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# Dissolution and fate of silver nanoparticles in the presence of natural aquatic organic matter

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## Abstract

Despite increasing interest in and use of nanoparticles (NP), the environmental consequences of using NP are poorly understood because most relevant studies have not taken the effects of natural coatings on NP into consideration. The aim of this study was to improve our understanding of the fates of NP in aquatic systems. The fates of silver NP (AgNP) capped with citrate and polyethylene glycol dispersed in ecotoxicological matrices in the presence of environmentally relevant components of natural water (humic substances and extracellular polymeric substances) were investigated. Interactions between AgNP and natural organic matter were evaluated by ultracentrifugation and electrophoretic mobility measurements to assess AgNP dissolution. Humic substances and extracellular polymeric substances both decreased the dissolution rate. The natural organic matter (humic substances and extracellular polymeric substances) provided conditions in which the medium stabilized the NP. The dissolution rate depended on the coating type (citrate or polyethylene glycol), dissolved organic carbon concentration, and particle concentration. The presence of algae and *Daphnia* affected AgNP conversion, demonstrating the value of research that takes environmentally relevant matrices into consideration. The results improve our understanding of the factors that affect the bioavailabilities of AgNP and therefore improve our ability to evaluate AgNP toxicity. Studies of other NP using the same strategy will improve our understanding of the fates of nanomaterials in the environment and biota.



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## Highlights

- Natural organic matter controls silver nanoparticle environmental dissolution/fate;
- Exopolysaccharides and aquatic humic substances both promote AgNP stabilization;
- Capping agents (citrate or polyethylene glycol) change the AgNP dissolution rate;
- AgNP behave differently when considering media for algae and *Daphnia* bioassays.

**Keywords:** Water, environmental chemistry, silver nanoparticles, exopolysaccharides, humic substances

## INTRODUCTION

The current expansion of nanoscience and nanotechnology and the use of large amounts of nanoscale manufactured particles (particles with at least one dimension measuring 1-100 nm) in commercial products have increased the concentrations of nanoscale materials in the environment. Nanoparticles (NP) are in a state between being molecular and being bulk material, so they have distinct magnetic, electrical, optical, mechanical, chemical, and structural properties caused by their large surface area to volume ratios (i.e., NP have high proportions of surface atoms). Because of their unique characteristics, NP are used in a wide range of industries, including the medical, environmental, computer, textile, and energy industries<sup>[1]</sup>.

Coatings of NP, whether man-made or natural, strongly affect how the NP behave in the environment. Man-made coatings include stabilizers added by manufacturers to increase NP mobility and stabilize the NP size. Natural coatings form through interactions between NP and materials present in the environment and biota. NP behaviors will be different in environmental systems with different physical and chemical properties. Various NP may contain components that can undergo (i) reduction and/or oxidation<sup>[2-4]</sup>; (ii) photoreactions<sup>[5]</sup>; (iii) dissolution and sulfidation<sup>[6-8]</sup>; and (iv) homoaggregation (i.e., aggregation of the same NP) and/or heteroaggregation (i.e., aggregation of NP with other colloids)<sup>[7,9,10]</sup>.

The fates, behaviors, and toxicities of NP are poorly understood, so attention is currently being paid to developing materials called capped NP<sup>[11]</sup>. In fact, much effort has been made by the academic and industrial communities worldwide to combine studies into a reliable framework for assessing NP safety and decreasing the dangers associated with NP disposed of in the environment<sup>[12]</sup>. Nanomaterial use by various industries is increasing, and NP may be emitted into the environment during production, use, and disposal. The health of humans could be harmed through unintentional exposure to NP in the environment, such as after engineered nanomaterials (ENMs) have been dumped in freshwater systems or wastewater treatment facilities<sup>[13,14]</sup>. In this situation, the environmental risks posed by NP should be assessed, taking into account (i) the shapes of the NP in commercial products, including the presence of manufactured coatings; (ii) the likelihood that the material is released into the environment; and (iii) transformations of the material that may affect the exposure of biota to the material and human health.

Interactions between NP and natural colloids, including natural organic matter (NOM) as extracellular polymeric substances (EPS) and humic substances (HS), in water are some of the most important NP modifications that can occur, but such interactions have previously been given little attention. Natural colloids can bind to the surface of a NP and form a coating around the NP. This will affect the physical-chemical properties and biocompatibility of the NP and therefore affect the reactivity of the NP and the ways the NP can interact with cells and whole organisms. The effects of NOM are complex and difficult to

predict, but it is extremely important to assess these interactions because NOM is present at concentrations orders of magnitude higher than NP concentrations and can therefore markedly affect the properties and behaviors of NP<sup>[15]</sup>. In the study presented here, electrophoretic mobility (EPM), dissolution, and speciation experiments were performed to evaluate the fates of silver NP (AgNP) coated with citrate (Cit) and polyethylene glycol (PEG) dispersed in ecotoxicological matrices containing environmentally relevant natural components (aquatic HS and EPS).

## EXPERIMENTAL

Commercially available chemicals and solvents (analytical grade) were used in the experiments. Ultra-high purity water ( $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ) was used throughout the experiments. Test media typically used in ecotoxicology studies, L.C. Oligo Media and soft reconstituted water, were prepared following guidelines described in protocols for studies using the microalga *Raphidocelis subcapitata*<sup>[16,17]</sup> and the microcrustacean *Daphnia* sp.<sup>[18,19]</sup>. The characteristics of the algae medium and *Daphnia* medium are shown in [Supplementary Table 1](#).

### Producing NOM

#### *EPS extracted algae*

An axenic culture of a *Raphidocelis subcapitata* strain was placed in a chamber that was kept at 25 °C and exposed to light at 4000 lux. The algae were cultivated in algae media following the ABNT standard<sup>[17]</sup>. The flasks, tools, and media solution used were sterilized in a Q190M autoclave (Quimis, Diadema, Brazil) at 121 °C for 15 min before use.

A 2 L aliquot of L.C. Oligo Media (pH 7.0-7.2) was added to each of a series of Erlenmeyer flasks and then *R. subcapitata* was added to give an initial algal cell density of  $\sim 10^5$  cells  $\text{mL}^{-1}$  to produce EPS. The microalgae were cultured for 15 d with pumped air aeration and continual illumination at  $\sim 4000$  lux at 25 °C until the organisms entered the mature phase. The algae mixture was then centrifuged at  $2500 \times g$  for 15 min, then the supernatant was carefully removed and the cells discarded (with sodium hypochlorite added to act as an algicide). The EPS were collected by passing the solution through a cellulose nitrate filter ( $0.45 \mu\text{m}$  pores; Sartorius, Goettingen, Germany) in a vacuum filtration system to remove any remaining cells with diameters  $> 0.45 \mu\text{m}$ . The EPS solution was freeze-dried when necessary (e.g., for characterization) or kept as a solution (e.g., for EPS-NP interaction tests). The dissolved organic carbon concentration was determined using a Multi N/C 3100 carbon analyzer (Analytik Jena, Jena, Germany), in which a sample was oxidized at 850 °C. A calibration curve was constructed by analyzing potassium hydrogen phthalate (Synth P.A., Diadema, Brazil), sodium hydrogen carbonate (Qhemis P.A., Jundiai, Brazil), and sodium carbonate (Synth P.A., Diadema, Brazil) as standards. Most test media defined in relevant guidelines contain ethylenediaminetetraacetic acid (EDTA), which is a chelating agent that may affect the behaviors of nanomaterials, so an Oligo media was used as culture medium for the algae<sup>[19]</sup>.

#### *Extracting HS from freshwater*

Freshwater samples from the Sorocabinha River in the city of Iguape, Brazil ( $24^{\circ}41'59''\text{S}$ ,  $47^{\circ}33'05''\text{W}$ ) were collected and HS in the samples were extracted. The HS was proceeded by chromatographic separation using Supelite DAX-8 resin (Sigma Aldrich, Darmstadt, Alemanha) following a method published by the International Humic Substances Society<sup>[20]</sup>. Briefly, river water was adjusted to pH 2 and then passed slowly through the DAX-8 resin column. Humic material was retained by the resin. The HS adsorbed to the resin were eluted with  $0.1 \text{ mol L}^{-1}$  sodium hydroxide (Dinamica P.A., Indaiatuba, Brazil). The results of *in situ* physicochemical measurements are shown in [Supplementary Table 2](#). The HS solution was adjusted to pH 5.8. The metal concentrations in the water samples were determined and the total, dissolved, and free

concentrations are shown in [Supplementary Figure 1](#).

### NOM characterization

The concentrations of various metals (aluminum, cadmium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, silver, strontium, and zinc) in both types of isolated NOM (HS and EPS) were determined by digesting each sample using a method published by the US Environmental Protection Agency<sup>[21]</sup> and analyzing the digested samples using an Agilent 720 inductively coupled plasma (ICP) optical emission spectrometer (Agilent Technologies, Santa Clara, CA, USA). The ICP optical emission spectrometer parameters were: axial torch; concentric nebulizer; RF generator frequency 40 MHz; RF power 1.1 kW; argon coolant gas flow rate 15.0 L min<sup>-1</sup>; auxiliary gas flow rate 1.5 L min<sup>-1</sup>. The results of the digestion and analyses of the HS and EPS are shown in [Supplementary Table 3](#). The carbon and nitrogen contents of the HS and EPS were determined using a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The freeze-dried HS and EPS samples were weighed using an MX5 microbalance (Mettler-Toledo, Greifensee, Switzerland), and the masses were 3-10 mg. A calibration curve was established by analyzing standards containing 2,5-bis(5-*tert*-butyl-benzoxazol-2-yl)thiophene (C<sub>26</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>S) (Thermo Fisher Scientific). The types and concentrations of functional groups in the HS and EPS were determined using an acid-base titration method [[Supplementary Figure 2](#)]. Solutions containing HS or EPS at a concentration of 1.0 g L<sup>-1</sup> were titrated with NaClO<sub>4</sub> at concentrations of 0.005, 0.01, 0.1, 0.3, and 0.5 mol L<sup>-1</sup>. An electrostatic parameter model was established using the acid-base potentiometric titration data for at least three ionic strengths. The FIT package<sup>[22]</sup> was used to determine the NICA-Donnan parameters for the model. Fourier-transform infrared (FT-IR) spectroscopy was used to identify the organic materials from the functional groups present<sup>[23]</sup>. The powdered freeze-dried materials were analyzed using a Spectrum 65FT-IR spectrometer (PerkinElmer, Waltham, MA, USA) using a resolution of 4 cm<sup>-1</sup> and a wavenumber range of 4000-650 cm<sup>-1</sup>. The NOM characterization results are shown in [Supplementary Material](#).

### Interactions between NOM and AgNP

Transformations of NP (at concentrations between 1 and 100 µg L<sup>-1</sup>) in the algae and *Daphnia* media in the absence and presence of HS and EPS were investigated. Two commercially available AgNP with manufactured coatings of the neutral polymer PEG (AgPEG) and of single bonded Cit (AgCit) were used in the experiments. The AgPEG and AgCit were purchased from PlasmaChem (Berlin, Germany). The conditions used in the study were (i) pH 6.8-8.0; (ii) sodium and/or potassium concentration 2 × 10<sup>-4</sup>-2 × 10<sup>-3</sup> mol L<sup>-1</sup>; (iii) calcium concentration 1 × 10<sup>-4</sup>-4 × 10<sup>-4</sup> mol L<sup>-1</sup>; (iv) magnesium concentration 1 × 10<sup>-4</sup>-6 × 10<sup>-4</sup> mol L<sup>-1</sup>; (v) AgNP concentration 1-100 µg L<sup>-1</sup> total Ag; (vi) HS concentration 1-10 mg L<sup>-1</sup> freshwater HS<sup>[23]</sup>; and (vii) EPS concentration 1-5 mg L<sup>-1</sup> (the concentration produced by algae in 1-4 d, the exposure experiment durations).

As defined in the relevant alga bioassay protocol, the NP behavior was assessed at a specified time after exposure  $t_{\text{exp}}$ . The NP were exposed to the physicochemical conditions described above for between 4 and 7 or 8 d (to match the exposure study durations) because time was expected to strongly affect changes in the NP and needed to be carefully regulated.

### Charge density determined by EPM

Measurements of EPM were used to determine the NP charge density. The EPMs of 5 mL of the algae medium or *Daphnia* medium alone or containing 1 or 10 mg L<sup>-1</sup> of HS or 1 or 5 mg L<sup>-1</sup> of EPS and 2 mg L<sup>-1</sup> of AgCit or AgPEG were determined. The solutions were in the pH range of 3-8. The pH was adjusted by adding 0.1 mol L<sup>-1</sup> HNO<sub>3</sub> or 0.01 mol L<sup>-1</sup> NaOH. NP were added after the pH had been adjusted, and the samples were left overnight before EPM measurements were performed.

### *Calculations for the models*

MinteQ modeling was used to determine the species distributions of  $\text{AgNO}_3$  in the medias studied. MinteQ can be used to determine the concentrations of various chemical species at equilibrium from the equilibrium production constants for most inorganic species found in natural water under typical conditions. Estimates of  $\text{Ag}^+$  chemical speciation and NICA-Donnan organic matter model parameters were made and the results are shown in Table 1. The chemical compositions of both freshwater solutions were also included in the modeling. An algae medium exposure solution procedure<sup>[17]</sup> was used. The *Daphnia* medium was treated with soft reconstituted water<sup>[18]</sup>. Differences in the species present caused by the different media compared with the speciation determined by MinteQ were assessed using a solution of  $\text{AgNO}_3$  at a concentration of  $100 \mu\text{g L}^{-1}$  in ultrapure water and *Daphnia* and algae media in the absence and presence of HS at concentrations of  $1.0$  and  $10.0 \text{ mg L}^{-1}$  (called HS1 and HS2, respectively).

### *Centrifugation-based dissolution by ultrafiltration*

Dissolution of Ag NP was evaluated using a centrifugation-based ultrafiltration (CU) technique using an Amicon filter with 3 kDa pores (Millipore, Burlington, MA, EUA). A 20 mL aliquot of the algae or *Daphnia* medium was added to each of a series of 50 mL polypropylene falcon tubes and the tubes were allowed to stand for 0, 72, 96, 192, 360, and 720 h. Tests were performed using HS at concentrations of 1 and  $5 \text{ mg L}^{-1}$  and using dissolved organic carbon. AgCit and AgPEG were used in the tests at total Ag concentrations of 1 and  $100 \text{ g L}^{-1}$ <sup>[24]</sup>. The results for the CU tests are shown in Figure 1.

It can be difficult to effectively separate the soluble component and NP species when using coatings that can form metal complexes. The filtered fraction was equal to the amount of free silver ( $\text{Ag}^+$ ) plus all of the silver complexes with diameters smaller than the membrane pores or 1-2 nm.

In the filtration process, a 4 mL aliquot was centrifuged at approximately  $2500 \times g$  for 35 min. The sample was discarded and a fresh 4 mL aliquot from the same tube was then centrifuged and discarded. This process was performed four times in total, but the fourth sample was analyzed by high-resolution ICP mass spectrometry (HR-ICP-MS) using an Element instrument (Thermo Fisher Scientific)<sup>[25]</sup>. The HR-ICP-MS parameters were: cool gas flow  $16 \text{ L min}^{-1}$ ; auxiliary gas flow rate  $0.9 \text{ L min}^{-1}$ ; flush sample gas flow  $0.9 \text{ L min}^{-1}$ ; ignition power 1.1 kW; operating power 1.2 kW; final sample gas flow rate  $1.0 \text{ L min}^{-1}$ ; sample gas ramp time 90 s;  $m/z$  107 detected for Ag. The HR-ICP-MS data were processed using UFREASI software, the logic and mathematics behind which were described in detail by Tharaud et al.<sup>[26]</sup>.

## RESULTS AND DISCUSSION

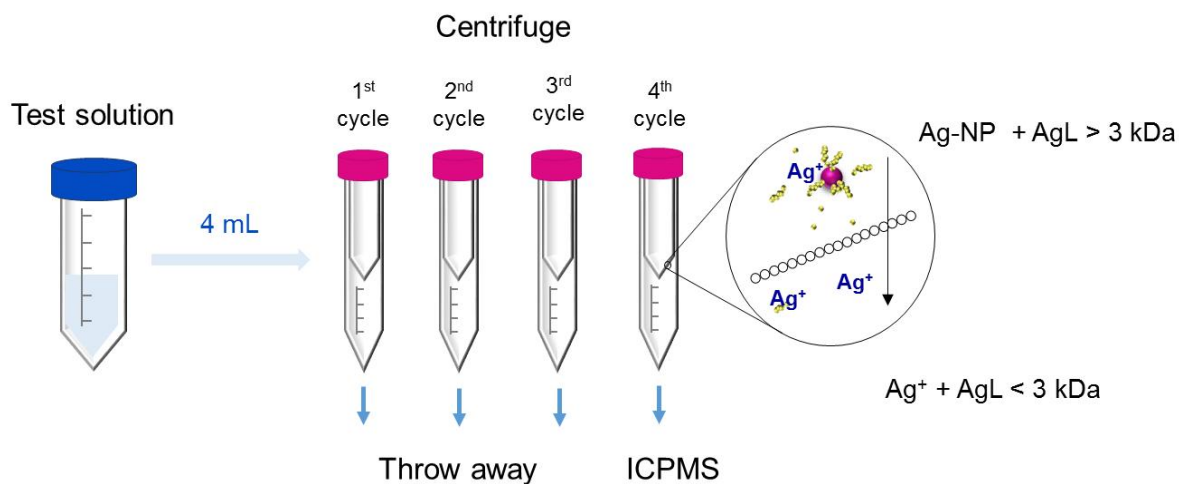
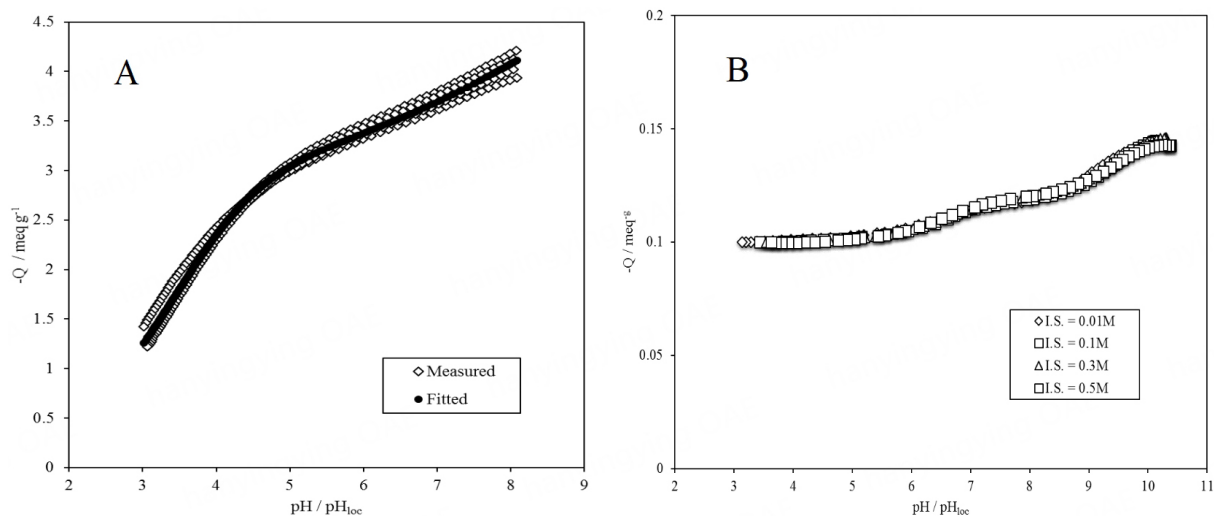
### **NOM characterization**

The carbon and nitrogen contents of the HS were  $43.95\% \pm 0.02\%$  and  $0.53\% \pm 0.01\%$ , respectively, and the carbon and nitrogen contents of the EPS were  $34.0\% \pm 0.1\%$  and  $20.5\% \pm 0.1\%$ , respectively. The carbon and nitrogen contents of the HS were consistent with previously published data. Humic compounds typically have carbon contents of 40%-60%. The nitrogen content of the EPS was similar to the nitrogen content previously found for *Mycrostitis aeruginosa*<sup>[27]</sup>.

A good master curve [Figure 2A] was obtained using the Donnan model for the HS data<sup>[28]</sup>. The amount of organic carbon in the EPS was less than required to establish a calibration curve for the titration method, so  $m_{\text{eq}}$  values ( $0.10$ - $0.15 \text{ g}^{-1}$ ) for the potentiometric EPS titration data shown in Figure 2B were not sufficient to establish a master curve. Titration data for  $\geq \text{pH } 3.5$  were therefore disregarded and were not used in the calculations.

**Table 1. Parameters describing the humic substances determined using the NICA-Donnan model**

Carboxylic site			Phenolic site			NICA-Donnan
$\log K_1$	$m_1$	$Q_{\max 1}$	$\log K_2$	$m_2$	$Q_{\max 2}$	
3.816	0.589	2.705	12.786	0.192	3.055	Data acquired

**Figure 1.** Flow diagram for the ultrafiltration by centrifugation process.**Figure 2.** (A) Master curve established using the NICA-Donnan model (closed symbols) and the experimental charge/pH data (open symbols) for the humic substances. (B) Data for potentiometric extracellular polymeric substance titrations at ionic strengths of 0.01, 0.1, 0.3, and 0.5 mol L<sup>-1</sup>.

The best-fit NICA-Donnan model parameters for the HS are shown in Table 1. The determination coefficient  $R^2$  was 0.994. The results indicated that the well-characterized HS could be used as analogs of natural HS<sup>[29]</sup>. The mean proton-affinity distributions of carboxyl and phenolic OH groups ( $Q_{\max,1}$  and  $Q_{\max,2}$ , respectively) and the widths of the proton-affinity distributions of the carboxyl and phenolic OH groups were represented by the NICA-Donnan model parameters ( $m_1$  and  $m_2$ , respectively). The empirical parameters indicated the relationship between the Donnan volume and ionic strength.

Similar values were found by Milne *et al.* using carboxyl and phenolic OH group content ( $Q_{\max}$ ) data<sup>[30]</sup>. Fulvic acid can contain carboxyl, phenolic hydroxyl, alcoholic hydroxyl, and carbonyl functional groups. Different fulvic acid samples contain different proportions of different functional groups. The approximate total acidity, carboxyl, phenolic hydroxyl, alcoholic hydroxyl, and carbonyl values per gram of acid were 12-14, 8-9, 3-6, 3-5, and 1-3, respectively. Small amounts of various methyl groups  $-CH_3$  may also have been present<sup>[31]</sup>.

The FT-IR spectra of the HS and EPS are shown in Figure 3. The absorbance peaks were assessed using data from previous studies. O-H stretching (H-bonds) of alcohols, phenols, carbon acids, and/or -NH proteins in the HS gave a broad peak close to  $3400\text{ cm}^{-1}$ <sup>[32,33]</sup>. The peak at  $1570\text{ cm}^{-1}$  may have been caused by asymmetric stretching of COO- and/or N-H bending in amino acids in proteins. The band at  $1700\text{-}1400\text{ cm}^{-1}$  was attributed to -COOH groups. The peak at  $1380\text{ cm}^{-1}$  was assigned to -OH, C-O stretching (in phenols, alcohols, and carboxylic acids), -CH (aliphatic), and symmetric stretching of COO-<sup>[34,35]</sup>. The presence of polysaccharides in the HS was supported by the peaks between  $950$  and  $1170\text{ cm}^{-1}$ . Incomplete carbohydrate removal during the isolation process would have resulted in carbohydrate structures being present in the HS<sup>[20]</sup>.

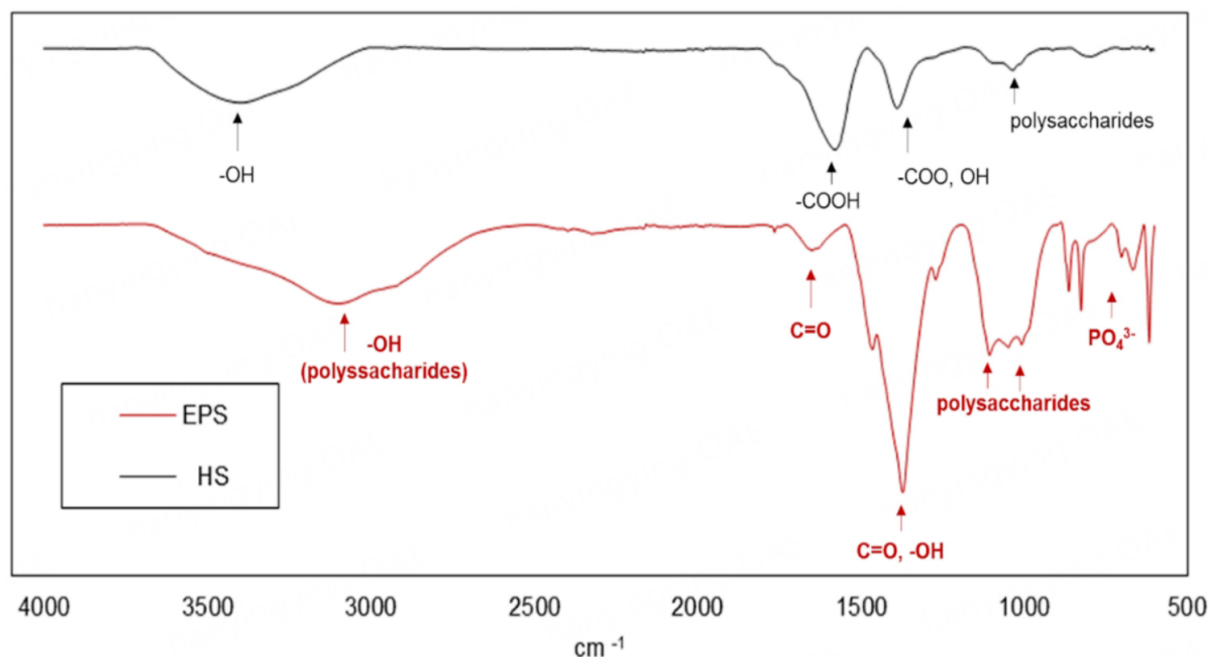
The broad peaks in the EPS spectrum were attributed to polysaccharide OH- stretching vibrations (H-bonds) and aliphatic -CH stretching ( $-CH_3$ ,  $-CH_2$ , and  $-CH$ )<sup>[31,32]</sup>. Similar peaks at  $2920\text{ cm}^{-1}$  were assigned to alkyl chains. Ketones, amides, and quinones in proteins were indicated by the peak at  $1630\text{ cm}^{-1}$ . The band at  $1440\text{ cm}^{-1}$  may have been related to contributions from amide III -CH bending vibrations and/or deformation vibrations of -CH in proteins and lipids. The prominent peak at  $1370\text{ cm}^{-1}$  could have been caused by stretching of C=O in carboxylate, -OH, C-O (phenolic), and/or CH (aliphatic). Amide III N-H group bending in proteins and C-N group stretching were indicated by the peak at  $1268\text{ cm}^{-1}$ . Polysaccharides could have caused the spectral band between  $1150$  and  $1000\text{ cm}^{-1}$ . Sun *et al.* also found peaks related to polysaccharide groups in an EPS FT-IR spectrum and they also found peaks around  $400$  and  $900\text{ cm}^{-1}$  that could be attributed to phosphate in nucleic acids<sup>[32]</sup>. The same zone was attributed by Zeng *et al.* to phosphate or sulfate groups<sup>[32-36]</sup>.

## Interactions between NOM and AgNP

### Charge density

The measured EPMS for the two Ag NP as a function of pH are shown in Figure 4. The AgNP EPM tended to be slightly negative when organic particles were present. The HS1 data shown in Figure 4 indicated that the EPM was more neutral for the *Daphnia* medium than the algae medium. For the HS1 data, a change in the pH between 3 and 8 strongly affected the EPMS of both AgCit and AgPEG [Figure 4]. In other words, the EPM was stable and somewhat negative throughout the pH range when AgCit or AgPEG was present. The same EPM was typically found for AgCit and AgPEG in the same medium. As shown in Figure 4B, similar results were found for HS2 and HS1, except that the EPM tended to be marginally more negative for HS2 than HS1. EPS at concentrations of 1 and 5 mg L<sup>-1</sup> (called EPS1 and EPS2, respectively) gave different EPMS in the same medium. The EPS1 in the algae medium and *Daphnia* medium had the same EPMS throughout the pH range of 3-8 at the same NOM concentration. The different EPMS for the AgCit and AgPEG in the presence of EPS1 [Figure 4C] and the different media indicated that the capping agent and solution strongly affected the EPM.

The EPMS for the HS were typically negative at all pH values, so higher negative EPMS were expected when the HS were in the algae and *Daphnia* media. The amount of charge at the interfaces between the particles and the solvent will strongly affect the conformation and mechanical and chemical stability of a colloidal



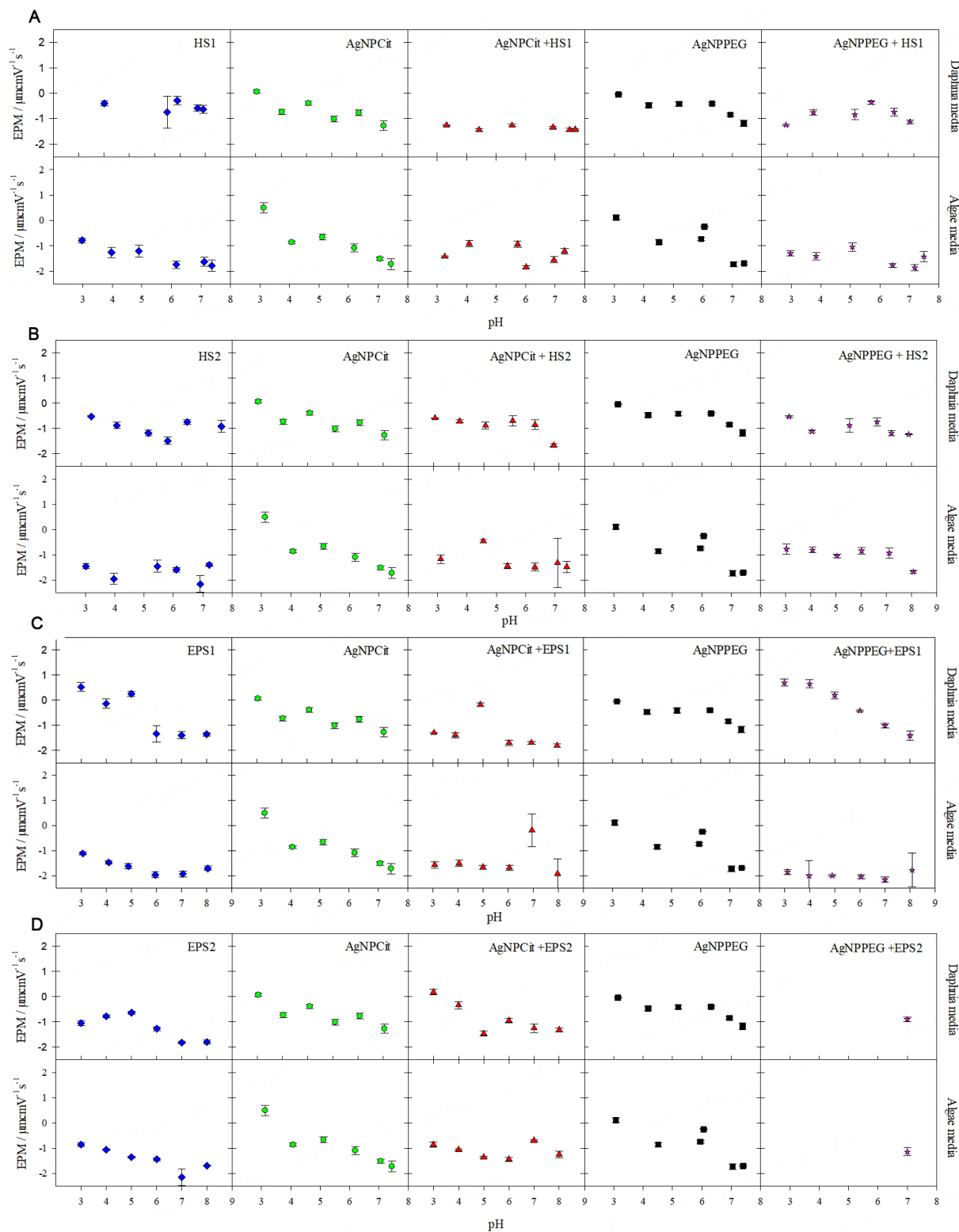
**Figure 3.** Fourier-transform infrared spectra of the humic substances (HS) extracted from Sorocabinha River water (upper, in black) and extracellular polymeric substances (EPS) extracted from the alga *Raphidocelis subcapitata* (lower, in red).

suspension. An understanding of the particle surface will allow the nature of the connection between the manufactured coating and the core of the AgNP to be assessed and to establish relationships between the efficacies of the manufactured coatings (whether simple ligands or neutral polymers) and the effects of the coatings on the behaviors of AgNP under environmental conditions<sup>[37]</sup>. EPM tests are typically used to indicate surface charge. The EPMs for various NP have been found to become more negative as the pH increases<sup>[38]</sup>. The ionic strength can affect the charge density, but as the ionic strength increases, the mobility decreases until it reaches zero. The importance of using a capping agent to assess the surface charge properties of NP was further demonstrated by the different EPMs for the AgCit and AgPEG in both media. Ionic strengths for the algae and *Daphnia* media were calculated using the Minteq tool and were found to be 0.1418 and 1.369 mol L<sup>-1</sup>, respectively, at pH 7.8 and 7.3, respectively. These results indicated that there were marked differences between the ionic strengths of the different solutions and that the composition of the medium may strongly affect the measured EPM. The ionic strength was higher for the algae medium than the *Daphnia* medium, but the concentrations of divalent cations (Ca<sup>2+</sup> and Mg<sup>2+</sup>) were higher in the *Daphnia* medium than the algae medium. It has previously been found that divalent cations can help organic matter and NP form complexes that can eventually form aggregates. Divalent cations can neutralize NOM (which is negatively charged) once the NOM has coupled to NP, and this will decrease the EPM and promote NP aggregation<sup>[39]</sup>. The effects of the pH have been assessed previously, and it has been found that the increase in OH<sup>-</sup> concentration as the pH increases means that OH<sup>-</sup> will more effectively compete for surface sites and cause negative surface charges at alkaline pH values<sup>[40]</sup>.

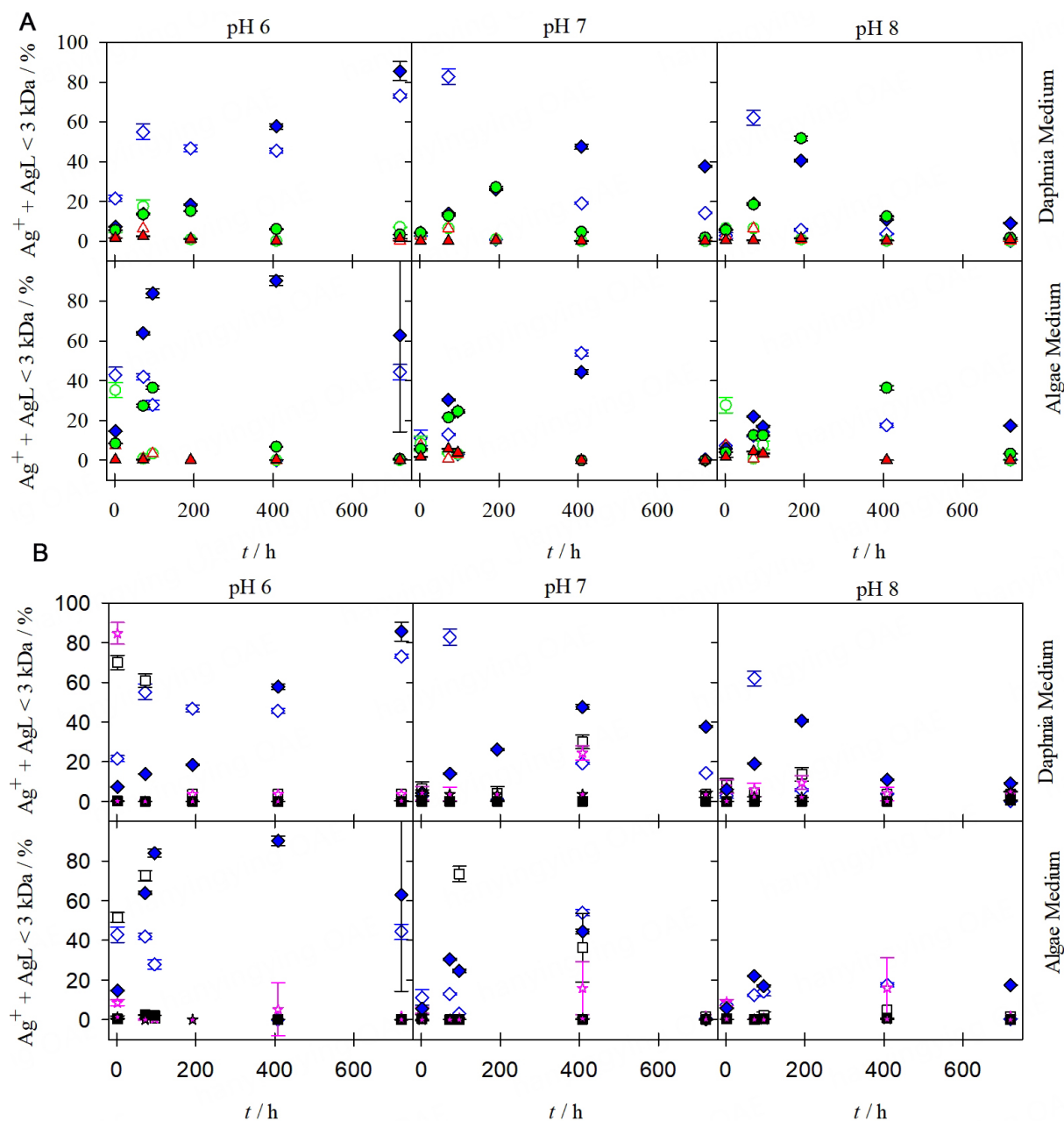
#### Dissolution

Dissolution data for AgCit and AgPEG in the presence and absence of HS and EPS are shown in Figures 5 and 6. The filtered fraction was equivalent to free Ag (Ag<sup>+</sup>) and all Ag complexes smaller than the membrane pores or 1-2 nm (i.e., Ag<sup>+</sup>, AgL, and AgCit). AgNP dissolved more readily at pH 6 and 7 and in the absence of HS than at other pHs and in the other media, particularly up to 408 h (17 d), after which the



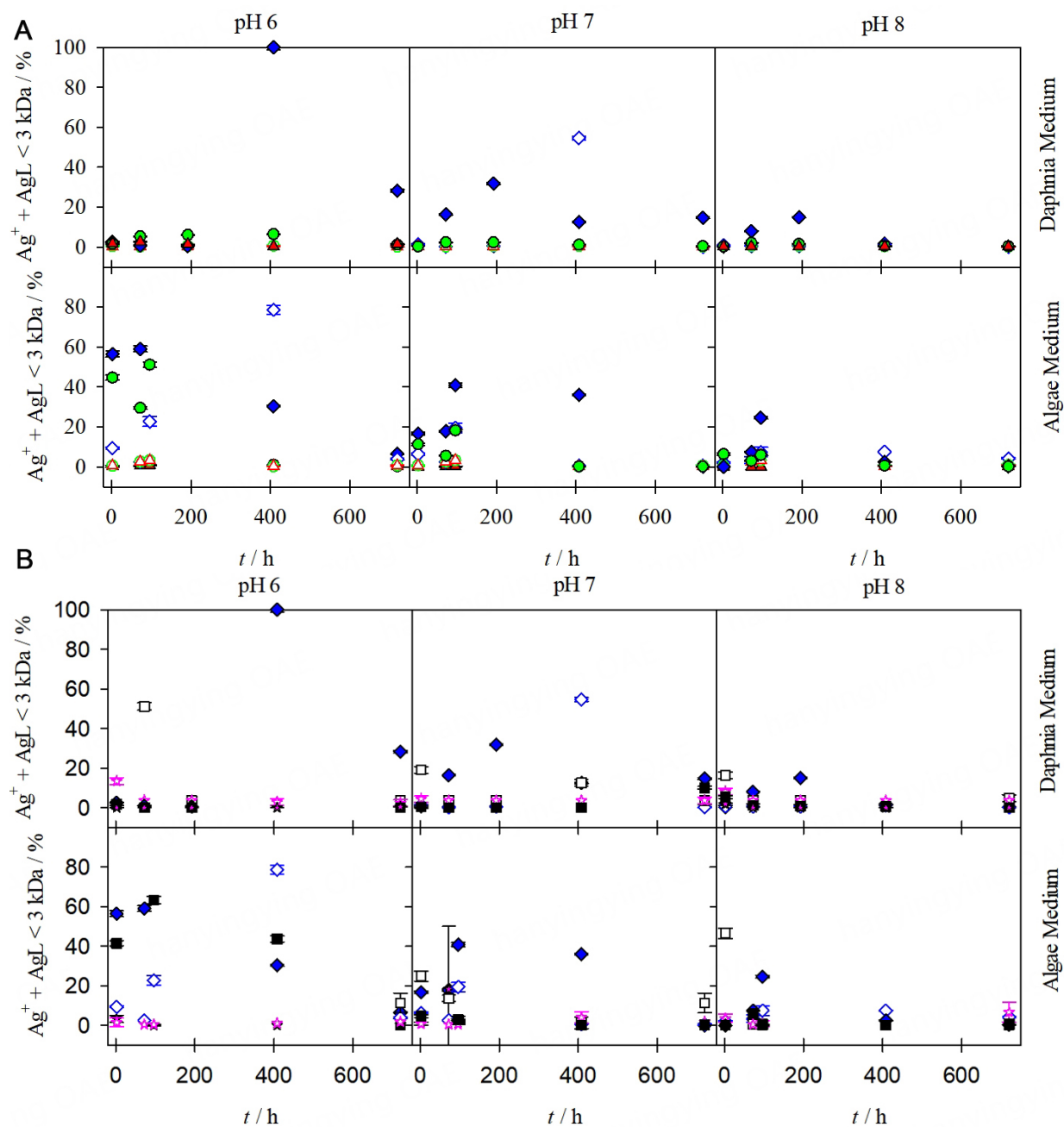


**Figure 4.** Electrophoretic mobility (EPM) data for (A) HS1 = 1 mg L<sup>-1</sup>, (B) HS2 = 1 mg L<sup>-1</sup>, (C) EPS1 = 1 mg L<sup>-1</sup>, and (D) EPS2 = 5 mg L<sup>-1</sup>. ◆ HS/EPS, ● AgCit = 2 mg L<sup>-1</sup>, ◆ AgCit = 2 mg L<sup>-1</sup> + HS/EPS, ■ AgPEG = 2 mg L<sup>-1</sup>, ★ AgPEG = 2 mg L<sup>-1</sup> + HS/EPS. HS = humic substances, EPS = extracellular polymeric substances, AgCit = silver nanoparticles capped with citrate, AgPEG = silver nanoparticles capped with polyethylene glycol.



**Figure 5.** Percentage of free silver ( $\text{Ag}^+$ ) and silver complexes ( $\text{AgL}$ ) smaller than the membrane pores ( $< 3$  kDa) plotted against exposure time for AgCit dispersed in algae and *Daphnia* media at pH 6, 7, and 8 in the absence and presence of (A) the HSs from the Sorocabinha River and (B) EPSs from *Raphidocelis subcapitata*.  $\diamond$  AgPEG =  $1 \mu\text{g L}^{-1}$ ,  $\circ$  AgPEG =  $1 \mu\text{g L}^{-1}$  + HS =  $1 \text{mg L}^{-1}$ ,  $\triangle$  AgPEG =  $1 \mu\text{g L}^{-1}$  + HS =  $10 \text{mg L}^{-1}$ ,  $\blacklozenge$  AgPEG =  $100 \mu\text{g L}^{-1}$ ,  $\bullet$  AgPEG =  $100 \mu\text{g L}^{-1}$  + HS =  $1 \text{mg L}^{-1}$ ,  $\blacktriangle$  AgPEG =  $100 \mu\text{g L}^{-1}$  + HS =  $10 \text{mg L}^{-1}$ ,  $\square$  AgPEG =  $1 \mu\text{g L}^{-1}$  + EPS =  $1 \text{mg L}^{-1}$ ,  $\star$  AgPEG =  $1 \mu\text{g L}^{-1}$  + EPS =  $5 \text{mg L}^{-1}$ ,  $\blacksquare$  AgPEG =  $100 \mu\text{g L}^{-1}$  + EPS =  $1 \text{mg L}^{-1}$ ,  $\blackstar$  AgPEG =  $100 \mu\text{g L}^{-1}$  + EPS =  $5 \text{mg L}^{-1}$ . The error bars indicate the standard deviations of triplicate measurements. HS = humic substances, EPS = extracellular polymeric substances, AgCit = silver nanoparticles capped with citrate.

AgNP dissolved less readily up to one month after exposure. AgPEG dissolved more over time and Ag was detected in the filtrate until 200 to 400 h, but then the amount of AgPEG that dissolved started to decrease. Unlike AgCit, AgPEG dissolved more readily at pH 7 than at pH 6 or 8 in the *Daphnia* medium. However, the Ag concentration in the filtrate was higher when the algae medium was used at the lowest pH, with the next highest at pH 7 and pH 8, and next highest at pH 9.



**Figure 6.** Percentage of free silver ( $\text{Ag}^+$ ) and silver complexes ( $\text{AgL}$ ) smaller than the membrane pores ( $< 3$  kDa) plotted against exposure time for AgPEG dispersed in algae and *Daphnia* media at pH 6, 7, and 8 in the absence and presence of (A) the HSs from the Sorocabinha River and (B) EPSs from *Raphidocelis subcapitata*.  $\diamond$  AgPEG =  $1 \mu\text{g L}^{-1}$ ,  $\circ$  AgPEG =  $1 \mu\text{g L}^{-1}$  + HS =  $1 \text{mg L}^{-1}$ ,  $\triangle$  AgPEG =  $1 \mu\text{g L}^{-1}$  + HS =  $10 \text{mg L}^{-1}$ ,  $\blacklozenge$  AgPEG =  $100 \mu\text{g L}^{-1}$ ,  $\bullet$  AgPEG =  $100 \mu\text{g L}^{-1}$  + HS =  $1 \text{mg L}^{-1}$ ,  $\blacktriangle$  AgPEG =  $100 \mu\text{g L}^{-1}$  + HS =  $10 \text{mg L}^{-1}$ ,  $\square$  AgPEG =  $1 \mu\text{g L}^{-1}$  + EPS =  $1 \text{mg L}^{-1}$ ,  $\star$  AgPEG =  $1 \mu\text{g L}^{-1}$  + EPS =  $5 \text{mg L}^{-1}$ ,  $\blacksquare$  AgPEG =  $100 \mu\text{g L}^{-1}$  + EPS =  $1 \text{mg L}^{-1}$ ,  $\blackstar$  AgPEG =  $100 \mu\text{g L}^{-1}$  + EPS =  $5 \text{mg L}^{-1}$ . The error bars indicate the standard deviations of triplicate measurements. HS = humic substances, EPS = extracellular polymeric substances, AgPEG = silver nanoparticles capped with polyethylene glycol.

Dissolution of both NP occurred more slowly in both media at pH 8 than at lower pH values. Essentially no Ag was detected in the filtrate containing HS at a concentration of 10 mg L<sup>-1</sup> regardless of the NP content or medium. The HR-ICP-MS quantification limit was ~10 ng L<sup>-1</sup>. Slightly more NP dissolution occurred in the presence of HS at a lower concentration (1 mg L<sup>-1</sup>) until around 200 h, then NP dissolution decreased. Even at the highest NP concentration, the presence of HS at the higher concentration (10 mg L<sup>-1</sup>, i.e., < 5%) did not markedly affect the Ag concentration in the filtrate. In general, less dissolution of AgCit occurred as the pH increased in both media and under all circumstances. The most NP dissolution occurred at pH 6, but the least dissolution occurred at pH 8. Excluding the first time point for the *Daphnia* medium in the absence of HS, maximum dissolution was found for AgCit at the highest NP concentration (100 µg L<sup>-1</sup>) in both the absence and presence of 1 mg L<sup>-1</sup> of HS in both media. Ag was not found at an appreciable concentration in the filtrate when the highest HS concentration was present, indicating that the NP concentration did not affect dissolution. More AgPEG dissolution occurred in the algae medium than in the *Daphnia* medium. This was more obvious at pH 6 than at higher pH values.

#### *Effects of organic matter*

Independently of the medium and pH, the HS affected the NP dissolution rate and profile. Even a small amount of HS (1 mg L<sup>-1</sup>) markedly slowed dissolution of the NP, but increasing the HS concentration to 10 mg L<sup>-1</sup> negligibly increased the Ag concentration in the filtrate. For NP in the *Daphnia* medium, the Ag concentration in the filtrate was higher when the pH was 8 than when the pH was 6. The algae medium appeared to be more conducive than the *Daphnia* medium to NP dissolution.

In the presence of HS, the dissolution rate was higher for the highest AgPEG concentration (100 g L<sup>-1</sup>) than for lower AgPEG concentrations. At the lowest HS concentration in the *Daphnia* medium, almost no Ag was detected in the filtrate. At 1 mg L<sup>-1</sup> of HS in the algae medium and at the highest NP concentration, some NP dissolution occurred at pH 6 and 7. However, Ag was not detected in the filtrate at the highest HS concentration.

The AgCit dissolution rate and profile were affected by the presence of EPS [Figure 6]. At an EPS concentration of 1 mg L<sup>-1</sup>, modest NP dissolution occurred for 400 h and then less dissolution occurred. In both media, almost no Ag was found in the filtrate throughout the exposure period in the presence of 5 mg L<sup>-1</sup> of EPS and at the maximum AgCit concentration. Ag was detected in the filtrate at the lowest AgCit concentration for up to 400 h. These results indicated the effects of the organic matter concentration on AgCit dissolution.

When AgCit was exposed to 1 mg L<sup>-1</sup> of EPS at pH 6 in either medium, the EPS had very little effect on dissolution. A small amount more dissolution occurred in the algae medium than in the *Daphnia* medium at pH 7 and 8 when the AgCit concentration was low. The low concentrations of dissolved Ag in tests using AgPEG indicated that the AgPEG NP were very stable [Figure 6]. The presence of EPS made it easy to detect a decrease in dissolution. The EPS decreased the dissolution rate at the maximum Ag concentration.

Usually, HS are associated with the presence of species such as alcohols, phenolic groups, carboxylic acid groups, N-H amino acids, proteins, and polysaccharides, and the species we detected in the HS and EPS by spectroscopic analyses were in agreement with species found in previous studies. Functional groups found in humic compounds in previous studies were also found<sup>[30,32,33]</sup>. We hypothesized that the EPS contained phenolic groups, polysaccharides, proteins, lipids, and sulfate/phosphate groups supplied by nucleic acids. Liu and Fang<sup>[41]</sup> found that EPS are made up of various compounds and that proteins and carbohydrates are the main constituents. Carbohydrates, lipids, organic acids, and amino acids were found to be components

of exudates from the alga *Cylindropermopsis raciborskii* by Tonietto *et al.*, Zeng *et al.* found that proteins and polysaccharides make sizable contributions to EPS and that the protein and polysaccharide contents are dependent on the processes involved in formation of the EPS<sup>[27,33]</sup>. The proteins in both the HS and EPS would have played important roles in the behaviors of the NP, taking into account the stabilities of ENM colloids, as mentioned by Sun *et al.*<sup>[42]</sup>.

### Speciation

The Ag speciation results determined using the NICA-Donnan model for the algae medium are shown in [Table 2](#).  $\text{AgCl}_{(\text{aq})}$  was the dominant Ag species regardless of the NOM concentration. The Ag speciation results for the *Daphnia* medium are shown in [Table 3](#). Free Ag ions contributed 21% of the total Ag concentration and  $\text{AgCl}_{(\text{aq})}$  contributed 62% of the total Ag concentration.

The adsorption results [[Figure 7](#)] indicated that  $\text{Ag}^+$  behaved differently in the solutions with different compositions. In theory, the overall concentration of ultrapure water did not change throughout the experiment. However, the overall Ag concentration decreased over time in the presence of algae or *Daphnia*. Zhang *et al.* found that the test medium composition strongly affected the fates and toxicities of ENMs<sup>[43]</sup>. AgCl formation (53% and 62% for the algae and *Daphnia* media, respectively) was related to the species distribution. In contrast to the modeling results, the  $\text{Ag}^+$  concentration decreased as the carbon concentration increased.

The model predicted that, in the presence of NOM, Ag would have a low affinity. Sikora and Stevenson<sup>[44]</sup> took phenolic and carboxylic groups into consideration and found decreased  $\text{Ag}^+$  affinities in the presence of humic compounds. They found, however, that N- and S-containing groups enhanced interactions between Ag and HS. FT-IR analyses indicated the presence of amine groups and indicated that the nitrogen content was higher for the EPS than the HS. The presence of N-containing groups may have caused interactions between the NOM and AgNP.

The results indicated that AgCit NP dissolve more readily than AgPEG NP in the absence or presence of HS at low or high concentrations and at all AgNP concentrations that were tested. Levard *et al.* found that AgCit NP dissolved more readily as the pH decreased<sup>[45]</sup>.

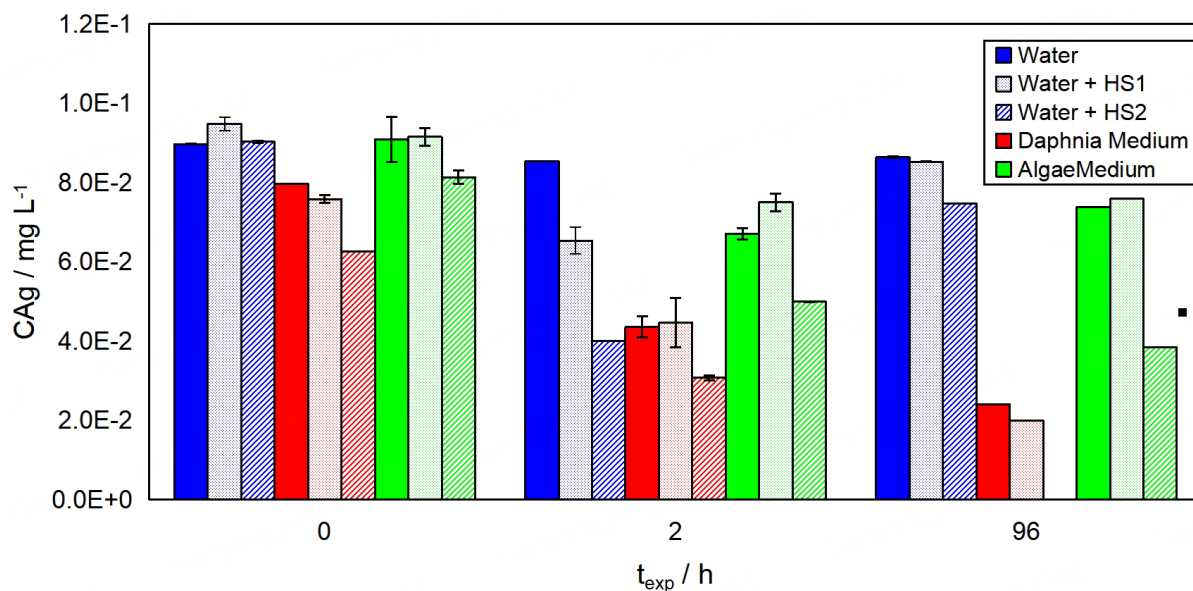
The HS and EPS decreased the dissolution rate. Adsorption of NOM to NP could explain this. HS and EPS can become attached to NP surfaces and create a coating on the NP. This would alter the physical-chemical and biological properties of the NP, change the surface reactivities of the NP, and change the ways the NP interact with biota. NOM could therefore make NP electrostatically or sterically stable or cause NP to flocculate. The formation of complexes will depend on the types of NP and organic matter present<sup>[9,46,47]</sup>. The availability of binding sites and the HS and EPS compositions determined by FT-IR spectroscopy indicated that there would be a strong potential for interactions between the NP and organic and inorganic compounds (e.g., phenol and carboxylic groups, polysaccharides, amines, and N-containing groups). Liu and Fang determined the binding capacities of EPS<sup>[41]</sup> and found electrostatic binding sites for EPS released by microbial communities of 10.88 and 16.44 mmol  $\text{g}^{-1}$ . They hypothesized that larger amounts of EPS may sorb to metals than bacterial cell surfaces. The algae and *Daphnia* media may both have aided interactions between NOM and the NP through cation bridging, taking into consideration the element concentrations in the media shown in [Supplementary Table 1](#). Divalent metal ions such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  can bridge functional groups on organic matter and nanomaterials and therefore facilitate sorption<sup>[48]</sup>. NP-NOM interactions could therefore be important. Diegoli *et al.* found that humic acid could sorb to gold NP with Cit caps and prevent aggregation through steric stabilization<sup>[49]</sup>. It is important to study NP dissolution rates because

**Table 2. Ag speciation determined using the NICA-Donnan model for the algae medium with organic matter present**

Species name		Ag <sup>+</sup>	AgCl <sub>(aq)</sub>	AgCl <sub>2</sub> <sup>-</sup>	AgCl <sub>3</sub> <sup>-2</sup>	AgSO <sub>4</sub> <sup>-</sup>	AgNH <sub>3</sub> <sup>+</sup>	AgNO <sub>3(aq)</sub>
Exposure condition		% of total concentration						
C <sub>Ag</sub> / M	C <sub>NOM</sub> / mgL <sup>-1</sup>							
9.35E-09	-	10	53	32	1	2	2	4
	1	10	53	32	1	2	2	4
	10	10	53	32	1	2	2	4
9.35E-07	-	10	53	32	1	2	2	4
	1	10	53	32	1	2	2	4
	10	10	53	32	1	2	2	4

**Table 3. Ag speciation determined using the NICA-Donnan model for the *Daphnia* medium**

Species name		Ag <sup>+</sup>	AgCl <sub>(aq)</sub>	AgCl <sub>2</sub> <sup>-</sup>	AgSO <sub>4</sub> <sup>-</sup>
Exposure condition		% of total concentration			
C <sub>Ag</sub> / M	C <sub>NOM</sub> / mgL <sup>-1</sup>				
9.35E-09	-	21	62	14	3
	1	21	62	14	3
	10	21	62	14	3
9.35E-07	-	21	62	14	3
	1	21	62	14	3
	10	21	62	14	3

**Figure 7.** Results of adsorption tests using an AgNO<sub>3</sub> solution (100 µg L<sup>-1</sup>) in ultrapure water (blue), *Daphnia* medium (red), and algae medium (green) in the absence of humic substances (HSs) (solid color), presence of HSs (1 mg L<sup>-1</sup>) (dots), and presence of HSs (10 mg L<sup>-1</sup>) (stripes).

various studies of the toxicities of ENMs have indicated that the main mechanism involved in the antibacterial activity of AgNP is dissolution of Ag<sup>+</sup> ions<sup>[50]</sup>. Zhang *et al.* published a comprehensive review indicating the need for studies of AgNP in the laboratory under realistic environmental conditions because of discrepancies between ENM toxicities to and effects in aquatic organisms commonly found in different

publications<sup>[43]</sup>. In some studies, it has been found that AgNP dissolution may affect the toxicities of AgNP to exposed organisms in aquatic systems and that NOM in the environment may affect AgNP dissolution<sup>[51-53]</sup>. It is important to investigate the hazards posed by AgNP at low concentrations, taking into account long exposure periods like in our study.

## CONCLUSIONS

The charge was affected by the pH and ions present in different media (algae or *Daphnia*) when electrostatic interactions between AgNP and NOM were considered. NOM strongly prevented AgNP dissolution. NP was stabilized in the media containing HS and EPS. Some types of NOM may have a high binding potential for AgNP. Dissolution was strongly affected by the type of coating (Cit and PEG) and by the dissolved organic matter and particle concentrations. AgNP transformations were also affected by the composition medium, indicating the importance of environmental matrices being taken into account. The results indicated how organic matter can affect the fates of AgNP, the bioavailabilities of AgNP, and predictions of AgNP dissolution and toxicity. The approach used here will further our understanding of the fates of AgNP and other nanomaterials in the environment and biota, which will help in the development of standards for regulating nanomaterials.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Watanabe CH, Domingos RF, Benedetti MF, Rosa AH

Performed data acquisition: Watanabe CH

Provided administrative, technical and material support: Domingos RF, Benedetti MF, Rosa AH

### Availability of data and materials

Data will be deposited into [Supplementary Material](#) in the journal.

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### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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