding AgNP transforma-
tions in lake mesocosms at environmentally relevant levels.
Both methods provide data on the concentrations and particle
sizes that can contribute to an understanding of AgNP dissolu-
tion and agglomeration. However, spICP-MS is currently limit-
ed to analysis of nanoparticles >30 nm and AF4-ICP-MS often
requires a compromise between the resolution of size separa-
tions and recovery. Varying recoveries through the AF4 channel
membrane with different surface coatings may also pose chal-
lenges for AgNP quantification using AF4-ICP-MS. This ana-
lytical technique is also less sensitive and subject to longer
analysis times relative to spICP-MS.

In the present study, particle size distributions determined
using AF4-ICP-MS indicated no decrease in AgNP size. Con-
versely, spICP-MS particle size distributions showed a decrease
in AgNP size over time. The spICP-MS analytical technique
uses Ag mass to determine the primary particle size, whereas
the AF4-ICP-MS method separates particles based on their hyd-
dynamic diameter. It is possible that the AF4-ICP-MS detected
Ag associated with other particles like DOC that increased
the hydrodynamic radius of AgNPs. The results highlight a
potential advantage of AF4-ICP-MS over spICP-MS for the analysis of small sized particles in the early eluting peak. In addition, AF4-ICP-MS may be a useful technique for monitoring the formation of Ag⁺ complexes through the analysis of small sized particles in the early eluting peak. Alternatively, the measurement of the primary size of AgNPs by spICP-MS is a useful indicator of dissolution when studying AgNPs at dilute concentrations (i.e. ng Ag L⁻¹) and when direct measurement of Ag⁺ is not possible because of partitioning to other environmental sinks such as sediment and biota. [36]

Sample preparation is another important consideration when analysing AgNPs. The extensive dilution required for spICP-MS analysis may not have maintained agglomerates, although the dilution was performed immediately before analysis to minimise alterations to particle behaviour. Also, before samples were analysed by spICP-MS and AF4-ICP-MS, the suspensions were sonicated and it is unclear whether this would have altered the agglomeration state. These results illustrate that in order to gain a full understanding of AgNP transformations in the environment, multiple analytical methods should be used to quantify different Ag forms and to overcome the limitations of each method.

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References


[41] G. Hartmann, T. Baumgartner, M. Schuster, Influence of particle coating and matrix constituents on the cloud point extraction efficiency of silver nanoparticles (Ag-NPs) and application for monitoring the formation of Ag-NPs from Ag<sup>+</sup>. *Anal. Chem.* 2014, 86, 790. doi:10.1021/AC403289D


