Stability of Citrate, PVP, and PEG Coated Silver Nanoparticles in Ecotoxicology Media

Mila Tejamaya,†‡ Isabella Römer,† Ruth C. Merrifield,† and Jamie R. Lead*,†

†School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT United Kingdom
‡School of Public Health, University of Indonesia, Kampus Baru UI Depok 16424, Jawa Barat, Indonesia

Supporting Information

ABSTRACT: Silver nanoparticles (AgNPs) are present in the environment and a number of ecotoxicology studies have shown that AgNPs might be highly toxic. Nevertheless, there are little data on their stability in toxicology media. This is an important issue as such dynamic changes affect exposure dose and the nature of the toxicant studied and have a direct impact on all (eco)toxicology data. In this study, monodisperse citrate, PVP, and PEG coated AgNPs with a core size of approximately 10 nm were synthesized and characterized; their behavior was examined in standard OECD media used for Daphnia sp. acute and chronic tests (in the absence of Daphnia). Surface plasmon resonance, size, aggregation, and shape were monitored over 21 days, comparable to a chronic exposure period. Charge stabilized particles (citrate) were more unstable than sterically stabilized particles. Replacement of chloride in the media (due to concerns over chloride-silver interactions) with either nitrate or sulfate resulted in increased shape and dissolution changes. PVP-stabilized NPs in a 10-fold diluted OECD media (chloride present) were found to be the most stable, with only small losses in total concentration over 21 days, and no shape, aggregation, or dissolution changes observed and are recommended for exposure studies.

INTRODUCTION

Nanoparticles (NPs) can be defined as material with at least one dimension between 1 and 100 nm1 or particles with novel properties that differ from its bulk material.2 NPs have been exploited in many consumer products such as in sunscreens, cosmetics, paint, appliances, computer devices, etc. Currently there are likely to be many thousands of nanoenabled products on the market, and this number is rapidly increasing.3 Due to their broad range of antimicrobial properties, silver nanoparticles (AgNPs) have become perhaps the most commonly used NP type. The rapid growth in commercialization has increased environmental exposure. For instance, AgNPs can be rapidly released from fabrics by washing and from building facades after rainfall events;4,5 exposure models have indicated measurable amounts in the environment (with predicted environmental concentration (PEC) in water of tens and perhaps hundreds of ng L\(^{-1}\)).6

In addition, AgNPs are potentially toxic both to humans and the environment as has been presented in a number of review papers.7–10 As with exposure, there are many uncertainties about mechanisms of action, dose measurement, dose–response relationships, and the physicochemical form of the AgNPs during and after exposure in a complex media. In particular, the change in exposure dose and the nature of the toxicant in (eco)toxicological media, due to aggregation, dissolution, shape, and surface chemistry changes, is poorly quantified.11,12 For AgNPs, there is little information about changes such as aggregation, dissolution, and shape at high ionic strengths and chloride concentrations relevant to such media.

Understanding both the dynamics of exposure concentration changes and the alteration in physicochemical properties of NPs during (eco)toxicology exposures is essential in the interpretation of dose–response relationships. Those changes, which are likely to occur over relevant exposure periods, are poorly accounted for in the literature to date.13,14 A few studies have shown that aggregation15,16 and dissolution17,18 of AgNPs have occurred in toxicology media with consequent changes in bioavailability and toxicity.8,19 Systematic investigations of temporal changes over exposure are required, and changes may be influenced by factors such as solution chemistry20,21 and light.22

This study examined the influence of media composition and concentration to the AgNPs stability. Three different capping agents which are representative and have been frequently used in other studies23 were utilized. They are citrate, which stabilizes by charge repulsion23 and is weakly bound to the

Special Issue: Transformations of Nanoparticles in the Environment

Received: November 2, 2011
Revised: February 8, 2012
Accepted: March 6, 2012
core Ag and polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP), which sterically stabilize nanoparticles and which are strongly bound to the core and potentially permeable to solutes and solvents. Standard OECD (Organisation for Economic Co-operation and Development) media for Daphnia sp. for acute and chronic studies, its dilution and variants by replacement of specific ions, were employed to examine NP changes over both acute and chronic exposure time scales.

**EXPERIMENTAL SECTION**

**Particle Synthesis.** Three types of AgNPs with similar core sizes (ca. 10 nm) were used in this study, which were coated in either citrate, PVP, or PEG. The synthesis method of the citrate coated AgNPs has been published previously and summarized in the Supporting Information (SI). Aliquots of citrate NPs were taken, which were converted to PEG-SH coated NPs by adding thiolated PEG (M<sub>w</sub> 5000, purchased from Sigma Aldrich) under vigorous stirring; and coatings were coated NPs by adding thiolated PEG (M<sub>w</sub> 10000, purchased from Sigma Aldrich) under vigorous stirring; and coatings were previously calculated as having 4 molecules of PEG-SH per nm<super>2</super>. The mixture was shaken overnight. The increase of particle size by dynamic light scattering (DLS) and flow-field flow fractionation (Fl-FFF), but not by transmission electron microscopy (TEM), and a shift in surface plasmon resonance (SPR) to a longer wavelengths confirmed that the citrate coated AgNPs has been converted into PEG coated AgNPs and no other changes were observed (e.g., shape, core size, and aggregation state).

PVP-stabilized AgNPs were prepared by reducing 60 mL of 1 mM silver nitrate (1 mM) with 180 mL of 2 mM sodium borohydride (>99% purity, Sigma Aldrich) in the presence of PVP10 (M<sub>w</sub> 10000, Sigma Aldrich) in a modification of the method developed by Mulfinger et al. (2007). Stirring was stopped after the addition of AgNO<sub>3</sub> and the suspension was left overnight. All particle suspensions were cleaned at least three times using a 1 kDa regenerated cellulose membrane and a diafiltration method to remove the excess reactants but prevent drying and subsequent aggregation and oxidation. The original concentration and volume were maintained by replacing the removed filtrate with solutions of citrate or original concentration and volume were maintained by preventing drying and subsequent aggregation and oxidation. The three times using a 1 kDa regenerated cellulose membrane and UV−vis spectrophotometer, with a 1 cm path length quartz cell. The SPR of the AgNPs absorption spectra were collected over a wavelength range of 200−800 nm a day after the synthesis. Suitable blanks and controls were measured.

TEM samples were prepared by partially drying a drop of the cleaned particle solution on a copper grid (300 mesh) coated with a continuous carbon support film (Agar Scientific) at room temperature. The grid was washed thoroughly with high purity water before the original sample dried and was only then fully dried. The core size of AgNPs was quantified by analyzing images captured by JEOL 1200EX (accelerating voltage 80 kV) and Tecnai F20 complemented with energy dispersive X-ray spectrophotometer (EDX) (accelerating voltage 200 kV). Size distributions were collected by measuring >100 NPs using a Gatan Digital Micrograph software package and ImageJ software. Mean hydrodynamic size was analyzed by DLS using a Zetasizer Nano (Malvern Instruments). At least five consecutive measurements were recorded and averaged to calculate a z average size.

Hydrodynamic diameters of the AgNPs were measured in an asymmetrical Fl-FFF (AF2000 Mid Temperature, Postnova Analytic) fitted with a uv detector operating at ca. 400 nm. Three independent replicates of 500 μL were injected per sample, analyzed and the mean calculated. Two different sizes of polystyrene nanoparticle standard (20 ± 2 nm and 40 ± 2 nm from Duke Scientific Corporation) were employed to calibrate the effective channel thickness for particle size conversion. Sample recovery was quantified by injecting the samples into the channel with zero cross-flow.

The concentration of silver nanoparticles in the stock solution was measured with an Agilent 7500 inductively coupled plasma-mass spectrometer (ICP-MS). After washing, an aliquot of AgNPs suspension was acidified with analytical grade HNO<sub>3</sub> to have 20% v/v, acid concentration. The mixture was shaken for at least 2 h and then was diluted further 10 times before analysis.

**Media.** A standard OECD media for Daphnia sp. acute and chronic test was used. This is denoted as CM-1; a 10-fold dilution of this media was denoted CM-10. Due to the potential impacts on dissolved silver speciation and modification of NP dissolution and characteristics, the chemical composition and concentration of the CM-1; NM-1 and SM-1 and pH value of all media are presented in Supporting Information (SI) Tables S1 and S2 respectively.

**Temporal Stability.** The stability of AgNPs with three different capping agents in different media chemistry and media concentration was investigated. High density polyethylene (HDPE) plastic vials were used for incubation which had been acid washed with 10% nitric acid (HNO<sub>3</sub>) for at least 24 h and rinsed with excess high purity water. No stirring was applied during incubation. The AgNPs colloids were analyzed regularly by UV−vis spectrophotometer and DLS over 21 days, which is equivalent in length of the OECD chronic daphnia test. The pH of the NPs-media suspension was also analyzed during 21 days using Orion 3 star pH meter which was calibrated with standard pH 4, 7, and 10 each time before measurement. At the end of the 21 days, the TEM images of particles in media as well as their EDX spectrum were taken for confirmation of size distribution, shape, and composition. The NPs were not exposed to light which is acceptable for OECD protocols, and no organisms were present in order to understand ionic strength and specific ion effects alone.

**RESULTS AND DISCUSSION**

**Characterization.** AgNPs with core size approximately 10 nm and stabilized with three different capping agents (citrate, PVP, and PEG) were synthesized and characterized. A summary of their physicochemical properties is presented in Table 1. Further details of characterization results are presented in Supporting Information (SI) (Figures S1−S4).

<table>
<thead>
<tr>
<th>instrument</th>
<th>citrate-AgNPs</th>
<th>PEG-AgNPs</th>
<th>PVP-AgNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLS (z average)</td>
<td>22.0 ± 1.0</td>
<td>36.0 ± 2.0</td>
<td>28.3 ± 0.8</td>
</tr>
<tr>
<td>DLS (PDI)</td>
<td>0.32</td>
<td>0.15</td>
<td>0.4</td>
</tr>
<tr>
<td>Fl-FFF</td>
<td>15.3</td>
<td>35.2</td>
<td>25.6</td>
</tr>
<tr>
<td>TEM</td>
<td>11.5 ± 3.3</td>
<td>10.3 ± 3.2</td>
<td>10.8 ± 3.3</td>
</tr>
</tbody>
</table>

dx.doi.org/10.1021/es2038596 | Environ. Sci. Technol. XXX, XXX, XXX−XXX
The suspensions of synthesized particles were yellow in color with an absorption peak at $\lambda_{\text{max}}$ 390 nm for citrate coated particles, 393 nm for PEG coated particles, and 400 nm for PVP coated AgNPs. In the latter case, a broader SPR peak indicated a broader size distribution, and this is confirmed by the higher polydispersity index (PdI) from DLS (Table 1) although the TEM gave the same core size and size distribution. For all studies, the concentration was 4.8, 2.4, and 3.0 mg L$^{-1}$ for PVP, citrate, and PEG capped AgNPs respectively.

A multmethod approach to reduce bias and increase information content was used which proved to be valuable. TEM measures only the core size of particles, so for coated particles TEM will give a smaller result than the measure obtained by FL-FFF or DLS. Since the size of PEG and PVP polymer was larger than the citrate ion, both DLS and FL-FFF showed larger sizes for PEG and PVP coated AgNPs than citrate coated AgNPs. Thus, as expected, our results show that DLS > FL-FFF > TEM for citrate coated Ag NPs but that DLS and FLFFF were in agreement and much larger than TEM sizes for the polymer capped NPs.

**AgNP Stability Investigated by Visual Observation, UV–Vis Absorption, and pH Measurement.** Visual observations revealed that citrate coated AgNPs were very unstable in concentrated media of all compositions (CM-1, NM-1, and SM-1). The characteristic yellow color of the citrate capped AgNPs changed to gray (CM-1), red (NM-1), and purple (SM-1) within seconds (Figure S4.a). The changes were then confirmed with SPR analysis after 2 h of mixing (Figure 1). A large decrease in absorbance of these AgNPs at a $\lambda_{\text{max}}$ 390–393 nm was observed in all concentrated media, corresponding to the loss of the yellow color. Interestingly, a new (broader) absorbance peak appeared at longer wavelength which indicates a scattered light from large aggregates. Aggregation was followed by significant sedimentation as rapid absorbance loss at $\lambda_{\text{max}}$ was detected within the first hour incubation (Figure S5 in the SI).

**Figure 1.** The SPR of citrate coated AgNPs in different media, measured 2 h after mixing and compared with SPR of the stock solution.

**Figure 2.** SPR stability of citrate coated AgNPs over 21 days in diluted media and within 3 days in concentrated media (— stock solutions, − 0 day, ---- 3 days, —— 7 days, ———— 14 days, (gray line) 21 days).
In the dilute media, after 2 h of mixing, different SPR changes was observed (Figure 1). There was a slight absorbance loss at \( \lambda_{\text{max}} \) and minor peak broadening of citrated coated AgNPs in CM-10. The full width at half-maximum (fwhm) increased by approximately 14% within 2 h which indicated possible aggregation. However, both NM-10 and SM-10 showed a more significant absorbance reduction at \( \lambda_{\text{max}} \) and appearance of a shoulder at around 470 nm suggesting other mechanisms as well as aggregation. Other mechanisms such as dissolution, shape transformation (see TEM result section), may also have occurred in NM-10 and SM-10.

After 1 day, there was a complete loss of color of the citrate coated AgNPs in all concentrated media. Two of the diluted media (NM-10 and SM-10) gave a red suspension after 24 h (Figure S4.b). No visually observable changes occurred in CM-10 suspension, and it retained its yellow color. There was no SPR change for the PEG-and PVP-AgNPs in any media after one day.

The SPR of citrate capped AgNPs in all media was then monitored for 21 days (Figure 2), equivalent to the time period for the OECD chronic daphnia toxicity test. In all media, the SPR changed over this time period. The CM-10 suspension was the only media where minor qualitative change of the SPR occurred, but the absorbance (peak area) decreased by approximately 40% over 21 days (Table S3). Both in NM-10 and SM-10 the absorbance at \( \lambda_{\text{max}} \) 390–393 nm continuously decreased and broadened with a shift to longer wavelengths (from 470 to 550 nm). In all concentrated media, there was a total loss of this peak between 0 and 3 days, indicating losses through aggregation or other changes. Thus for exposure studies using charge stabilized NPs, there are likely to be changes in NP exposure concentration and change in the nature of the toxicant species (from dispersed to aggregated form, possible shape changes, dissolution of NPs, etc.) over the course of the exposure, making dose–response relationships difficult to interpret. These data indicate such changes are likely to be very rapid, in agreement with previous studies.

The role of biota and their exudates on NP behavior also needs systematic investigation but was not investigated here. Although only small qualitative changes occurred in peak shape for the PEG capped AgNPs, the absorbance peak decreased significantly in all media over 21 days. The smallest decrease was again found in the dilute media and in the chloride containing media (Figure S6 and Table S3) with a ca. 30% decrease in the CM-10 and ca. 40% in the CM-1 over 21 days. However, in some cases essentially the entire peak disappeared (e.g., NM-1) indicating a complete loss of material. The observed significant differences at lower ionic strengths suggest a specific ion effect through preferential absorption, followed by aggregation and/or alteration of surface chemistry. Unlike the citrate and PEG stabilized AgNPs, the distinctive yellow color of PVP-AgNPs was preserved in all media until the end of the study, even at the highest ionic strength. This was confirmed by UV–vis absorbance (Figure 3), where minor decrease peak area (8–13%) were observed in the concentrated and diluted forms of all chloride free media (Table S3). No loss in absorbance was observed in the chloride containing media. Taken together, the results suggest that PVP
in dilute OECD media (containing chloride) may be the NP and media of choice for chronic exposure studies in agreement with our previous work over more limited media conditions and with fewer different types of NPs. From the changes in UV−vis peak area, estimates of the remaining NP concentration after 21 days were made (Table S3). In all concentrated media, the citrate NPs were completely lost, while ca. 40% was lost in all 10-fold diluted media. The PEG coated NPs were slightly more stable in concentrated media, although the NM-1 showed greater than 90% losses. However, the PVP coated NPs were extremely stable with a maximum peak loss of ca. 12%, while in a number of cases no loss in the UV−vis peak (and therefore no reduction in NP concentration) was observed.

**Size Changes over 21 Days.** To semiquantitatively investigate aggregation of the AgNPs in the different media types, z average was measured by DLS. Figure 4 compares the size of particles in media with its corresponding stock solution (in high purity water).

Figure 4a illustrates the results for citrate capped AgNPs in diluted media only, because in the concentrated media the size increased enormously (≫ 500 nm) due to aggregation and subsequent sedimentation, as indicated previously by color and SPR disappearance. In all diluted media, the size of citrate capped AgNPs increased significantly by approximately 2-fold compared to the stock solution. The size increased continuously in NM-10 and SM-10, but the trend was less clear-cut with the CM-10, most likely due to lack of sensitivity and accuracy of DLS in aggregated suspensions. However, taken together, the data indicate somewhat different behavior in CM-10 media.

The size of PEG coated AgNPs (Figure 4b) was stable in CM-1 and CM-10, but some aggregation was observed in other media. There was significant increase of particle size in NM-1 and SM-1 in agreement with changes observed in SPR. PVP coated AgNPs in all dilute media was very stable as measured by DLS (Figure 4c). However, some initial aggregation was observed in the concentrated media which reduced over time. The data are consistent with a small fraction of the NPs being
susceptible to aggregation and loss or with the formation of silver chloride and precipitation in concentrated media.\textsuperscript{35} precipitation is less likely to be an issue at exposure concentrations approaching realistic environmental levels (tens to hundreds of ng L\textsuperscript{-1}).\textsuperscript{36}

\textbf{pH Measurement Data.} The pH value of the AgNPs-media suspension was measured over 21 days (Table S4). There was a slight pH variation of all suspensions between from 6.6–7.7 with an average value of 7.1 ± 0.2 within 21 days. Taken as a whole, no significant change in pH was observed. However, this masks a consistent trend in increasing pH, which fell at later time points under specific conditions. According to previous studies\textsuperscript{27} dissolution of Ag NPs results in loss of protons with a 1:1 stoichiometry, indicating that the loss of Ag NPs was of the order of 0.01 mg L\textsuperscript{-1} of Ag lost by dissolution (i.e., < 1%). However, this figure does not include pH reduction due to ingress of CO\textsubscript{2} or reprecipitation, so this represents a minimum amount of dissolution.

\textbf{Shape Stability and Changes.} TEM images were taken after 21 days to assess the aggregation state and the shape of the particles (Figure S and S8–S11). Unlike the PEG and PVP capped AgNPs, spherical and dispersed citrate coated AgNPs were only found in CM-10 in agreement with SPR data, with some minor aggregation. Dispersed AgNPs were observed in all media with PEG as the capping agent and more frequently in the case of PVP coated particles.

In addition, many particles with core size apparently less than 5 nm were observed for all NPs in certain media, which were not been found in their stock solution (Figures S and S10). However, qualitative observations only were made. These small particles may be fragmented from larger spherical particles due to a partial dissolution and recrystallization.\textsuperscript{38} Anionic ligands (such as chloride, nitrate and sulfate) in the presence of oxygen may cause etching and dissolution of the spherical NPs followed by recrystallization (Note: in environmental compartments, dissolution may also occur but recrystallization is less likely after increases in salt concentrations due to likely dilution of the Ag.)

The TEM images also showed substantial shape changes, with triangular and hexagonal shapes, observed in citrate capped NPs in both NM-10 and SM-10 media, along with aggregates and very small particles (Figures S7–S9 and S11). Unlike the citrate capped NPs, those coated in PEG or PVP underwent little observable change, but some platelike particles were also observed in the SM-10 and SM-1 (Figures S7 and S8). Such shape transformations have been found in other studies,\textsuperscript{30,32,38} although their importance for exposure studies has not previously been recognized, to our knowledge.

It is clear that changes in surface chemistry, shape, aggregation, and dissolution occur as a function of AgNP surface coating, media composition, and ionic strength, potentially resulting in substantial changes during NP (eco)-toxicological exposures. Such changes quantitatively impact on exposure concentration and hence dose, along with the nature of the toxicant, from dispersed spherical NPs to irregularly shaped NPs, aggregates, small spheres, and dissolved Ag. Under such conditions, understanding of the dose–response relationship is very complex. However, these data show that all NP coatings may be suitable in dilute OECD daphnia media for acute (48 h) studies i.e. CM-10. However, replacement of chloride with other ions and use of undiluted media is not recommended, despite potential effects on dissolved Ag speciation. For chronic exposures (21 days), PVP coated AgNPs in CM-10 is recommended. Clearly, when silver is used with chloride, issues with AgCl complex formation and with precipitation at high concentrations need to be quantified in order to fully interpret toxicity data. In addition, the biological impacts of reduced media concentrations must be investigated.

\section{ASSOCIATED CONTENT}
\section{Supporting Information}
Additional information on summary of citrate coated AgNPs synthesis; the chemical composition and pH of media used for this study; AgNPs characterization result; SPR, pH and shape stability of AgNPs in ecotoxicology media. This material is available free of charge via the Internet at http://pubs.acs.org.

\section{AUTHOR INFORMATION}

\textbf{Corresponding Author}
*Phone: +44 121 414 8147. E-mail: j.r.lead@bham.ac.uk.

\textbf{Notes}
The authors declare no competing financial interest.

\section{ACKNOWLEDGMENTS}
We would like to thank Directorate General of Higher Education of Republic of Indonesia (DIKTI), Centre for Environment, Fisheries and Aquaculture Science (CEFAS), The Natural Environment Research Council (NE/D004942/1; NE/H013148/1 and the Facility for Environmental Nanoscience Analysis and Characterisation) and the University of Birmingham for financial support.

\section{REFERENCES}
\(\textsuperscript{(3)}\) The Project on Emerging Nanotechnologies Website. http://www.nanotechproject.org/inventories/consumer/analysis_draft/.