Invited article

Water chemistry controlled aggregation and photo-transformation of silver nanoparticles in environmental waters

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The inevitable release of engineered silver nanoparticles (AgNPs) into aquatic environments has drawn great concerns about its environmental toxicity and safety. Although aggregation and transformation play crucial roles in the transport and toxicity of AgNPs, how the water chemistry of environmental waters influences the aggregation and transformation of engineered AgNPs is still not well understood. In this study, the aggregation of polyvinylpyrrolidone (PVP) coated AgNPs was investigated in eight typical environmental water samples (with different ionic strengths, hardness, and dissolved organic matter (DOM) concentrations) by using UV–visible spectroscopy and dynamic light scattering. Raman spectroscopy was applied to probe the interaction of DOM with the surface of AgNPs. Further, the photo-transformation and morphology changes of AgNPs in environmental waters were studied by UV–visible spectroscopy, inductively coupled plasma mass spectrometry, and transmission electron microscopy. The results suggested that both electrolytes (especially Ca²⁺ and Mg²⁺) and DOM in the surface waters are key parameters for AgNP aggregation, and sunlight could accelerate the morphology change, aggregation, and further sedimentation of AgNPs. This water chemistry controlled aggregation and photo-transformation should have significant environmental impacts on the transport and toxicity of AgNPs in the aquatic environments.

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Introduction

Silver nanoparticles (AgNPs) are one of the most commonly used engineered nanomaterials due to their high electrical conductivity and optical and anti-microbial properties (Jain et al., 2008; Kim et al., 2007). Because of the toxicity of AgNPs to microorganisms, algae, plants, and animals (Navarro et al., 2008; Marambio-Jones and Hoek, 2010; Asharani et al., 2008), the inevitable release of AgNPs into aquatic environments has drawn great concern about their environmental toxicity and safety (Benn and Westerhoff, 2008; Gottschalk and Nowack, 2011). Once released into aquatic environments, AgNPs transform physicochemically in terms of
aggregation and chemical speciation, which not only modify their properties but also have great impacts on their transport, fate, and toxicity (Levard et al., 2012). Aggregation of AgNPs could decrease their toxicity to bacteria (Kvitek et al., 2008) and rainbow trout (Kalbassi et al., 2013). The oxidative dissolution of AgNPs to silver ion (Ag⁺) will increase the toxicity of AgNPs to organisms (Xu et al., 2012; Pratsinis et al., 2013), while the reduction of Ag⁺ into AgNPs decreases the toxicity to protozoan tetrahymena thermophila (Juganson et al., 2013). Therefore, in recent years, study on the aggregation and chemical transformation of AgNPs is becoming a hotspot in the environmental fate of AgNPs.

Besides the characteristics of AgNPs such as size (Liu et al., 2010; Ma et al., 2012; Zhang et al., 2011a) and coating (Ma et al., 2012; El Badawy et al., 2010; Tejamaya et al., 2012), various environmental factors also play crucial roles in the aggregation and chemical transformation of AgNPs in aquatic environments. Generally, increasing ionic strength could destabilize AgNPs, and divalent electrolytes (Ca²⁺ and Mg²⁺) are more efficient in destabilizing AgNPs than monovalent electrolytes (i.e. Na⁺) (Huynh and Chen, 2011; Baalousha et al., 2013; El Badawy et al., 2012), while addition of dissolved organic matter (DOM) (i.e. humic or fulvic acids) could provide additional electrostatic repulsion and enhance the stability of AgNPs (Huynh and Chen, 2011; Baalousha et al., 2013). In natural waters, electrolyte type and concentration also play important roles in the aggregation of AgNPs (Picciapetra et al., 2012; Chinnapongse et al., 2011). However, the combined effects of electrolytes and DOM in natural waters on the stability of AgNPs are still not well understood (Gao et al., 2009). In addition to aggregation, AgNPs also encounter chemical and morphology changes in simulated or natural waters. Decreasing particle size (Ma et al., 2012; Zhang et al., 2011a) and solution pH (Li et al., 2010) could accelerate the oxidative dissolution of AgNPs to Ag⁺.

In ecotoxicology media (standard OECD media), morphology change, including formation of smaller or larger new AgNPs, was observed for engineered AgNPs with various coatings (Tejamaya et al., 2012). Importantly, it was found that light irradiation has great impact on the inter-species-transformation of Ag⁺ and AgNPs. Light exposure could accelerate the oxidative dissolution of AgNPs to Ag⁺ and dissolution of AgNPs to Ag⁺ (Grillet et al., 2013). On the other hand, Ag⁺ could also be reduced into zero-valent AgNPs by DOM under sunlight (Yin et al., 2012; Hou et al., 2013). Because of the redox instability of silver (φAg⁺/Ag⁻ = 0.8 V), in our previous study, the cycle of oxidation of AgNPs to Ag⁺ and reduction of Ag⁺ to form new AgNPs was observed for AgNPs in DOM-solution under simulated and natural sunlight, which could induce changes in the morphology of AgNPs in aquatic environments (Yu et al., 2014). A related study also discovered that under sunlight, AgNPs with different coatings irreversibly aggregated and formed much larger nanoparticles or “chain”-like nanostructures in de-ionized water (Cheng et al., 2011). However, since most of these previous studies were performed in de-ionized water or synthesized water (i.e. DOM solution), there is still a lack of knowledge on the aggregation and photo-transformation of AgNPs in natural waters (Li and Lenhart, 2012).

The major objectives of this study were to evaluate how the water chemistry (i.e. ion composition and concentration, DOM) of real environmental waters influences the aggregation and photo-transformation of engineered AgNPs. To this end, eight environmental water samples with different ion compositions and DOM concentrations were collected, and the aggregation of polyvinylpyrrolidone (PVP) coated AgNPs in these environmental waters was investigated by UV–visible spectroscopy and dynamic light scattering (DLS). The aggregation behavior of AgNPs was probed by Raman spectroscopy in simulated and natural waters. Further, the photo-transformation of AgNPs was studied in selected environmental waters by using UV–visible spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), and transmission electron microscope (TEM) characterization.

1. Materials and methods

1.1. Chemicals

Silver nitrate (AgNO₃), sodium hypophosphite (NaH₂PO₂·H₂O), and sodium hexametaphosphate ((NaPO₃)₆), of analytical grade, were purchased from Beijing Chemicals (Beijing, China). NaClO₄, CaCl₂·2H₂O, MgCl₂·6H₂O, PVP (molecular weight 3500), and benzotriazole were from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Suwannee River Natural Organic Matter (SRNOM), from an aquatic source, was purchased from the International Humic Substances Society (IHSS, St. Paul, MN, USA).

1.2. Synthesis and characterization of AgNPs

Considering PVP is widely used as a stabilizer and capping agent to control the shape of AgNPs and other nanoparticles (Moran et al., 2011), PVP-coated AgNPs were selected as a model to study the aggregation and photo-transformation of AgNPs in environmental waters. The AgNPs were synthesized as follows: AgNO₃ (3.40 g) was added into 20 mL de-ionized water and pre-heated at 50 °C in a water bath. The reductant was prepared by adding 1.325 g NaH₂PO₂·H₂O, 0.43 g sodium hexametaphosphate, 3.40 g PVP and 2.5 mL H₂SO₄ (1.0 mol/L) into 125 mL de-ionized water. Then, at 50 °C in the water bath, the reductant was stirred vigorously and the above prepared AgNO₃ solution was added at 20–30 drops per minute. Then, the mixture was stirred vigorously for another 30 min, and a reddish brown AgNP solution was formed. For purification of AgNPs, 150 mL of 2% (W/V) benzotriazole was added into the AgNP solution, and after 20 min incubation, the solution was centrifuged at 12,000 r/min (14,972 × g) for 30 min. The precipitate was washed by acetone and ethanol, and dried in vacuum at 35 °C. Before use, the AgNP powder was re-dissolved in de-ionized water by ultrasonic dispersion (600 W for 5 min, KQ-600DE, Kunshan Ultrasonic, Shanghai, China). The AgNPs were then characterized by UV–visible spectroscopy, zeta potential measurement, and TEM.

TEM images were obtained from a Hitachi H-7500 (Hitachi, Japan) or JEOL 2011 (Herts, England) transmission electron microscope. The specimen for TEM was prepared by slow evaporation of solution onto a carbon-coated copper grid (T11031, Xinxing Bairui, Beijing, China) in a vacuum desiccator at room temperature.

1.3. Sampling and characterization of environmental water samples

Environmental waters, including river water, lake water, and spring water samples, were collected from Beijing. All the
water samples were filtered through 0.2 μm glass fiber filters. The pH, conductivity, cations, and dissolved organic carbon (DOC) were determined before the experiment. DOC in environmental waters was determined with a Phoenix 8000 total organic carbon analyzer (Tekmar-Dohrmann, Cincinnati, OH, USA). The pH was determined by an Orion pH/ISEmeter, model 710A (Thermo Fisher Scientific, Waltham, MA, USA). The sampling and characterization information for the environmental waters is given in Table 1.

1.4. Characterization of the aggregation of AgNPs in environmental and simulated waters

UV–visible spectra were recorded on a Shimadzu UV–Vis-NIR Spectrophotometer (UV-3600, Kyoto, Japan). DLS measurements were performed on a Zetasizer Nano ZS (Malvern, Worcestershire, UK), in which aqueous solutions of AgNPs were analyzed using disposable plastic cuvettes.

Raman spectroscopy was used to characterize the aggregation and surface change of the AgNPs. For the environmental water, 100 mg/L AgNPs (as Ag) were spiked into river water. For the simulated water samples, the concentrations of AgNPs and SRNOM were 100 mg/L and 20 mg C/L, respectively. The electrolytes (NaClO4, Ca(ClO4)2, and Mg(ClO4)2) were spiked to give final concentrations of 5 or 10 mmol/L. The prepared water samples were then dropped on the silicon wafer and dried in a vacuum desiccator at room temperature. The Raman spectra of samples were obtained by using a Leica microscope in a confocal Raman spectroscopy system (Renishaw InVia Raman microscope, Britain). Spectra were measured using a 50× objective lens and sixty-second scan between 155.48 and 1132.18 cm⁻¹ with a 785 nm laser (5 mW) and 2400 lines/mm grating.

1.5. Photo-transformation of AgNPs in environmental waters

The experiments were performed in 100 mL quartz glass bottles with quartz caps under natural sunlight. The intensity of ambient photo-synthetically active radiation (PAR) was measured at 15 min intervals by an LI-192 Quantum Sensor (LICON Biosciences, Lincoln, NE, USA). Three mL of solution was sampled at specific time intervals for UV–visible spectrometry characterization. After 3 hr solar irradiation, the resulting precipitate were also collected for further TEM characterization.

2. Results and discussion

2.1. Characterization of AgNPs and environmental water samples

The synthesized AgNPs were characterized first by TEM and UV–visible spectroscopy (Fig. 1). The TEM image (Fig. 1a) showed that the AgNPs are spherical with an average diameter of 27.15 nm. The size distribution of AgNPs (Fig. 1b) showed that the AgNPs are polydispersed with a wide distribution of particle size. The analysis of UV–visible spectroscopy (Fig. 1c) showed the characteristic surface plasmon resonance (SPR) of AgNPs at 402 nm for the PVP coated AgNPs. The Z-average diameter and zeta potential of the AgNPs were measured by DLS as 33.35 nm and –23.1 mV, respectively.

Eight typical fresh surface water samples were collected in Beijing, including spring water, lake water and river water. After filtration, the characteristics and chemical composition of water samples were measured (Table 1). All the waters were alkaliescent, with pH from 7.37 to 8.33. The conductivities ranged from 0.212 to 1.239 mS/cm, with Ca²⁺ from 29.3 to 87.0 mg/L and Mg²⁺ from 2.9 to 60.9 mg/L. The DOM concentrations varied from 2.22 to 77.38 mg C/L. By comparison, FHL-S had low conductivity, hardness, and DOM concentration, whereas WYH-R, YDH-R, and GBD-L, had high conductivity, hardness and medium DOM concentration. FH-L and JM-R had medium conductivity, hardness, and DOM concentration. CBH1-R and CBH2-R had medium conductivity, hardness, and high DOM. Generally, a wide range of ionic strength, hardness and DOM concentrations were represented in the sampled environmental waters.

2.2. Aggregation of AgNPs in environmental waters

As the synthesized AgNPs have a characteristic SPR absorbance at 402 nm, the aggregation of AgNPs in environmental waters could decrease the SPR absorbance at λmax of single AgNPs (Chinnapongse et al., 2011). Therefore, the aggregation of AgNPs in surface waters was first investigated by using UV–visible spectroscopy. The evolution of SPR absorbance of AgNPs at λmax in de-ionized water and environmental waters is given in Fig. 2. In de-ionized water, the absorbance of at λmax remained essentially the same in the investigated time (23 hr), indicating the AgNPs are stable in de-ionized water without electrolyte. However, in FHL-S, the absorbance of at λmax decreased significantly, suggesting a decrease in the concentration of single AgNPs with time. Inorganic ions, especially divalent Ca²⁺ and Mg²⁺, could possibly account for the aggregation of AgNPs (Huynh and Chen, 2011). For CBH1-R and CBH2-R, containing high concentrations of DOM, the absorbance at λmax only slightly decreased. As the ionic strength and hardness of CBH1-R and CBH2-R were higher than those of FHL-S, the slight variation of absorbance at λmax in CBH1-R and CBH2-R was ascribed to the stabilization effect from NOM (Huynh and Chen, 2011). The electrosteric interaction provided by the DOM adsorbed on AgNPs could inhibit the aggregation of AgNPs induced by ionic strength and Ca²⁺/Mg²⁺ (Huynh and Chen, 2011; El Badawy et al., 2012). For water with high ionic strength and hardness (WYH-R, YDH-R and GBD-L), the decrease of absorbance at λmax was much faster than for the others, suggesting a fast depletion of single AgNPs from the solution. For FH-L and JM-R, although the ionic strength and hardness were a little higher than those of FHL-S, the occurrence of DOM could partially inhibit the aggregation of AgNPs. Thus, the decrease of absorbance at λmax was similar for FH-L and JM-R to that for FHL-S.

DLS was further used to probe the evolution of the hydrodynamic radius of AgNPs in these surface waters (Fig. 3). The hydrodynamic radius of AgNP in de-ionized water was
stable over 11 hr, which accords well with the UV–visible results. For FHL-S, JM-R, and FH-L, the hydrodynamic radii of AgNPs only slightly increased. Similarly, the hydrodynamic radii of AgNPs in CBH1-R and CBH2-R were very stable and no significant change was observed. However, for water with high ionic strength and hardness, including WYH-R, GBD-L, and YDH-R, the aggregation of AgNPs was much faster than that in other surface waters. In the first 100 min, the hydrodynamic radii of AgNPs increased almost linearly, while periodic fluctuations in hydrodynamic radii were observed after 100 min incubation of AgNPs with natural waters. This periodic fluctuation was also observed by Zhang et al. (2011b) for citrate-coated AgNPs in electrolyte solution with 7.8 mg L\(^{-1}\) of dissolved oxygen. As this periodic fluctuation was inhibited by deoxygenation, this phenomenon was ascribed to dissolved oxygen-induced oxidation and dissolution of AgNPs, which could further change the surface energy of AgNPs (Zhang et al., 2011b). However, there is still a lack of direct evidence of the role of dissolved oxygen, and further study is needed to elucidate this phenomenon. Nevertheless, the periodic fluctuation of the hydrodynamic radius of AgNPs possibly has great influence on the transport and subsequent transformation of AgNPs in surface waters.

The combined results of UV–visible spectra and DLS suggested that concentrations of both electrolytes (especially Ca\(^{2+}\) and Mg\(^{2+}\)) and DOM in the surface waters are key parameters for AgNP aggregation, and the combined effect of electrolyte and DOM should be considered for nanoparticle aggregation in complex natural waters (Gao et al., 2009). In addition, compared with DLS, the UV–visible spectrum is more sensitive to the aggregation of single AgNPs, while DLS could provide additional information (i.e. the fluctuation of hydrodynamic radii). Therefore, the use of multiple techniques is proposed for characterization and monitoring the aggregation behavior of AgNPs.

Further, Raman spectroscopy was used to probe the variation of the surface of AgNPs in contact with the constituents of environmental and simulated waters (Fig. 4). Fig. 4a shows that after mixing with CBH2-R, the intensities of characteristic Raman frequencies arising from the PVP coating (shear vibration of CH\(_3\) group in polyethylene chain at 1399 cm\(^{-1}\)) and breathing vibration of pyrrole rings at 1007 cm\(^{-1}\)) (Zhu et al., 2004) decreased, indicating that the DOM in the environmental water could displace PVP from the surface of AgNPs (Lau et al., 2013). Accordingly, two new Raman frequencies at 994 cm\(^{-1}\) (ring breathing vibration of the pyridine-like rings) (Yang and Chase, 1998; Wang et al., 1999) and 855 cm\(^{-1}\) (out-of-plane deformations of aromatic C-H) (Alvarez-Puebla et al., 2004) from DOM demonstrated the adsorption of DOM on the surface of AgNPs. Further, the aggregation behavior of AgNPs in the

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**Fig. 1** – Characterization of the synthesized silver nanoparticles (AgNPs). (a) Transmission electron microscope (TEM) image, (b) particle size distribution, and (c) UV–visible spectrum. Concentration of AgNPs was 6 mg/L for the UV–visible spectroscopy characterization.

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### Table 1 – Characteristics of sampled environmental waters.

<table>
<thead>
<tr>
<th>Water sample</th>
<th>Latitude and longitude</th>
<th>pH</th>
<th>K(^{+}) (mg/L)</th>
<th>Na(^{+}) (mg/L)</th>
<th>Ca(^{2+}) (mg/L)</th>
<th>Mg(^{2+}) (mg/L)</th>
<th>Conductivity (mS/cm)</th>
<th>DOC (mg C/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water 1 from Chaobai river (CBH1-R)</td>
<td>N40°10’13.66&quot; E116°40’39.79&quot;</td>
<td>7.53</td>
<td>14.8</td>
<td>111.2</td>
<td>34.4</td>
<td>22.7</td>
<td>0.835</td>
<td>77.38</td>
</tr>
<tr>
<td>River water 2 from Chaobai river (CBH2-R)</td>
<td>N40°10’20.56” E116°40’39.24&quot;</td>
<td>7.79</td>
<td>14.5</td>
<td>95.8</td>
<td>31.0</td>
<td>24.7</td>
<td>0.805</td>
<td>67.52</td>
</tr>
<tr>
<td>River water from Wenyu river (WYH-R)</td>
<td>N40°03’49.33” E116°31’52.39&quot;</td>
<td>7.37</td>
<td>17.8</td>
<td>96.9</td>
<td>77.2</td>
<td>30.1</td>
<td>1.108</td>
<td>13.40</td>
</tr>
<tr>
<td>River water from Yongding river (YDH-R)</td>
<td>N39°56’56.04” E116°05’59.67&quot;</td>
<td>8.32</td>
<td>9.4</td>
<td>99.2</td>
<td>85.0</td>
<td>60.9</td>
<td>1.239</td>
<td>37.83</td>
</tr>
<tr>
<td>Lake water from Gaobeidian (GBD-L)</td>
<td>N40°00’28.39” E116°20’14.37&quot;</td>
<td>7.78</td>
<td>17.1</td>
<td>95.4</td>
<td>87.0</td>
<td>35.4</td>
<td>1.149</td>
<td>18.65</td>
</tr>
<tr>
<td>Lake water from Fuhai (FH-L)</td>
<td>N40°10’13.14” E116°18’16.19”</td>
<td>7.60</td>
<td>19.9</td>
<td>108.3</td>
<td>36.9</td>
<td>29.6</td>
<td>0.948</td>
<td>37.75</td>
</tr>
<tr>
<td>River water from Jingmi canal (JM-R)</td>
<td>N40°00’13.66” E116°15’52.60&quot;</td>
<td>8.33</td>
<td>4.5</td>
<td>24.0</td>
<td>57.6</td>
<td>23.6</td>
<td>0.575</td>
<td>26.70</td>
</tr>
<tr>
<td>Spring Water from Fenghuangling (FHL-S)</td>
<td>N40°00’00.49” E116°23’21.64&quot;</td>
<td>8.25</td>
<td>0.7</td>
<td>11.3</td>
<td>29.3</td>
<td>2.9</td>
<td>0.212</td>
<td>2.22</td>
</tr>
</tbody>
</table>
presence of SRNOM and mono- and divalent electrolytes was studied in simulated waters. Fig. 4b shows that with the addition of 5 mmol/L NaClO₄, the intensities of characteristic Raman frequencies from PVP increased significantly (Zhu et al., 2004), revealing that 5 mmol/L NaClO₄ could induce the aggregation of AgNPs. However, with the addition of 20 mg C/L SRNOM, the Raman signal of PVP decreased, even in the presence of 50 mmol/L NaClO₄, which suggested that DOM could inhibit the aggregation of AgNPs. Similarly, in the presence of Ca(ClO₄)₂ and Mg(ClO₄)₂, enhanced aggregation of AgNPs was observed, while DOM could inhibit the Ca²⁺ and Mg²⁺-induced aggregation (Fig. 4c and d). By comparison, from the intensity of Raman signal of PVP, we can also see that divalent Ca²⁺ and Mg²⁺ could induce the aggregation of AgNPs more effectively than monovalent Na⁺, which is consist with a previous study (Huynh and Chen, 2011).

2.3. Photo-transformation of AgNPs in environmental waters

Recently, solar irradiation was found to play important roles in the transformation and toxicity of AgNPs (Cheng et al., 2011; Shi et al., 2013). Our previous study suggested that oxidation of Ag⁺ and reduction of Ag⁺ in DOM-containing simulated water could induce chemical and morphological change in AgNPs (Yu et al., 2014). However, the photo-transformation of AgNPs in real environmental waters and the possible impacts of water chemical factors are still not well understood. In this study, the photo-transformation of PVP coated AgNPs in four selected environmental waters was further investigated by using UV-visible spectroscopy, ICP-MS and TEM. After 3 hr sunlight irradiation, the AgNP solutions of FHL-S, JM-R, and YDH-R turned from yellow to gray, and after further irradiation, a black precipitate was observed. However, the tint of AgNPs in CBH1-R remained yellow in color. A similar precipitation phenomenon was also observed in the photo-reduction of Ag⁺ to AgNPs in environmental waters in our previous study (Yin et al., 2012). Accordingly, UV-visible spectroscopy (Fig. 5) showed that when compared with results in dark conditions, the SPR peak of AgNPs decreased more significantly under 1–3 hr sunlight irradiation for all the studied environmental waters. These results clearly demonstrated that sunlight irradiation could accelerate the photo-transformation of AgNPs in environmental waters. In addition, generally the SPR peak slightly shifted to shorter wavelength (i.e. from 422 to 407 nm for JM-R) under sunlight, indicating larger sized AgNPs could be removed more effectively than smaller ones (Willets and Van Duyne, 2007). Moreover, for FHL-S, JM-R, and CBH2-R, the enhanced absorbance at longer wavelength (>600 nm) suggested the formation of AgNP aggregates or larger Ag nanostructures (Chinnapongse et al., 2011). Comparably, the decrease in the SPR of singly dispersed silver is not significant for CBH2-R, which revealed that DOM could inhibit both the aggregation and photo-transformation of AgNPs in the environmental waters. One the other hand, the evolution of UV–visible spectra for JM-R and YDH-R revealed that ionic
strength or hardness could accelerate the aggregation and photo-transformation of AgNPs in the environmental waters.

After sunlight irradiation for 3 hr, the solution was centrifuged at 1000 r/min for 10 min, and then the upper solution was determined for total silver by ICP-MS. The result (Fig. 6) showed that under dark conditions, most of the AgNPs (>61%) could suspend in environmental waters, while the suspended AgNPs were decreased significantly under sunlight for FHL-S, YDH-R, and JM-R. Especially, the total silver in CBH2-R was only slightly decreased under sunlight, which is consistent with our results from UV-visible spectroscopy.

The morphology changes of AgNPs in environmental waters under sunlight were further characterized by TEM (Fig. 7). As shown in Fig. 7a, d, and g, in the upper solution, although aggregation of AgNPs was observed, the AgNPs still kept their initial morphologies. However, for the precipitates, significant morphology changes of AgNPs, including formation of larger and smaller “new” AgNPs (Fig. 7b, e, and h) and the cross-linking and fusion of AgNPs (Fig. 7c, f, and i), were evident. These results, combined with our previous study in simulated water (Yu et al., 2014), suggested the morphology change mechanism of dissolution–reduction–fusion–growth. Dissolved oxygen (Zhang et al., 2011b) and sunlight (Grillet et al., 2013) could enhance the oxidation of AgNPs to Ag⁺, and subsequently these free or adsorbed Ag⁺ could be re-reduced back to Ag⁺ by DOM under sunlight, which resulted in larger and smaller “new” AgNPs. Although the ubiquitous Cl⁻ could form AgCl with the dissolved Ag⁺, these AgCl particles could also transform into AgNPs under irradiation (Badireddy et al., 2014). Ca²⁺ and Mg²⁺ in environmental waters could induce AgNPs in close proximity, and the reduction of Ag⁺ on the surface or nearby of AgNPs formed nanobridges between AgNPs (as shown in Fig. 7c and i) (Yu et al., 2014). The cross-linking of AgNPs could further accelerate the aggregation and precipitation of AgNPs. However, as NOM could prevent AgNPs from coalescing, the re-reduction of Ag⁺ on AgNP surfaces to form nanobridged and cross-linked nanostructures was relatively sparse in the presence of NOM at high concentration, compared with that in the presence of Ca²⁺ and Mg²⁺. Therefore, AgNPs in the presence of higher concentrations of NOM (i.e., in CBH2-R) are more stable under both light and dark conditions.

As discussed above, morphology change and precipitation of AgNPs in environmental waters under sunlight were induced not only by physical aggregation, but also by chemical transformation, i.e. the oxidation of AgNPs and reduction of Ag⁺.

2.4. Environmental implications

Our results suggest that the water chemistry of environmental waters has great impact on the aggregation and photo-
transformation of AgNPs, which should have significant environmental implications for the transport and toxicity of AgNPs in aquatic environments. Simulated experiments have demonstrated that aggregation of AgNPs could enhance the retention of AgNPs in porous media (Liang et al., 2013; Mitzel and Tufenkji, 2014). Therefore, it is reasonable that the aggregation of AgNPs could also decrease the mobility of AgNPs in real environments, such as sediment and sandy soil. Aggregation of AgNPs could also possibly reduce environmental transformation of AgNPs (i.e. sulfidation and Ag⁺ dissolution) and decrease the uptake of AgNPs by organisms (Levard et al., 2012). For example, large agglomerates of AgNPs cause significantly less hemolytic toxicity than small agglomerates (Zook et al., 2011). Similarly, agglomeration of AgNPs reduces the acute toxicity of AgNPs to juvenile rainbow trout (Kalbassi et al., 2013). Another report revealed that aggregation of AgNPs could result in less sulfidation of AgNPs and thus enhance the toxicity on the growth of E. coli (Reinsch et al., 2012). Solar irradiation could further accelerate the aggregation and morphology transformation of AgNPs, and also has similar impacts on the transport and toxicity of AgNPs. The sunlight-induced sedimentation

![Graphs showing UV-visible spectra of AgNPs in environmental waters under darkness and sunlight.](image)

**Fig. 5** – The evolution of UV-visible spectra of AgNPs in environmental waters under darkness and sunlight. Concentration of spiked AgNPs was 6 mg/L. The accumulative PARs were 4.9, 11.0, and 17.7 E/m² for 1, 2, and 3 hr sunlight irradiation, respectively.

![Bar graph showing the concentration of total silver in upper solution after 3 hr incubation with environmental waters.](image)

**Fig. 6** – The concentration of total silver in upper solution (centrifuged at 1000 r/min for 10 min) after 3 hr incubation (accumulative PARs 17.7 E/m²) of AgNPs with environmental waters. Concentration of spiked AgNPs was 6 mg/L.
could transfer AgNPs from surface water to sediment, which could potentially influence the toxicity of AgNPs to benthic organisms. Considering the water chemistry (ionic composition and strength, DOM) differs greatly for different sites and temporal trends, the aggregation and photo-transformation of AgNPs in environmental waters should highly depend on the sites studied.

3. Conclusions

In conclusion, the water chemistry has great impact on the aggregation and photo-transformation of AgNPs in environmental waters. Electrolytes and hardness could enhance the aggregation of AgNPs, while DOM could inhibit aggregation. In real environmental waters, the aggregation of AgNPs was controlled by the combined effects of electrolytes and DOM. We also have observed that sunlight could induce the fusion, morphology change, and sedimentation of AgNPs in environmental waters, and electrolyte-induced aggregation could further accelerate this transformation process. This study could shed light on the transport, transformation, and toxicological effects of AgNPs in aquatic environments.

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