SUPPORTING INFORMATION

Freezing Facilitates Formation of Silver Nanoparticles under Natural and Simulated Sunlight Conditions

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Total pages: 22
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Chemicals and Materials.

An Ag⁺ stock solution (1000 mg/L) was purchased from the National Institute of Metrology (Beijing, China). A stock solution of poly(vinylpyrrolidone) and chitosan co-stabilized AgNPs (PVP/CTS-AgNPs, 20 nm, 10,000 mg/L) was obtained from Shanghai Huzheng Nanotechnology Co., Ltd. (Shanghai, China). ¹⁰⁷Ag-enriched silver foil (purity > 99%) was obtained from Trace Sciences International (Ontario, Canada). Suwannee River NOM powder was purchased from the International Humic Substances Society. The ¹⁰⁷Ag⁺ and NOM stock solutions were prepared by dissolution and filtration, as described in our previous study,¹ and full details of the preparation are described in next section. Other chemicals used such as boric acid, sodium borate, and sodium perchlorate (NaClO₄) were of analytical reagent grade from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All aqueous samples were prepared using ultrapure water (18.3 MΩ) produced with a Milli-Q gradient system (Millipore, Billerica, MA).

Preparation of NOM and ¹⁰⁷AgNO₃ Solutions.

The stock NOM Solutions (1 g/L) was prepared by dissolving 50 mg of SRNOM powder (Suwannee river natural organic matter) in 500 mL of ultrapure water. After being shaken overnight, the solution was filtered through 0.22 μm membrane filter (mixed cellulose esters, Millipore, Billerica, MA). The total organic carbon (TOC) in the filtrates was quantified by a TOC analyzer (Phoenix 8000, Tekmar-Dohrmann, Cincinnati, OH). To prepare the ¹⁰⁷AgNO₃ stock solution, the required amount of ¹⁰⁷Ag-riched silver foil (> 99%) (Ontario, Canada) was firstly dissolved with concentrated HNO₃ in a 20 ml brown glass bottle. Then, the redundant HNO₃ was evaporated by a hot plate at 160 °C. Finally, the ¹⁰⁷AgNO₃ left at the bottom of poly(tetrafluoroethylene) crucible was redissolved with ultrapure water. The total Ag concentration in the ¹⁰⁷AgNO₃ stock solution was determined...
by Agilent 7700 ICPMS (Santa Clara, CA). Both of the two stock solutions were stored at 4 °C before use.

**Natural Water Sample Collection.**

Two environmental water samples were collected from the Chaobai River (CBR, Beijing, China) and Hun River (HR, Shenyang, China), respectively. Despite the fact that the heteroaggregation between AgNPs and other small-sized particulate matters may occur in the natural water samples, the samples were sequentially filtered through quantitative filter paper and a 0.22 μm mixed cellulose ester membrane filter to eliminate the interferences of most large-sized particulate matters (e.g., bacteria and clay minerals) in the samples on the following formation and stability of AgNPs. Evaluating the filtration procedure on the formation and stability of AgNPs is beyond the scope of the study. The filtered effluents were stored in 500 mL Teflon bottles at 4 °C prior to use. The main physiochemical parameters of these eluents are listed in Table S2.

**Separation of AgNPs and Ag⁺.**

Separation of dissolved Ag⁺ species and AgNPs was achieved by ultrafiltration using centrifugal ultrafilter units (Amicon Ultra-15 30 kD, Millipore, MA) as previously described. Briefly, diluted AgNP suspensions as well as 10 mM Na₂S₂O₃ and 0.1% (w/v) Triton X-114 were added into the ultrafilter unit. Na₂S₂O₃ was used to dissolve AgCl, whereas Triton X-114 served to protect AgNPs from dissolution. After shaking and centrifugation, the upper solution retained in the ultrafilter unit was used for AgNP characterization and the ionic Ag⁺ in the filtrate was collected for further quantification.

**Characterization and Quantification of AgNPs.**

UV–vis spectra measurement was performed using a Shimadzu UV-3600 spectrophotometer (Kyoto, Japan), between 300 and 800 nm, with pure water as the blank
in a 1 cm quartz cuvette.

Transmission electron microscopy (TEM) results were obtained using an H-7500 transmission electron microscope (Hitachi, Japan) at 80 kV, and high-resolution TEM (HRTEM) with selected area electron diffraction (SAED) was performed on a JEOL JEM-2100 (Japan) transmission electron microscope at 200 kV. TEM/HRTEM samples were prepared by drop-drying the sample solution onto ultrathin carbon-coated copper grids at room temperature in a vacuum oven (DZF 6090, Yiheng Technology Co., Ltd., Shanghai, China).

The mass concentration of AgNPs was measured using an Agilent 7700 inductively coupled plasma mass spectrometry (ICPMS) (Santa Clara, CA) after sample digestion with 65% HNO₃. For calibration, a series of diluted Ag⁺ stock solutions were determined with ¹¹⁵In as the internal standard, and main parameters of ICPMS were summarized in Table S4.

**Determination of AgNPs by Single Particle-ICPMS (SP-ICPMS).**

To obtain size information of AgNPs at environmentally relevant concentration levels, an Agilent 7900 ICPMS was used for data acquisition in single particle mode with 0.1 ms as dwell time and 60 s as sample analysis time based on our previously reported optimization.¹ The calibration method was referred to a recent study.³ Briefly, the transport efficiency was determined using AuNP NIST reference materials 8012 with a nominal diameter of 30 nm. Data were acquired and processed using MassHunter software (Agilent Technologies). Main parameters of SP-ICPMS were summarized in Table S4.

**Quantification of Stable Ag Isotopes in the Mixture of Ag⁺ and AgNPs.**

Herein, isotope-enriched ¹⁰⁷Ag⁺ and commercial AgNPs were added into the real water samples to monitor the effect of engineered AgNPs on the formation of new AgNPs under freezing treatment and sunlight irradiation. The ¹⁰⁷Ag and ¹⁰⁹Ag content in nanosized and
ionic Ag were determined by ICPMS and calculated to evaluate the redox reaction between
Ag⁺ and AgNPs. Based on the $^{107}\text{Ag}$ and $^{109}\text{Ag}$ content in the filtrate, the concentration of
ionic Ag⁺ arising from AgNPs (${[\text{Ag}^+]_{\text{AgNPs}}}$) or $^{107}\text{AgNO}_3$ ($^{107}[\text{AgNO}_3]$) could be respectively
calculated. The oxidation of AgNPs and reduction of $^{107}\text{Ag}^+$ could then be tracked by
quantifying the increase in $[\text{Ag}^+]_{\text{AgNPs}}$ ($\Delta[\text{Ag}^+]_{\text{AgNPs}}$) and decrease in $^{107}[\text{AgNO}_3]$
($\Delta^{107}[\text{AgNO}_3]$). According to our previous studies,¹⁴ the calculation method was described
as follows.

Given that $^{107}\text{Ag}$ and $^{109}\text{Ag}$ are the only two stable isotopes of element Ag, their
concentrations in the mixture solution of $^{107}\text{AgNO}_3$ and AgNPs can be obtained by ICPMS.
Contributions of $^{107}\text{AgNO}_3$ and AgNPs to $^{107}\text{Ag}^+$ and $^{109}\text{Ag}^+$ were calculated by eqs 1 and 2:

$$^{[107]\text{Ag}^+]} = P^{107} \times [\text{Ag}^+]_{\text{AgNPs}} + P \times [^{107}\text{AgNO}_3] \quad (1)$$

$$^{[109]\text{Ag}^+]} = P^{109} \times [\text{Ag}^+]_{\text{AgNPs}} + (1-P) \times [^{109}\text{AgNO}_3] \quad (2)$$

Where $[\text{Ag}^+]_{\text{AgNPs}}$ is the concentration of Ag⁺ released from AgNPs, and $^{[107]\text{AgNO}_3]}$ is the
residue $^{107}\text{AgNO}_3$ in the aqueous solution. P stands for the purity of $^{107}\text{Ag}^+$ in $^{107}\text{AgNO}_3$
spiked (e.g., 98 % in this study), and and $P^{107}$ and $P^{109}$ are the natural abundances of $^{107}\text{Ag}$
i.e., 51.8%) and $^{109}\text{Ag}$ (i.e., 48.2%).

Compared with the Ag⁺ concentration before and after treatments, the increase of
$[\text{Ag}^+]_{\text{AgNPs}}$ ($\Delta[\text{Ag}^+]_{\text{AgNPs}}$) and decrease of $^{107}[\text{AgNO}_3]$ ($\Delta^{107}[\text{AgNO}_3]$) can be used to
quantify oxidation of AgNPs and reduction of $^{107}\text{AgNO}_3$ as in eqs 3 and 4:

$$\Delta[\text{Ag}^+]_{\text{AgNPs}} = [\text{Ag}^+]_{\text{AgNPs}} - \{[\text{Ag}^+]_{\text{AgNPs}}\}_{\text{initial}} \quad (3)$$

$$\Delta^{107}[\text{AgNO}_3] = ^{107}[\text{AgNO}_3] - ^{107}[\text{AgNO}_3]_{\text{initial}} \quad (4)$$

Where $\{[\text{Ag}^+]_{\text{AgNPs}}\}_{\text{initial}}$ and $^{107}[\text{AgNO}_3]_{\text{initial}}$ stand for the concentrations of original
dissolved Ag⁺ in the mixture solution before reaction, respectively.
Figure S1. Temperature (A) and sunlight intensity (B) profiles during the days of natural frozen treatments (December 23-25, 2016).
Figure S2. Experimental set-up (C: refrigerated and heated immersion circulators) and operating procedures.
Figure S3. Identification and characterization of AgNPs formed in the mixtures solution of NOM (10 mg/L) and Ag⁺ (10 mg/L): TEM (A, E), HRTEM (B, F), EDS (C, G), and SAED (D, H) images of AgNPs in test samples under natural sunlight irradiation (A, B, C, and D) and control groups under dark conditions (E, F, G, and H).
**Figure S4.** Identification and characterization of AgNPs formed in the Ag⁺ spiked HR samples (10 mg/L): TEM (A, E), HRTEM (B, F), EDS (C, G), and SAED (D, H) images of AgNPs in test samples under natural sunlight irradiation (A, B, C, and D) and control groups under dark conditions (E, F, G, and H).
Figure S5. TEM (A), HRTEM (B), EDS (C), and SAED (D) images of AgNPs in control groups under dark conditions.
Figure S6. EDS image of AgNPs in test samples under natural sunlight irradiation.
Figure S7. TEM images of AgNPs in NOM solutions under dark conditions after different treatments: freeze–thaw cycling from −20 °C to 4 °C (A), storage at −20 °C (B), and storage at 4 °C (C).
Figure S8. Temperature profiles of slow (0.07 °C/min), medium (0.1 °C/min), and fast (0.2 °C/min) freezing rates during three freeze–thaw cycles.
Figure S9. Effects of three freezing rates of 0.07 °C/min (slow), 0.1 °C/min (medium), and 0.2 °C/min (fast) on the concentrations of AgNPs and Ag⁺ in the mixture solution of NOM (10 mg/L) and Ag⁺ (10 mg/L) under simulated sunlight irradiation and after one (A) and three (B) freeze–thaw cycles.
Figure S10. Effect of ionic strength on the concentrations of AgNPs and Ag⁺ in the mixture solution of NOM (10 mg/L) and Ag⁺ (10 mg/L) under simulated sunlight irradiation and three freeze–thaw cycles at a freezing rate of 0.1 °C/min.
Figure S11. Characterization of AgNPs formed in the PVP/CTS-AgNPs and Ag$^+$ (both at 10 mg/L) spiked CBR water samples (A, C, E and G) and HR water samples (B, D, F and H): TEM (A, B, E and F) and HRTEM (C, D, G and H) images of AgNPs in test samples under natural sunlight irradiation (A, B, C, and D) and control groups under dark conditions (E, F, G, and H).
Figure S12. Signal intensity frequencies of the mixture of Ag⁺ and AgNPs (both at 50 ng/L) together with NOM (10 mg/L DOC) before (A) and after the freeze–thaw treatment in the dark (B) and under simulated sunlight irradiation (C).
### Table S1. Analytical techniques used in the experiments.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Identification and characterization of AgNPs</th>
<th>Quantification of AgNPs and Ag⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV–vis</td>
<td>TEM/HRTEM</td>
</tr>
<tr>
<td>Formation of AgNPs in NOM-rich artificial and natural waters during natural freezing process</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Reduction of Ag⁺ to AgNPs by NOM under controlled freezing treatments and simulated sunlight irradiation.</td>
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<td>√</td>
</tr>
<tr>
<td>Effects of the presence of commercial AgNPs on sunlight-induced formation of AgNPs</td>
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<td>√</td>
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*Both AgNPs and Ag⁺ were at 50 ng/L.
Table S2. Characteristic of two river water samples.

<table>
<thead>
<tr>
<th></th>
<th>DOC (mg/L)</th>
<th>pH (mg/L)</th>
<th>Na⁺ (mg/L)</th>
<th>K⁺ (mg/L)</th>
<th>Ca²⁺ (mg/L)</th>
<th>Mg²⁺ (mg/L)</th>
<th>Cl⁻ (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>CBR*</td>
<td>14.2</td>
<td>8.4</td>
<td>117.6</td>
<td>18.4</td>
<td>28.4</td>
<td>25.3</td>
<td>112.0</td>
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<tr>
<td>HR*</td>
<td>249.5</td>
<td>6.7</td>
<td>68.6</td>
<td>7.4</td>
<td>65.1</td>
<td>24.5</td>
<td>32.0</td>
</tr>
</tbody>
</table>

* CBR, Chaobai river; HR, Hun river.
Table S3. Detailed quantitative results of SP-ICPMS for AgNPs in samples collected before and after freeze–thaw treatment.

<table>
<thead>
<tr>
<th></th>
<th>Detection limit (nm)</th>
<th>Diameter (nm)</th>
<th>Number concentration × 10^7 /L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>25.2 ± 0.7</td>
<td>15.3</td>
<td>33.2 ± 1.9</td>
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<tr>
<td>Dark</td>
<td>31.1 ± 0.6</td>
<td>3.6 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>3.8 ± 0.4</td>
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<td></td>
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</tbody>
</table>

^aCalculated based on 3 times the standard deviation of the background.

^bValue: mean ± standard deviation (SD), n = 3.
**Table S4. Operational parameters of ICPMS.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters</th>
<th>Parameter values</th>
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</thead>
<tbody>
<tr>
<td>Agilent 7700</td>
<td>RF Power</td>
<td>1500 W</td>
</tr>
<tr>
<td></td>
<td>Plasma gas flow rate</td>
<td>15 L/min</td>
</tr>
<tr>
<td></td>
<td>Nebulizer flow rate</td>
<td>0.4 ml/min</td>
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<tr>
<td></td>
<td>Sample depth</td>
<td>9.0 mm</td>
</tr>
<tr>
<td></td>
<td>Dwell time</td>
<td>50 ms</td>
</tr>
<tr>
<td></td>
<td>Analyte monitored</td>
<td>$^{107}\text{Ag}$, $^{109}\text{Ag}$, $^{115}\text{In}$</td>
</tr>
<tr>
<td>Agilent 7900</td>
<td>RF Power</td>
<td>1550 W</td>
</tr>
<tr>
<td></td>
<td>Nebulizer flow rate</td>
<td>0.4 ml/min</td>
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<tr>
<td></td>
<td>Dwell time</td>
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<td></td>
<td>sample analysis time</td>
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<tr>
<td></td>
<td>Analyte monitored</td>
<td>$^{107}\text{Ag}$, $^{115}\text{In}$</td>
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</tbody>
</table>

**REFERENCES**


