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Single Particle ICP-MS as a Tool for Determining the Stability of Silver Nanoparticles in Aquatic Matrixes Under Various Environmental Conditions, Including Treatment by Ozonation

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Abstract

Silver nanoparticles (AgNPs) are used in a large number of consumer products due to their antimicrobial and antifungal properties, and these materials may be discharged into municipal wastewater. Wastewater treatment, including advanced oxidation processes (AOPs) may modify the forms of silver in wastewater before they are discharged into surface waters. In addition, little is known about the changes in AgNPs that occur in natural waters under different environmental conditions. In this project, we utilized single particle ICP-MS (spICP-MS) and Dynamic Light Scattering (DLS) analytical techniques to evaluate changes in the number and size of AgNPs in laboratory experiments with milliQ water under different environmental conditions, as well as during ozonation. Changes in the number and size of AgNPs determined by spICP-MS were evidence of altered stability of the nanoparticles. Increased rates of dissolution occurred under extremes of pH. Lower temperature decreased the rate of dissolution of AgNP relative to the dissolution in treatments at room temperature. The addition of chloride resulted in the loss of AgNPs from suspension due to agglomeratiuon and precipitation. Ozonation led to a rapid decline in the number and size of AgNPs, as indicated by both spICP-MS and DLS analysis. An increase in the concentration of dissolved silver in the ozone treatments was evidence that changes in particle size were a result of oxidative dissolution of AgNPs to silver ion.

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Introduction

Because of their antimicrobial and antifungal activity, silver nanoparticles (AgNPs) are being incorporated into a large number of consumer products, such as socks, sports clothing, deodorants, additives to washing machines, food packaging and wound dressings [1]. Because AgNPs can be released from these products and then enter domestic wastewater, there is a growing concern about the toxicity of these nanoparticles [2]. However, little information is available on the behavior of AgNPs in wastewater treatment plants (WWTPs), or in surface waters, once they are discharged. There is evidence that a high proportion of AgNPs are transformed into insoluble species (e.g. Ag_2S) in conventional WWTPs and will be incorporated into sewage sludge, and thus only a small amount of the AgNPs will be discharged from WWTPs into receiving waters [3-5]. Yang et al. predicted that AgNP concentrations in treated municipal wastewater would range between 0.028 and 5.5 μg/L [6]. Dumont et al. used models to predict that the long-term average concentrations of AgNP in European surface waters would be in the range of 0.002 ng/L to 0.3 ng/L [7].

Little is known about the effects of oxidants used for disinfection and advanced oxidative treatment of wastewater on the fate of AgNPs. Ho et al. observed rapid dissolution of AgNPs in the presence of H_2O_2 [8]. Mitrano et al. reported rapid dissolution of AgNPs in chlorinated tap water [9]. Thalmann et al. investigated the effect of ozone on silver sulfide (Ag_2S), which is formed under anaerobic conditions during wastewater treatment, and concluded that Ag_2S will dissolve during ozonation [10]. A similar environmental fate might be expected for AgNPs exposed to ozone, resulting in oxidative dissolution that will release silver ions. Free silver ion has been shown to be highly toxic to aquatic organisms [11]. Kittler et al. reported that the toxicity of AgNPs increased during storage due to slow dissolution of the nanoparticles to silver ion [12].

The fate of AgNPs released into surface waters is also not fully understood. It has been shown that AgNPs dissolve rapidly at low pH, but this process is dependent on the size of the original particle [13-14]. The electrolyte composition in the AgNP suspension has been shown to affect the rate of particle agglomeration and dissolution [15]. Ionic strength and the presence of dissolved organic carbon (DOC) are also parameters that affect the dissolution of AgNPs in aquatic matrixes [16]. All of these studies, however, have been carried out at aqueous concentrations much higher than the part per billion or part per trillion levels expected in wastewater and in surface waters. These limitations are due to the low sensitivity

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of established analytical methods such as transmission electron microscopy (TEM) and dynamic light scattering (DLS). Investigations using more sensitive analytical techniques at environmentally relevant concentrations are needed to develop a more complete understanding of the fate of AgNPs in aquatic matrices.

A promising method for characterizing the size and number of nanoparticles at low concentrations in aquatic matrices is single particle inductively coupled plasma mass spectrometry (spICP-MS). Originally developed by Degueldre et al. [17], this analytical technique has now been applied in a range of laboratory and field studies to evaluate the fate of nanoparticles in aquatic matrixes [18-20]. Mitrano et al. reported that spICP-MS can be used to track dissolution of AgNPs in suspensions at environmentally relevant concentrations, since they found a correlation between increasing concentrations of silver ion (Ag^{+}) and declining mean sizes of AgNPs [9].

In this study, we evaluated changes in the number and size of AgNPs in milliQ water under varying conditions of temperature, pH, and Cl^{-}, as well as under conditions typical for ozonation of wastewater. The diameter of the particles was monitored by means spICP-MS, as well as DLS. The concentrations of dissolved silver (dAg) in the aquatic matrixes were monitored using ICP techniques with MS (ICP-MS) or with optical emission spectroscopy (ICP-OES) detection.

Experimental

Chemicals

Suspensions of AgNPs with spherical shape and mean diameters reported by the manufacturer as 50 nm and 80 nm, respectively, were purchased from NanoComposix (San Diego, CA, USA). The particles were coated with citrate or polyvinyl pyrrolidone (PVP) capping agent. The suspensions were stabilized with 378 mg/L citrate and 1-3 μg/L PVP, respectively. An aqueous Ag(I) standard for ICP-MS analysis (1000 mg/L) was purchased from SPC Science (Baie D'Urfé, QC, Canada). Nitric acid (65%) and potassium iodide (10% w/v) were purchased from Thermo-Fisher Scientific (Mississauga, ON, Canada). Extra dry oxygen (MEGS, Canada) was used as feed gas for the ozone generator. All chemicals were used in the highest quality available. Deionized water was prepared with a Milli-Q Element system (Millipore, Billerica, MA, USA).

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Stability experiments

AgNP suspensions

Working suspensions of AgNP at a nominal concentration of 20 µg/L were prepared by diluting the NanoComposix stock suspensions with MilliQ water. These suspensions were made fresh daily, kept at 4°C when not in use and were manually shaken for 1 minute before being aliquoted. All stability experiments were conducted in polypropylene tubes covered with aluminum foil, with 50 mL of test medium in each tube. The test media were prepared first, and then the AgNP suspension was added to the mixture. Four sets of experiments were conducted to determine the stability of AgNPs under various environmental conditions. In each set, AgNPs with a mean diameter of 80 nm with either citrate or PVP capping were tested. The experiments were carried out with nominal silver concentrations of C_{Ag} = 0.1 µg/L and C_{Ag} = 1 µg/L.

In experiments to determine the stability of AgNPs under different temperatures, AgNP suspensions were added to MilliQ water maintained at room temperature (21±2^{o}C) and at 4°C, respectively. In other experiments conducted at room temperature, AgNP suspensions were added to MilliQ water containing chloride at a nominal concentration of 200 µg/L, as a mixture of NaCl (199 µg/L), KCl (24 µg/L), and $MgCl_2$ (116 µg/L) . In these solutions, the pH value was not altered. In other experiments, the pH of the MilliQ water was adjusted to values of 5, 7 and 7.6 with acetic acid and sodium hydroxide solution, respectively. The samples for t=0 measurements were collected for analysis directly after AgNP addition. Over the next week, samples were collected at intervals for monitoring of changes in AgNP diameter and the concentrations of dAg.

Ozonation experiments

AgNP suspensions

A working suspension of AgNP at a nominal concentration of 100 mg/L was prepared by diluting the NanoComposix stock suspension with MilliQ water. The working suspension was kept at 4°C and was manually shaken for 1 minute prior to each use. A 20 mL aliquot of the stock suspension was placed into twelve glass Erlenmeyer flasks covered with aluminum foil, which were used as the ozonation reaction vessels.

Ozonation

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Ozone was generated by passing oxygen at 10 psi through an Ozonia (Duebendorf, Switzerland) TOGC2 ozone generator using a corona discharge. A 1 L stock solution was produced by bubbling an oxygen/ozone mixture (OOM) through a porous ceramic diffuser in MilliQ water. Excess OOM was vented out to a quenching solution of potassium iodide. Formation and presence of OOM was visually noticeable by a colour change of the quenching solution from light yellow to dark brown. This colour change was not observed when pure oxygen was bubbled through the quenching solution. The ozone concentration was verified by measuring absorption of UV with wavelength of 258 nm [21], and the dissolved concentrations were approximately 180 µM.

Appropriate volumes of 100 to 600 µL of ozone solution were added to three of the AgNP suspensions contained in the Erlenmeyer flasks to create a reaction mixtures with ozone/AgNP molar ratios of 0:1, 1:1 and 3:1. A control treatment consisted of 0:1 ozone/AgNP mixture generated by adding 500 µL of a solution of dissolved oxygen. The oxygen solution was made by bubbling pure oxygen through the ozonation apparatus without turning on the ozone generator. The oxygen was left to equilibrate in the water for 5 minutes to ensure saturation. The quenching solution was checked to see if there was no colour change, as this would indicate presence of ozone in the gas phase.

All the flasks were kept covered and at room temperature (21±2°C) for the duration of the experiment. Three of the twelve reactions vessels were exposed to ozone and stored for 168 hours prior to analysis. The remaining nine unreacted solutions were kept under the same condition. After 96 hours, three more flasks were exposed to ozone using the same three doses and then stored for 72 hours. The experiment was repeated until all flasks were treated with ozone. Volumes of 1 L of ozone solutions were made fresh and then spiked into the AgNP suspension. Subsequently, the ozone concentrations were determined and the volumes of the samples adjusted in order to achieve the same ozone doses in each vessel. The experimental plan was designed so that all samples would be ready for analysis at the same time after different storage times of 45 min, and 24, 72 and 168 hours.

Analysis

Ultrafiltration

In order to determine the concentrations of Ag in the dissolved phase, water samples were subjected to ultrafiltration using the centrifugal ultrafiltration method reported by

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161 Hadioui et al. [22]. The optimized method was conducted using Microsep Advance
162 Centrifugal Filter Units (CFU) with 3 kDa average pore diameter purchased from Pall Science
163 (Montreal, QC, Canada). Water samples were pre-filtered through 0.45 µm (25 mm) nylon
164 syringe filters (Canadian Life Sciences, Peterborough, ON, Canada) before being passed
165 through the CFU.
166 A volume of 4 mL of sample was transfered into the CFU, and centrifuged in a Sorvall
167 Legend X1R centrifuge (ThermoFisher Scientific, MA, USA) at room temperature at 4,000 g
168 for 30 min, and this was repeated for 4 consecutive cycles. As dissolved Ag (dAg) needs to
169 equilibrate with ultrafiltration membrane, the first three cycles of centrifugation are regarded
170 as membrane equilibration steps. The term dAg is operationally defined as Ag^+ and any
171 soluble Ag complexes that are not retained by the 3 kDa filter, which corresponds to a pore
172 size of approximatey 1 nm. The filtrate was digested by adding 80 µL of nitric acid to the 4
173 mL of filtrate, and allowing digestion for 48 hours at room temperature before analysis by
174 ICP-OES.

175 *ICP-OES analysis*
176 ICP-OES (iCAP 6000 Series, Thermo Scientific) was used to determine the
177 concentration of dAg. The optimized operating parameters for the instrument were: RF
178 power, 1150 W; Pump rate, 50 rpm; Auxiliary gas flow, 0.6 L/min; Nebulizer gas flow, 0.5
179 L/min; Coolant gas flow, 12 L/min; Purge gas flow, 1 L/min. The calibration of the
180 instrument was done using multiple standard silver solutions prepared with 1% (v/v) nitric
181 acid using PlasmaCAL (SCP Science) silver standard at 1 ppb, 10 ppb, 50 ppb, and 500 ppb
182 and a blank.

183 *DLS analysis*
184 The hydrodynamic diameter of the AgNPs was analysed by Dynamic Light Scattering
185 (DLS) using a Malvern Zetasizer (Malvern, U.K.) NanoZ instrument. The sample was run
186 with 15 runs, 20 seconds per run. A disposable sizing cuvette (DTS0012) was used.

187 *spICP-MS analysis*
188 Analysis by spICP-MS analysis was carried out on a ThermoFisher (Bremen, Germany)
189 XSeries 2 ICP-MS. The limit of particle sizes detectable under the operating conditions with
190 this instrumentation was ~40 nm for this instrumentation. However, in presence of dissolved
191 Ag(I) ions, this value increases. The samples were introduced into the plasma with a

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192 borosilicate glass conical nebulizer (AHF, Tübingen, Germany) at a rate of 200 µL/min *via* a
193 conical spray chamber with impact bead. Prior to measurements, the operating conditions
194 were optimized with an Ag(I) ICP-MS standard (5 µg/L) for best signal intensity. The
195 nebulizer gas flow was set to 0.85 L/min. The plasma was run at a RF power of 1450 W. Cool
196 gas was operated with a flow of 15 L/min. The spray chamber was cooled externally to 4°C.
197 The optimal flow rate of the nebulizer was determined daily. For all measurements of
198 calibration standards and samples, the intensity of ^{107}Ag was determined with a dwell time of
199 5 ms, and with a run time of 300 s.
200 Data from the ICP-MS were processed using PlasmaLab software, version 2.5.9.300
201 (ThermoFisher). Raw data were exported as Thermo Electron Glitter Format V1.1. The
202 numerical results were subsequently imported into the software OriginLab8 (OriginLab Corp.,
203 Northampton, MA, USA) for further processing.
204 NP diameters were calculated for single intensity data points as described by Pace et al.
205 [23]. The transport efficiency of the sample introduction system was determined by
206 measuring the pulse frequency of a reference nanoparticle suspension (80 nm, PVP coated,
207 200 ng/L) in single particle mode. With the known particle number in the suspension and the
208 known sample flow rate, a transport efficiency of ca. 3% was calculated.
209 Ag(I) standard solutions (C_{Ag} = 0, 0.10, 0.25, 0.50, 0.75, 1.00 and 2.00 µg/L) were also
210 analyzed in single particle mode. By taking into account the calculated transport efficiency
211 and the dwell time, the respective intensities could be transformed into a mass flux calibration
212 curve, with intensity plotted as a function of mass per measuring point (in this case per
213 particle). With use of the known density of silver, the volume and subsequently the diameter
214 of the particles were calculated.
215 For the determination of mean diameter of the AgNPs, all diameters of one run were
216 plotted as histograms with increments of 2 nm. The histograms were then fitted to a Gaussian
217 function to determine the mean diameter of the nanoparticles in the samples. The particle
218 concentration was calculated by counting all detected NP peaks (above the respective
219 detection limit) per run and extrapolating to the respective sample volume by taking the
220 transport efficiency into account.
221
222 **Results and Discussion**
223 *Experimental approach*

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The greatest advantage of spICP-MS as an analytical techique is that is can provide detailed qualitative and quantitative information about the particle size distribution and number, and the mass concentration of dAg and AgNPs. Furthermore, it has detection limits for AgNP that cover expected environmental concentrations. Isotope dilution techniques using ^{109}Ag-enriched standards even allow for reliable characterization in complex matrixes [24]. The largest limitation, however, is the size detection limit, which is, depending on the mass spectrometer, around 20-30 nm [25]. However, recent advances in instrumentation and manipulation of the data generated by spICP-MS are expanding the lower range of sizes that can be detected [26-27]. Since spICP-MS analysis generates data from which the size of the particle core is determined, it is useful to simultaneously use other analytical techniques, such as DLS to determine the hydrodynamic size of the particles. However, DLS is not a sensitive as spICP-MS and so higher concentrations are required in order to be detectable using this instrumentation.

The initial concentrations of AgNPs in suspension for stability experiments were in the range of 0.1 to 1 µg/L, which are relevant to concentrations in municipal wastewater [3-6], but is probably higher than the concentrations of AgNPs expected in surface waters [7]. However, AgNP concentrations used in the ozonation experiments were in the mg/L range. The higher concentrations were necessary to allow complementary analysis using DLS instrumentation, in addition to spICP-MS. The ozonation levels used in these experiments are relevant to ozone concentrations typically used for wastewater disinfection [21].

Stability in aqueous matrixes

The behaviour of AgNPs with different particle sizes and coatings was monitored over time in aqueous solutions with varying pH, temperature, and chloride concentrations. Analysis by spICP-MS of samples from the experiments at room temperature showed that there was a steady decline in the mean diameter of AgNPs over the first 14 days (i.e. 336 h) of the experiment, with the rate of decline showing a curvilinear response (Figure 1a). The declines in mean diameter of AgNPs were assumed to occur as a result of oxidative dissolution to Ag^+. The particle concentration of the respective suspensions are documented in table 1. The particle concentration did not change in the first hours of the experiment, although the particle diameter decreased. However, after one and two weeks, respectively, a significant decrease in particle concentration was observed. In the low temperature

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experiment, the AgNP was more stable (Figure 1a and table 1), both in diameter as well as particle concentration. The rates of dissolution from these experiments were more rapid than the rates reported by Mitrano et al. using a similar experimental approach with natural surface waters, but were similar to the dissolution rates they observed for AgNPs suspended in deionized water [9]. In the present study, a lower temperature (i.e. 4^{o}C) reduced the rate of decline of the mean size of the AgNPs occurring through dissolution of the nanoparticles (Figure 1a). These results indicate that AgNPs in suspension in natural waters may be much more stable during colder months of the year in temperate climates. Experiments with AgNPs (80 nm) with the citrate coating showed similar results.

Neutral pH (i.e. pH=7.0) also promoted the stability of AgNP, relative to a more rapid decline in the mean diameter of AgNPs at higher, and especially lower pH values (Figure 1b). These findings can be explained by the mechanism of the dissolution. According to Liu and Hurt [28], the reaction stoichiometry of the AgNP dissolution under aerobic conditions in aqueous solutions with no other reactants must be:

$$2Ag_{(s)} + \tfrac{1}{2}\, O_{2(aq)} + 2H^{+}_{(aq)} \leftrightarrow 2Ag^{+}_{(aq)} + H_2O_{(l)}$$

The thermodynamic equilibrium is therefore a function of pH. The higher the H^+ concentration, the faster the reaction. It is also possible that the impact of basic pH values on AgNP stability can be explained by destabilization of the coating at high pH, resulting in exposure of an unprotected silver core. However, even though the negatively charged citrate coating has different properties than the neutral PVP coating, no significant differences were observed between the results for citrate coated and PVP coated AgNPs. Mitrano et al. also concluded that the capping agent of AgNPs has a negligible effect on the dissolution rate of AgNPs in suspension [9]. Therefore, more research is required to determine whether destabilization of the coating is a factor in the dissolution of AgNPs at extremes in pH.

The concentration of AgNPs in the test suspension was an important variable, as the rate of decline in the mean size of AgNPs in suspensions at concentrations of C_{Ag} = 0.1 µg/L was much faster than in AgNP suspensions at concentrations with C_{Ag} = 1.0 µg/L (Figure 1c). This is reasonable when looking at the reaction stoichiometry, as the proportion of protons to particles will be higher at low AgNP concentrations.

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Figures 1d shows the change in mean diameter of 80 nm AgNPs coated with PVP stored in suspensions spiked with chloride. The mean diameter of AgNP decreased very rapidly in the chloride enriched solution, such that, after one day, AgNPs were no longer detected. The rapid change in particle number shown in Table 1 is consistent with that observation. This is most likely caused by the rapid precipitation of AgCl out of suspension. However, agglomeration was not observed. These results are not in agreement with dissolution experiments conducted by Mitrano et al. in which chloride stabilized the suspension and slowed the rate of dissolution; especially in a solution of 1 mg/L Cl^- [19]. The differences between the present study and the Mitrano et al. study may be due to differences in the aqueous matrix in which the AgNPs were suspended [19]. Li et al. observed that addition of Cl^- to a suspension of AgNPs caused aggregation and precipitation of the nanoparticles out of suspension; consistent with the present study [15].

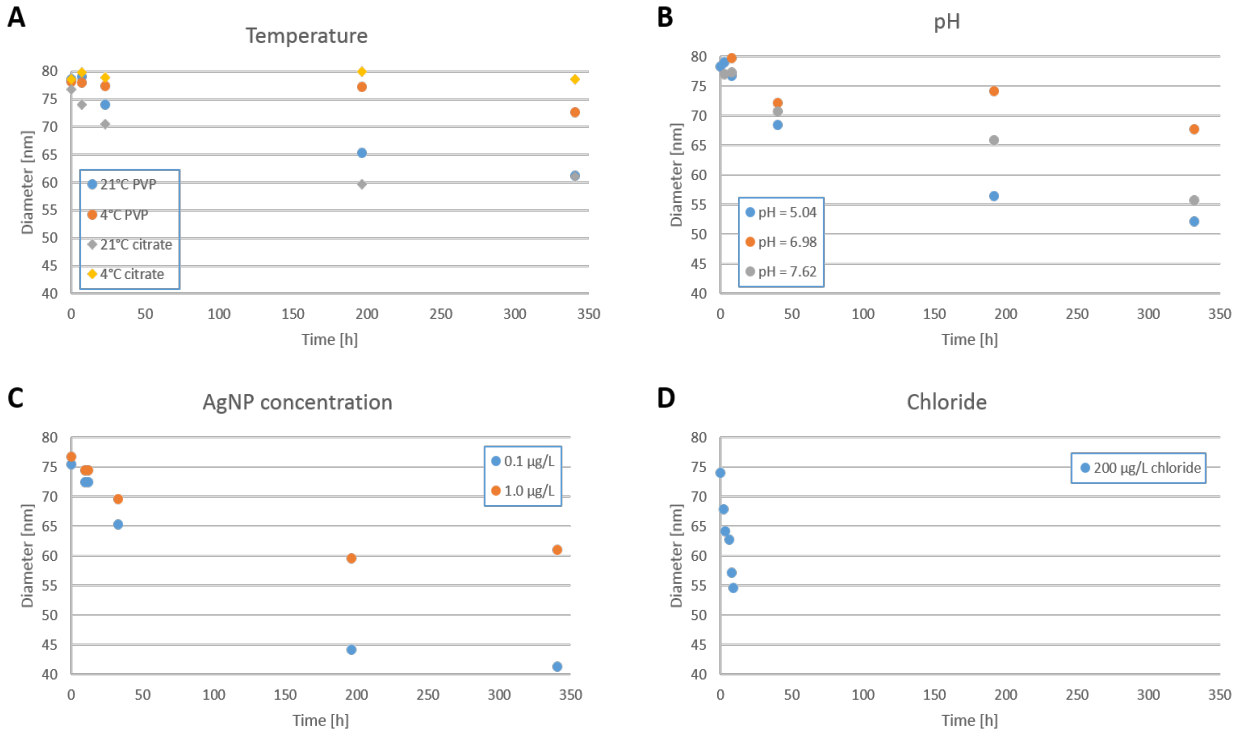

Figure 1: Changes over 2 weeks in the mean diameter of AgNPs (80 nm). A) stored at temperatures of 4°C and 21°C (PVP or citrate capped, C_{Ag} = 1 µg/L). B) stored at pH values of 5, 7 and 7.6 (PVP capped, C_{Ag} = 1 µg/L). C) stored at different concentrations of C_{Ag} = 0.1 µg/L and C_{Ag} = 1 µg/L (citrate capped). D) stored in aqueous solutions containing chloride at a nominal concentration of 200 ppb (PVP capped, C_{Ag} = 1 µg/L).

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These experiments in milliQ water showed a trend of declining mean size and particle concentration in treatments with AgNPs exposed to extremes of pH and at higher temperatures, presumably as a result of rapid dissolution of the nanoparticles. Addition of chloride appeared to remove AgNPs from suspension through precipitation. Additional studies are required to investigate the effects of a range of water quality parameters to better predict the fate of AgNPs in surface waters.

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316 Table 1: Changes over 2 weeks in the particle concentration (particles/L) of AgNP suspensions (80
317 nm, PVP or citrate capped) stored at different temperatures, concentrations, and at different pH values.
318

time [h]	particle concentration [particles/L]								
	21°C, PVP (1.0 µg/L)	**21°C, citrate (0.1 µg/L)**	**21°C citrate (1.0 µg/L)**	**4°C PVP (1.0 µg/L)**	**4°C citrate (1.0 µg/L)**	**pH = 5.04**	**pH = 6.98**	**pH = 7.62**	**200 µg/L chloride**
0	3.31E+08	4.62E+07	3.88E+08	3.14E+08	3.20E+08	3.83E+08	4.25E+08	3.28E+08	2.85E+08
7	3.36E+08	4.04E+07	3.63E+08	3.67E+08	3.88E+08	2.97E+08	3.96E+08	3.91E+08	1.82E+08
23	3.56E+08	2.98E+07	3.30E+08	3.11E+08	3.03E+08	2.38E+08	3.42E+08	1.60E+07	0
196.5	8.58E+07	1.10E+06	6.20E+07	3.58E+08	3.90E+08	3.88E+07	1.22E+08	1.14E+08	0
340.5	4.03E+07	2.84E+05	3.82E+07	3.27E+08	2.59E+08	1.88E+06	4.39E+07	3.70E+07	0

319

320 *Ozonation*

321 Figure 2 shows the mean diameters of AgNPs in experiments at room temperature
322 conducted with 50 and 80 nm (PVP capped) particles, respectively, with various ozone doses
323 and incubation times. These data on the hydrodynamic diameter of AgNPs were generated by
324 DLS analysis. The hydrodynamic diameters did not vary significantly over time in the control
325 treatments (i.e. oxygen addition only). In experiments with an ozone to AgNP molar ratio of
326 1:1, a slight decrease in average hydrodynamic diameter of the AgNPs was observed. A
327 significant decline in the hydrodynamic diameter of the AgNPs was observed in samples
328 collected from treatments with a 3:1 molar ratio (Figure 3). The same trends were observed
329 for changes in the mean diameter of AgNPs with both 50 and 80 nm sizes (Figure 3).

330 Hydrodynamic diameter refers to the size of the silver particle plus the capping material
331 and is determined by DLS, as opposed to the diameter of the silver core, which is calculated
332 from spICP-MS analysis. Note that the mean hydrodynamic diameters determined by DLS
333 (Figure 2) were approximately the same as the nominal 50 nm and 80 nm core diameters
334 reported by the commercial suppliers of the particles, respectively. It would have been
335 expected that the mean hydrodynamic diameters would be somewhat greater than the nominal
336 core diameters. However, DLS instrumentation is subject to generating data that are not
337 accurate in terms of mean hydrodynamic diameter, as has been reported previously [29-30].
338 Therefore, the absolute values for mean hydrodynamic diameter generated by DLS should be
339 interpreted with caution. The data do indicate however, that the mean hydrodynamic diameter
340 declined in the treatments with ozone.

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Figure 3 shows the size distribution of the AgNPs (80 nm) in treatments with the 1:1 molar ratio in the ozonation experiment at 45 min and 1 and 3 days, as measured by spICP-MS. Due to the presence of high concentrations of ionic silver in solution, the size detection limit using this method was slightly larger than 50 nm, so no particles were detected with sizes >50 nm at Day 3 (Figure 3). The bimodal distribution may originate from particle coincidence; meaning that two particles reached the detector at the same monitoring time, leading to a signal with a higher intensity. The higher peak may represent the signal from the single particles and the smaller peak (i.e. 50-60 nm) may represent the signals generated from two coincident smaller particles. This may have been avoided by further dilution of the samples, although the treatments were at environmentally relevant concentrations. In contrast, DLS analysis showed that particles with mean hydrodynamic diameters of approximately 50 nm were still present at Day 3 of the experiment.

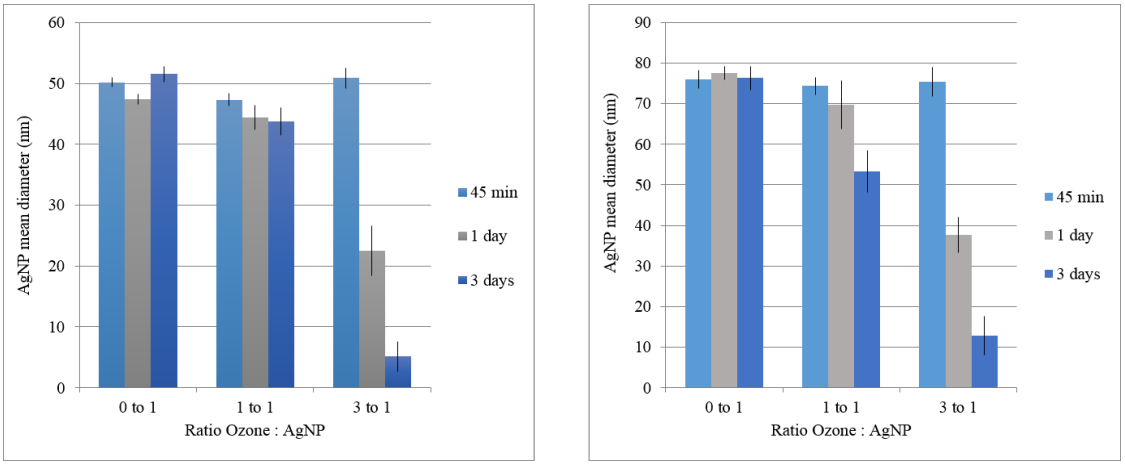

Figure 2: Mean and SD (n=3) of measurements of the hydrodynamic size (nm) of AgNPs in ozonation experiments at various AgNP to ozone ratios for experiments conducted with 50 nm (left) and 80 nm (right) PVP capped AgNP. These data were generated by DLS analysis of samples collected at 45 min, 1 day and 3 days of incubation.

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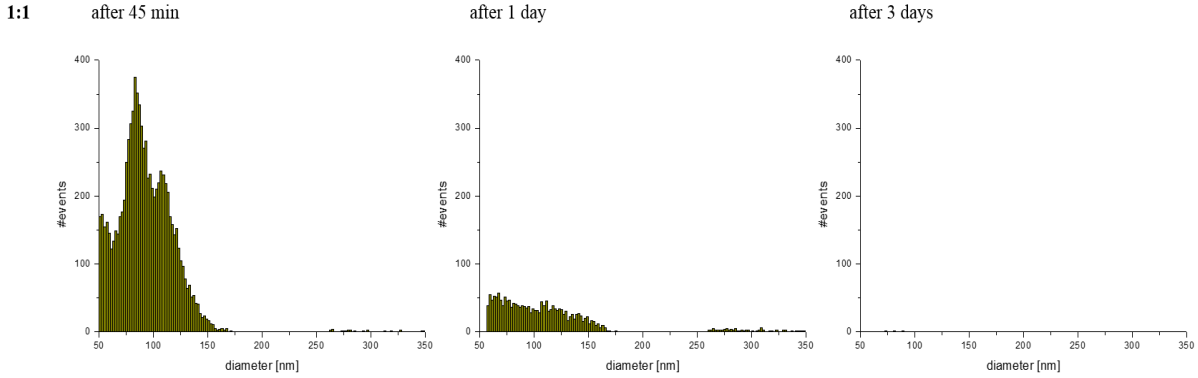

Figure 3: Distribution of sizes (nm) of AgNPs (80 nm) in the ozonation experiment at an AgNP to ozone ratio of 1:1, as measured at 45 min, 1 day and 3 days after addition of ozone. These data were generated by spICP-MS analysis of samples collected at 45 minutes, 1 day and 3 days of incubation.

Figure 4 shows the Ag concentration determined by ICP-OES analysis of the filtrate after ultrafiltration of the samples collected during ozonation experiments. The dAg present in the filtrate could be ionic silver (Ag^+) or silver ion complexed with organic ligands. The increase in the concentration of dAg in treatments with both 50 nm and 80 nm AgNPs (PVP capped) indicates that ozonation promotes the formation of dAg as a result of dissolution of AgNPs. The dissolution of the particle reduces the size of the silver core; thereby causing a decline in the mean size of the AgNPs.

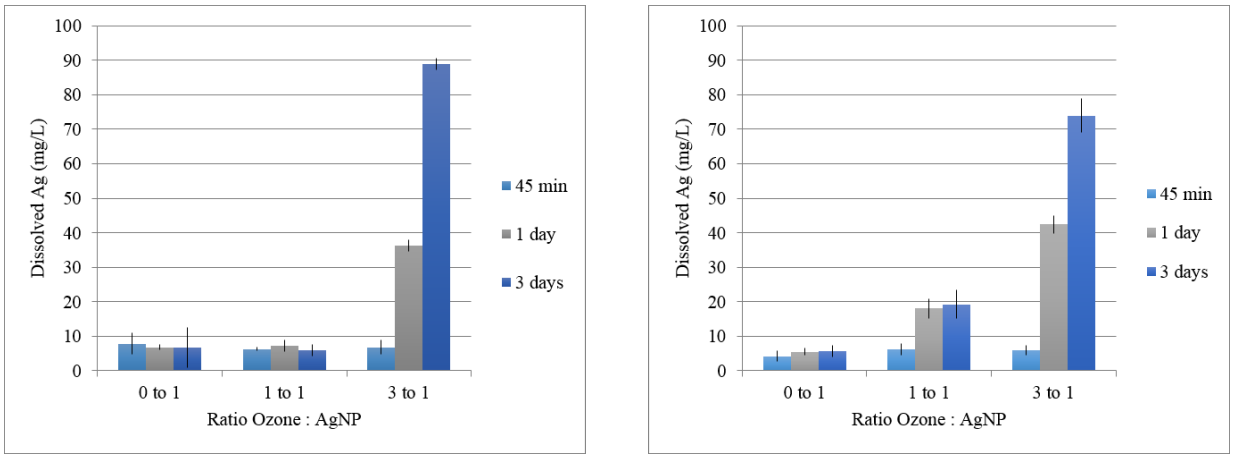

Figure 4: Mean and SD (n=3) of the dissolved silver concentration (mg/L) as a function of time and ozone dose for experiments with 50 nm (left) and 80 nm (right) PVP capped AgNPs. These data were generated by ICP-OES analysis of the filtrate after centrifugal ultrafiltration of samples.

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Particle concentrations (particles per mL) determined by spICP-MS analysis of samples nm are shown in Figure 5. Very little variation in particle numbers over time were observed in the control treatment (i.e. oxygen). Samples collected from the treatment with a 1:1 molar ratio showed a marked decline in particle concentration after one day, with no particles detected at 3 days. In ozonation treatments at a molar ratio of 3:1, no particles were detected at Day 1 or Day 3 of the experiment (Figure 5). Due to the high dAg concentrations in solution, the size limits of detection in these treatments was >50 nm. This limitation of the instrumentation, and the fact that particle coincidence may have biased the accuracy of the particle number determination (Figure 3) complicates data evaluation. However, the trend is consistent with the results of hydrodynamic diameter determination and the dissolved silver concentration, which is that the higher the ozone concentration, the faster the dissolution. These results show the challenges associated with spICP-MS analysis, but recent advances in data processing [26] and instrumentation [27] may overcome some of these shortcomings.

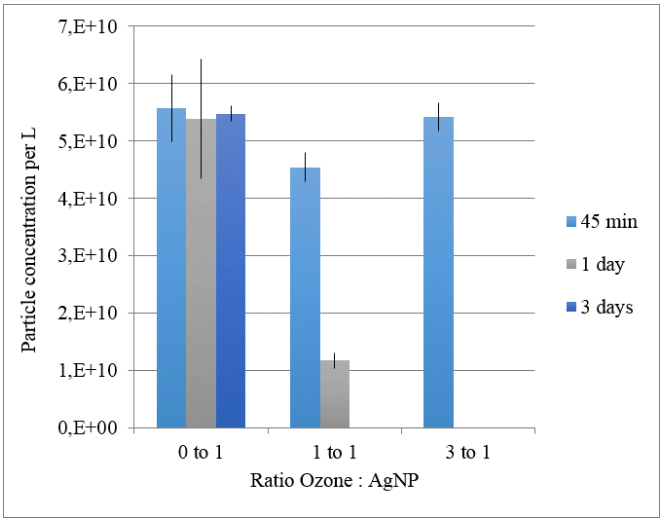

Figure 5: Mean concentrations of AgNPs (particles/mL) in suspension as a function of ozone dose for ozonation experiments with 80 nm PVP capped AgNPs. These data were generated by spICP-MS analysis of samples collected at 45 minutes, 1 day, and 3 days of incubation.

The complementary results of analysis using DLS, ICP-OES, and spICP-MS show that the addition of oxygen in the control treatments had no effect on removal of AgNPs over the 3 day incubation period, either through dissolution or particle agglomeration. Treatments with ozone, however, led to rapid removal of AgNPs from suspension through dissolution, as

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shown by a decline in the mean size of the AgNPs and increasing dAg concentrations. An examination of the data generated by spICP-MS analysis of the suspensions during ozonation indicates that extensive agglomeration did not take place, which would be visible as the appearance of large particles.

Comparing the results of the experiments conducted with different ozone/AgNP ratios, it is obvious that the higher the ozone concentration, the faster the dissolution process. Ozone is a highly reactive oxidant, which can degrade the capping agent, as well as the silver core of the AgNPs. Oxidative reactions between AgNPs and ozone at the 1:1 and 3:1 molar ratios were, based on experience with ozonation experiments, almost certainly completed long before the first samples were collected for analysis (i.e. 45 min of incubation). However, rapid declines in the number and size of particles continued throughout the 1 and 3 days of incubation. Therefore, it is likely that the initial oxidative damage to the capping agent and/or silver core caused by the reaction with ozone promoted the continued dissolution of the AgNPs over time.

As these experiments were carried out in MilliQ water, it is difficult to predict how a wastewater matrix will affect the reaction of ozone with AgNPs. Ozone might preferentially react with other compounds in the complex wastewater matrix. However, it is possible that ozonation of wastewater containing AgNPs will lead to enhanced dissolution of the nanoparticles and the formation of high concentrations of dissolved Ag. This introduces concerns regarding the formation of the toxic free silver ion, which could affect aquatic organisms in surface waters.

Conclusions

The data from this study shows that AgNPs in deionized water at environmentally relevant concentrations are not stable in acidic or basic environments because of rapid dissolution to dAg. Lower temperatures increased the stability of the suspensions by reducing the rate of dissolution. Chloride ion concentrations similar to those in natural waters decreased the stability of AgNPs, probably through the formation of insoluble AgCl complexes. Ozonation markedly increased the rate of dissolution of AgNPs. This process is dependent on the ozone concentration relative to the concentration of AgNPs, as the rate of disappearance of AgNPs from suspension and formation of dAg was about three times greater in treatments with a 3:1 molar ratio in comparison to treatments with a 1:1 ratio. These results indicate that advanced

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wastewater treatment by means of ozonation could increase the concentration of toxic silver ions in the effluent. This has to be taken into account when predicting the impact of ozonation on the toxicity of treated wastewater.

This study demonstrates the utility of spICP-MS for evaluating the fate of nanoparticles in aquatic matrixes. However, the maximum size range for analysis of AgNPs (i.e. 40-50 nm) at the time of this experiment was a barrier to a more complete analysis of the fate of these nanoparticles. Since spICP-MS analysis generates data from which the size of the particle core is determined, it is useful to use other analytical techniques such as DLS to determine changes in the hydrodynamic size of particles. Complementary analysis of dAg can also provide information on the dissolution of AgNPs. Therefore, spICP-MS can be a valuable analytical tool in conjunction with other analytical techniques to evaluate the fate of nanoparticles in aqueous matrixes.

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