Speciation Analysis of Labile and Total Silver(I) in Nanosilver Dispersions and Environmental Waters by Hollow Fiber Supported Liquid Membrane Extraction

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Supporting Information

ABSTRACT: Hollow fiber supported liquid membrane (HFSLM) extraction was coupled with ICP-MS for speciation analysis of labile Ag(I) and total Ag(I) in dispersions of silver nanoparticles (AgNPs) and environmental waters. Ag(I) in aqueous samples was extracted into the HFSLM of 5%(m/v) tri-*n*-octylphosphine oxide in *n*-undecane, and stripped in the acceptor of 10 mM Na₂S₂O₃ and 1 mM Cu(NO₃)₂ prepared in 5 mM NaH₂PO₄–Na₂HPO₄ buffer (pH 7.5). Negligible depletion and exhaustive extraction were conducted under static and 250 rpm shaking to extract the labile Ag(I) and total Ag(I), respectively. The extraction equilibration was reached in 8 h for both extraction modes. The extraction efficiency and detection limit were (2.97 ± 0.25)% and 0.1 μg/L for labile Ag(I), and (82.3 ± 2.0)% and 0.5 μg/L for total Ag(I) detection, respectively. The proposed method was applied to determine labile Ag(I) and total Ag(I) in different sized AgNP dispersions and real environmental waters, with spiked recoveries of total Ag(I) in the range of 74.0–98.1%. With the capability of distinguishing labile and total Ag(I), our method offers a new approach for evaluating the bioavailability and understanding the fate and toxicity of AgNPs in aquatic systems.

Engineered metal/metal oxide nanomaterials are widely used in various areas and have been detected in the environment.^{1–5} Due to their oxidation and dissolution, metal/metal oxide nanomaterials in environments coexist with metal ions that may be present in the form of free ions, complexes with the capping agents or ligands in the matrixes, and adsorbed on the particles.^{6–8} Even a simple colloid of silver nanoparticles (AgNPs) consists of three silver forms: Ag⁰ nanoparticles, soluble Ag⁺ and surface-adsorbed Ag⁺.⁹

While it is generally believed that the free/labile metal ions are responsible for the toxicity of metals in environment, and the metal ions associated with matrix (such as particles and nature organic matter) are not bioavailable,^{10–12} the case for nanoparticles is much more complicated because the particles could enter the cells by a Trojan-horse type mechanism.^{13,14} Therefore, determination of nanoparticulate and ionic species of metals in metal/metal oxide nanomaterial dispersions and environment waters is crucial for exploiting the applications, as well as for understanding and elucidating the environmental transportation, transformation, and toxicity properties of these materials.^{15–18}

Various techniques, such as centrifugation,¹⁹ centrifugal ultrafiltration,²⁰ dialysis,²¹ ultrafiltration,^{22,23} cloud point extraction,^{24–26} and magnetic solid phase extraction,²⁷ were

proposed for the separation and determination of nanoparticulate and ionic metals in various samples. Ion selective electrode (ISE)^{17,20,28–30} was adopted for measuring the free metal ions in nanomaterials. While the ISE method is quick and low-cost, it has limited sensitivity and reproducibility for nanomaterial samples. Moreover, this technique suffered from the lack of proper electrodes for some target metal ions. Although diffusive gradients in thin films (DGT) was used to measure labile Ag(I),²⁰ it can only distinguish the metal ions and relatively large nanoparticles, as small nanoparticles are reported to accumulate in the DGT device.³¹ Inductively coupled plasma mass spectrometry (ICP-MS) was performed in single-particle mode, but it can only distinguish metal ions from nanoparticles of over 20 nm.^{32–34} ICP-MS was also coupled with field-flow fractionation (FFF)^{35–38} for selective determination of AgNPs in the presence of Ag⁺, and coupled with reversed-phase liquid chromatography (LC)^{39,40} or capillary electrophoresis (CE)⁴¹ for speciation test of AgNPs and dissolved monovalent silver (Ag(I)), which includes Ag⁺ and its

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complexes. However, these methods are incapable of distinguishing labile Ag(I) and total Ag(I).

Supported liquid membrane (SLM), also named as permeation liquid membrane (PLM), has been demonstrated an advantageous technique to evaluate the bioavailability/toxicity of chemicals including metals^{12,42–48} and organic compounds.^{49,50} In SLM, target chemicals were extracted into a liquid membrane, a hydrophobic organic solvent or a mixture of hydrophobic organic solvents/carrier supported on a microporous membrane (planar or hollow fiber), and re-extracted to the strip solution. It has been shown that either free metal ion concentration or the total concentration of labile complexes can be measured by tuning the extraction conditions.⁴⁴ This advanced separation technique combines selective extraction, re-extraction and preconcentration of the target species into one step, thus provides high sensitivity and selectivity for speciation analysis. Additionally, it mimics somehow the biological membrane in uptake of chemicals and had been adopted in evaluation of the bioavailability/toxicity of chemicals especially metal species.^{12,44–49} However, SLM has neither been exploited in the speciation analysis nor the bioavailability/toxicity evaluation of nanomaterials.

Herein, we report the development of a novel analytical method for the determination of labile and total Ag(I), respectively, by coupling hollow fiber based SLM (HFSLM) with ICP-MS. Labile Ag(I) and total Ag(I) in dispersions of AgNPs and water samples were extracted by the negligible depletion extraction and the exhaustive extraction mode, respectively. The established methods were applied to analyze labile Ag(I) and total Ag(I) in various sized AgNPs dispersions and environmental waters.

■ EXPERIMENTAL SECTION

Chemicals and Materials. Tri-*n*-octylphosphine oxide (TOPO, 98%) and *n*-undecane (99%) were purchased from Alfa Aesar (Massachusetts). Silver nitrate, sodium thiosulfate, copper nitrate, disodium hydrogen phosphate dodecahydrate, and sodium dihydrogen phosphate dehydrate were analytical-reagent grade or above and obtained from Sinopharm Chemical Reagent Beijing (Beijing, China). Nitric acid (65%) was obtained from Merck (Darmstadt, Germany). Stock solutions of Ag⁺ (100 mg/L) and Cu²⁺ (0.1 mol/L) were prepared by dissolution of AgNO₃ and Cu(NO₃)₂ in 0.2 M HNO₃, respectively. The 50/280 Accurel PP polypropylene hollow fiber tubing (50 μm wall thickness, 280 μm inner diameter, and 0.1 μm pore size) was bought from Membrana GmbH (Wuppertal, Germany). Ultrapurified water (EASY-pure LF, Barnstead International, Dubuque, IA) was used throughout the experiments.

Preparation and Characterization of AgNPs. Polyvinylpyrrolidone (PVP) capped AgNP (AgNP-1) with particle size of 37.5 ± 6.0 nm was synthesized by reduction of AgNO₃ with sodium hypophosphite as described in our previous study,²⁴ while PVP capped AgNPs with particle size of 16.2 ± 5.4 nm (AgNP-2) and 48.9 ± 12.1 nm (AgNP-3) were synthesized by reduction of AgNO₃ with hydroxylamine hydrochloride. Another PVP coated AgNPs (AgNP-5) with particle size of 9.12 ± 2.22 nm and the glutathione protected silver nanoclusters about 1 nm (AgNP-6) were synthesized by reduction of AgNO₃ with sodium tetraethylborate as described as our previous work.^{17,40} Details on their synthesis are shown in Supporting Information (SI). Stock suspensions of the four PVP capped AgNPs were prepared by redispersion of the PVP

capped AgNPs with water. AgNP-4 was obtained by soaking a piece of wound dressings (ACTICOAT Flex, Smith & Nephew, Canada), which contains AgNPs (Silcryst nanocrystalline silver), with water for 20 h. TEM images and size distribution of AgNPs except for AgNP-1 are shown in SI Figures S1 and S2.

The absorbance spectra of AgNPs were recorded with a multimode microplate spectrophotometer (Varioscan Flash, Thermo). Transmission electron microscope (TEM) was carried out with H-7500 (Hitachi, Japan) at 80 kV, while field emission scanning electron microscopy (FESEM) was performed with Su-8020 (Hitachi, Japan) coupled with energy dispersive spectrometer (EDS, Oxford, UK) at 15 kV. TEM samples were prepared by loading 5 μL aliquots of the aqueous sample onto carbon-coated grid sample holders. The size distribution of the nanoparticles was estimated using Image-Pro plus software and Gaussian fitting. The particles counted from multipicture were over 200 for AgNP-2, AgNP-3, and AgNP-5, and over 100 for AgNP-4. For FESEM observation, the hollow fiber after extraction was treated with liquid nitrogen and fractured immediately. All the AgNPs stock dispersions were kept in the dark and quantified by ICP-MS. The free Ag⁺ in the samples was determined by ISE as described in our previous study.²⁴

Water Sample Collection. The lake water was collected from Peking University, and the river water was collected upstream of Yongding River in the northwest of Beijing (China). Municipal sewage effluent was collected at Qinghe wastewater treatment plant (WWTP) in the west part of Beijing. All the water samples were filtered with 0.22 μm membrane before analysis.

HFSLM Extraction and ICP-MS Determination of Ag(I). For HFSLM extraction, 5% (m/v) TOPO dissolved in *n*-undecane was used as liquid membrane, whereas 5 mM NaH₂PO₄–Na₂HPO₄ buffer (pH 7.5) containing 10 mM Na₂S₂O₃ and 1 mM Cu(NO₃)₂ as acceptor. Hollow fiber membranes were manually cut into pieces of 30 and 60 cm, and looped for preparing the extraction device for the negligible depletion extraction and exhaustive extraction mode, respectively, with procedures modified from our previous study.^{49,51} Briefly, into the lumen of the hollow fiber was filled with the acceptor by using a syringe, and then the hollow fiber membrane was dipped into the 5% (m/v) TOPO in *n*-undecane for a 2 min to form the liquid membrane in the micropores of the membrane wall. The two ends of the hollow fiber membrane were folded and sealed with a strip of aluminum foil. After washing away the surplus membrane liquid on the surface of the fiber with water, the HFSLM devices were ready for extraction.

The labile Ag(I) was extracted by immersing the 30 cm HFSLM device into 50 mL of sample solution placed in a 50 mL flask and keep static for 8 h, while the total Ag(I) was extracted by immersing the 60 cm HFSLM device into the 1.5 mL of sample solution placed in a 2.0 mL vial, which was then shaken at 250 rpm for 8 h. After that, the acceptor phase in the hollow fiber was flushed into a clean sample vial, and diluted to 0.5 to 2.0 mL based on the concentration with 2% (v/v) HNO₃ for ICP-MS analysis. Based on the weight of the collected acceptor, the volume ratio of sample to acceptor (V_s/V_a) was (50 mL: 0.017 mL) and (1.5 mL: 0.032 mL) in the extraction of labile Ag(I) and total Ag(I), respectively. Certified reference material (1000 mg/L Ag, GBW08610, National Institute of

Metrology, China) was diluted with 3% (v/v) HNO₃ for preparing calibration solutions.

The ICP-MS determination was mainly conducted by using the 7500ce ICP-MS (Agilent Technologies, Palo Alto, CA) equipped with a glass nebulizer at a flow rate of 0.4 mL/min. However, a 7700x ICP-MS (Agilent) equipped with a PFA nebulizer with a flow rate of 0.1 mL/min when high sensitivity was required.

RESULTS AND DISCUSSION

Mechanisms of Extracting Labile and Total Dissolved Ag(I) by HFSLM. In this study, TOPO was adopted as carrier because it is stable and commonly used as carrier in SLM, while a mixture of Cu(NO₃)₂ and Na₂S₂O₃ was employed as acceptor as the complex constant of S₂O₃²⁻ with Cu²⁺ is lower than that with Ag⁺ but higher than that with other metal complexes, and therefore provides high extraction selectivity for Ag(I). Figure 1

Figure 1. Mechanism diagram of HFSLM extraction of Ag(I).

shows the principle for HFSLM extraction of dissolved Ag(I), including Ag⁺ and its various complexes. Briefly, the transport of dissolved Ag(I) in HFSLM occurs by (i) Ag⁺ and anion X⁻, which can be OH⁻, Cl⁻ or coating agents of AgNPs that are able to associate with Ag⁺ to form complexes with lower complex constants than that of [Ag₂S₂O₃], in the sample solution diffuses to the donor-membrane interface and associates with TOPO to form complex [AgX·mTOPO] through solvating reaction; (ii) [AgX·mTOPO] then diffuses through the membrane toward the membrane-acceptor interface where exchange reaction with CuS₂O₃ occurs to form new complexes [Ag₂S₂O₃]; (iii) [Ag₂S₂O₃] is trapped in the acceptor due to its higher complex constant than AgX, and TOPO diffuses back to the donor-membrane interface for repeating the processes as depicted by eqs 1 and 2,⁴⁸

in which (aq) represents the aqueous phase, and (org) represents the organic liquid membrane phase. This mechanism was verified by the molar ratio of Ag in the acceptor to Cu in donor phase at extraction equilibrium (8 h), which was determined to be 1.8:1 (agreed with the theoretical value of 2:1) by using ICP-MS.

Two extraction modes of HFSLM,⁵² the negligible depletion extraction⁴⁹ and the exhaustive extraction,⁵¹ were coupled to ICP-MS for analyzing the labile and the total Ag(I) content, respectively. In the negligible depletion HFSLM, in order to avoid disturbing the equilibrium between the labile Ag(I) and

other forms of dissolved Ag(I) such as nanoparticle-adsorbed Ag(I), the extracted Ag(I) was controlled to below 5% of the total Ag(I) by using large phase volume ratio of donor to acceptor and low enrichment factor of Ag(I) in the acceptor. For sensing the total Ag(I), the exhaustive extraction mode of HFSLM was conducted by using low volume ratio of donor to acceptor and long extraction time, which helps the complete transportation of Ag(I) from donor into the acceptor. The enrichment was calculated by ratio of Ag(I) concentration in the acceptor after extraction to initial Ag(I) concentration in the sample.

Optimization of the Extraction Conditions. Parameters that usually influence the extraction efficiency were optimized with 20 μg/L Ag(I) (AgNO₃) standard solutions, and the enrichment factor of Ag(I) (the ratio of final Ag(I) concentration in the acceptor to the initial Ag(I) concentration in the sample) was used to evaluate the extraction efficiency.

Membrane Liquid. Although both dihexyl ether and *n*-undecane were widely used as organic membrane liquid, *n*-undecane was adopted in this study as dihexyl ether might interact with AgNPs through the Ag–O interaction. While bis(2-ethylhexyl) phosphate (DEPHA) and TOPO are usually used as carrier in SLM of metal ions, our previous success in SLM of Hg(II) with TOPO⁴⁸ encouraged us to use TOPO in this study. Experiments showed that the enrichment factor of Ag(I) increased sharply with TOPO concentration in the range of 1–4% (m/v) and then increased very slowly (SI Figure S3), thus 5% (m/v) TOPO in *n*-undecane was used as the optimum liquid membrane.

Acceptor Solution. The enrichment factor of Ag(I) increased with Na₂S₂O₃ concentration up to 10 mM and then decreased with the further increase of Na₂S₂O₃ concentration (SI Figure S4). The reduced enrichment factor of Ag(I) at high Na₂S₂O₃ concentration might be ascribed to the salt out effect, which reduced the partitioning of Ag₂S₂O₃ from the liquid membrane phase to the acceptor phase. The Cu²⁺ concentration in the acceptor was optimized from 0.02 to 2 mM, and result showed that the enrichment factor of Ag(I) increased with Cu²⁺ concentration up to 0.2 mM and then remained constant until 2 mM (SI Figure S5). The acceptor pH was optimized in the range of 5.0–9.0 with 5 mM phosphate buffer, and it was found that the maximum enrichment factor was obtained at pH 7.5 (SI Figure S6). Consequently, 10 mM Na₂S₂O₃ and 1 mM Cu(NO₃)₂ prepared in 5 mM Na₂HPO₄–NaH₂PO₄ buffer (pH 7.5) was adopted as acceptor.

Shaking Rate. Agitation such as shaking improves the diffusion rate and thus the extraction efficiency in cases that diffusion of analytes through the aqueous diffusion layer is the rate limiting process of SLM, but has no effect in cases that the diffusion of metal-carrier complex across the membrane is the rate-limiting step.⁵³ In the extraction of labile Ag(I), enhancement of the extraction was not observed by increasing the shaking rate from 50 to 200 rpm (SI Figure S7), thus diffusion through the membrane is the rate limiting process and static extraction was adopted. While for the exhaustive HFSLM extraction of total Ag(I), the effect of shaking rate was evaluated in the range of 0–250 rpm. Results (SI Figure S8) showed that the 250 rpm provided the highest extraction efficiency and was adopted in this study. With the progress of exhaustive extraction, depletion of Ag(I) in the aqueous phase occurs and diffusion of Ag(I) through the aqueous diffusion layer becomes the rate limiting step. Therefore, high shaking rate can enhance the extraction efficiency.

Figure 2. Effects of time on the extraction of labile Ag(I) (A) and total Ag(I) (B) in AgNO₃ standard solution (20 µg/L Ag) or AgNP-1 dispersion (1 mg/L Ag). The acceptor solution contains 10 mM Na₂S₂O₃, 1 mM Cu²⁺, and 5 mM Na₂HPO₄–NaH₂PO₄ buffer (pH 7.5); the liquid membrane is 5% (m/v) TOPO in *n*-undecane. The sample volume and length of HFSLM device are 50 mL and 30 cm for extraction of labile Ag(I), and 1.5 mL and 60 cm in extraction of total Ag(I), respectively. Each point represents the mean values $\pm$ standard deviation for three replicates. Note the extracted Ag amount instead of enrichment factor was used as Y-axis, as the enrichment factor cannot be calculated for the lack of exact concentration of labile and total Ag(I) concentration in the AgNP dispersions.

Extraction Time. Preliminary studies (3 h extraction) indicated that, for samples in the studied range of pH 6.0–8.0, the lowest extraction efficiency was observed at pH 6.0–7.0. Therefore, Ag(I) standard solutions (~20 µg/L) and AgNP dispersions (1000 µg/L AgNPs) with the same pH (6.5) and comparable Ag(I) concentration were used to test the equilibration time under the above optimized conditions. Figure 2 shows the profiles of the uptake of Ag(I) in the acceptor which indicate that for both the labile and total Ag(I), the extraction reached equilibration in 8 h. Although the highest enrichment factor and thus method sensitivity can be obtained by using equilibrium extraction, the relatively long equilibration time (8 h) might give rise to artifacts due to the release of Ag⁺ from Ag nanoparticles during the extraction process. While kinetic extraction with short extraction time (e.g., 15 min) can avoid the artifacts, it provides low enrichment factor and thus high detection limit. More importantly, strict control of extraction conditions is required in kinetic extraction, which is more complicated to handle than equilibrium extraction.⁴⁹ Experiments showed that for the samples in this study, the release of Ag⁺ from Ag nanoparticles during the extraction is limited, thus equilibrium extraction (8 h) was employed as optimum in the following studies to obtain high enrichment factor. For samples that extended extraction might vary the Ag⁺ contents, kinetic extraction with strictly controlled extraction conditions is recommended.

Sample pH. Under the optimum extraction time, the effect of sample pH on the enrichment factor of labile Ag(I) in a 20 µg/L Ag(I) solution was tested by adjusting the sample pH with 5 mM Na₂HPO₄–NaH₂PO₄ buffer. As shown in Figure 3, the enrichment factor kept constant in the entire studied range of pH 5.5–9.0, suggesting that this proposed method extracts labile Ag(I) rather than only free Ag⁺. As free Ag⁺ is expected to varied with sample pH values, the enrichment factor should vary with pH variation if only free Ag⁺ is extracted. The result also indicates that in determination of labile Ag(I) by this proposed method, samples with pH 5.5–9.0 can be calibrated with a common calibration curve prepared with standard solutions at pH 6.5.

Method Selectivity Toward Ag(I). In this study, the method selectivity to Ag(I) relies on its selective extraction of Ag(I) from AgNP dispersions, that is, the AgNPs should not be extracted. To test if the AgNPs were transported through the liquid membrane and trapped into the acceptor, 20 mg/L AgNP-1 and 10 mg/L AgNP-5 dispersions were extracted,

Figure 3. Effect of sample pH on the extraction of Ag(I). The 50 mL of AgNO₃ solutions (20 µg/L Ag) was extracted with 10 mM Na₂S₂O₃ and 1 mM Cu²⁺ filled in the 30 cm HFSLM device for 8 h. The liquid membrane is 5% (m/v) TOPO in *n*-undecane. Each point represents the mean values $\pm$ standard deviation for three replicates.

respectively, under the above optimized conditions for total Ag(I). Figure 4E shows the respective UV–vis spectra of the sample before and after extraction, and the acceptor after extraction. The absence of the characteristic maximum peak absorbance of AgNPs (~410 nm) in the acceptor indicates that the AgNPs were not extracted into the acceptor. Although the hollow fiber membrane has micropores of 100 nm, the AgNPs are not able to transfer through the membrane due to the fact that the membrane pores are filled with membrane liquid into which the AgNPs are unable to distributed. The slight reduction of peak absorbance (412 nm) of the sample after extraction (Figure 4E) suggests that a small part of the AgNPs were adsorbed on the surface of the container or the hollow fiber membrane. To verify these results, we detected the acceptor solution with TEM, and the cross section and the surface (both inner and outer) of the hollow fiber wall with FESEM-EDS after extraction (Figure 4A–D). The absence of nanoparticles in the TEM image of the acceptor (data not shown) again verified that AgNPs were not extracted. Although Ag signal was observed in the EDS of the outer wall of the hollow fiber, the absence of Ag signal in the EDS of the inner wall and the cross section of the hollow fiber indicates that the AgNPs cannot diffuse into the membrane (Figure 4D). The presence of intense signal of Na, P, and S in EDS stemmed from TOPO, Na₂S₂O₃ and phosphate buffer. In order to further verify that small sized (<10 nm) AgNPs were not extracted into acceptor phase, the AgNPs and Ag(I) in the sample solutions (9.12 nm AgNP-5 and ~1 nm AgNP-6, 10 mg/L) before

Figure 4. Evidence for excluding the coextraction of AgNPs in proposed HFSLM procedures. The extraction was conducted under the optimized conditions for extracting the total Ag(I) in 20 mg/L and 10 mg/L of AgNPs. (E) The UV-vis spectra of sample (20 mg/L AgNP standard) before and after extraction, and the acceptor after extraction; (A, B, C) FESEM images of the outer wall (A), inner wall (B) and cross section (C) and their corresponding EDS spectra (D) of hollow fiber after extraction. (F) Chromatograms of Ag(I) standard, acceptor blank, and the sample phase before extraction and the acceptor phase after extraction of 10 mg/L AgNP-5 (9.12 ± 5.4) and AgNP-6 (~ 1 nm). The chromatographic separation was performed with an amino column of 500 Å poresize, and a 0.7 mL/min mobile phase containing 0.1% (v/v) FL-70 and 2 mM $\text{Na}_2\text{S}_2\text{O}_3$.

extraction and the acceptor after extraction were determined, respectively, with the HPLC-ICPMS method reported in our previous work.⁴⁰ This method is very sensitive and can detect AgNPs at sub $\mu\text{g/L}$ level. Results shown in Figure 4F indicated that while the AgNP peaks were detected in the sample solutions, no AgNP peaks were detected in the acceptor after extraction, demonstrating small AgNPs even silver clusters were not extracted. Combining all the above results, it is safe to say that AgNPs were not extracted into the acceptor, and silver content in the acceptor determined by ICP-MS stems from the Ag(I) only.

Analytical Performance. Table 1 shows the analytical performance including linear range, calibration curve, correla-

Table 1. Analytical Performance of the Proposed Methods

	labile Ag(I)	total Ag(I)
linear range ($\mu\text{g/L}$)	0.3–100	1.5–100
calibration equation	$y = 80.54x + 92.71$	$y = 0.9130x + 0.2779$
R^2	0.996	0.999
RSD (% , $n = 6$)	12	2.4
LOD ($\mu\text{g/L}$)	0.1	0.5
extraction efficiency (%)	2.97 ± 0.25	82.3 ± 2.0
enrichment factor	93.5 ± 11.4	26.4 ± 0.9

tion coefficient (r^2), relative standard deviation (RSD), enrichment factor, limit of detection (LOD) and limit of quantification (LOQ). The linearity was tested by extracting 7 AgNO_3 standard solutions containing 0, 2, 5, 10, 20, 50, and 100 $\mu\text{g/L}$ Ag. The concentration of Ag ($\mu\text{g/L}$) in donor phase was used as x axis, while the Ag(I) concentration ($\mu\text{g/L}$) and total Ag mass (ng) in acceptor phase were used as y axis for preparation of calibration curve of labile Ag(I) and total Ag(I), respectively. The extraction efficiency and reproducibility were determined by extracting 6 AgNO_3 standard solutions containing 20 $\mu\text{g/L}$ Ag. The extraction efficiency of labile Ag(I) (2.97%) was below 5%, indicating that it was a negligible depletion extraction and did not disturb the equilibrium

between the various silver species in the sample.⁴⁹ LOD and LOQ were calculated based on three times of baseline noise ($S/N = 3$) and ten times of baseline noise ($S/N = 10$) for the determination of labile and total Ag(I), respectively. Thanks to the enrichment in the HFSLM procedure and the high sensitivity of ICP-MS, this proposed method shows low detection limits for labile and total Ag(I). It is noteworthy the method LOD and LOQ were strongly dependent on the instrument used. The data shown in Table 1 were obtained by using the 7500ce ICP-MS equipped with a glass nebulizer with a flow rate of 0.4 mL/min. However, by using the 7700x ICP-MS equipped with a PFA nebulizer with a flow rate of 0.1 mL/min, the LOD for labile and total Ag(I) were decreased to 0.05 $\mu\text{g/L}$ and 0.2 $\mu\text{g/L}$, respectively.

Sample Analysis. The labile and total Ag(I) in five AgNP dispersions were determined with results shown in Table 2. The total Ag(I) determination was verified by spiked recovery, which was in the range of 74.0–98.1% at spiking levels of 10 and 20 $\mu\text{g/L}$, respectively (Table 2). The proposed method for labile Ag(I) determination cannot be verified by spiked recovery, as the species of Ag(I) might be modified after spiking. However, for all the samples, the fact that the values of labile Ag(I) located between the determined total Ag(I) and the free Ag^+ in sample dispersions determined by ISE indicates that the proposed method is reliable.

To further evaluate the applicability of the proposed method to real environmental waters, the labile and total Ag(I) were determined before and after adding AgNP-5 with the same concentration to lake water, river water and effluent of WWTP, respectively. Results shown in Table 3 indicated that the apparent extraction efficiency of total Ag(I) was between 74.2% and 93.1% for the three environmental water samples, demonstrating the common ions and DOC in environmental waters showed no effect on the extraction of total Ag(I) by the proposed HFSLM method.

In summary, the labile and total Ag(I) in AgNP dispersions and real environmental waters were determined by coupling

Table 2. Labile Ag(I), Total Ag(I), Free Ag⁺ and Spiked Recoveries of Total Ag(I) (Mean $\pm$ s, n = 3) in AgNP Dispersions Determined by the Proposed HFSLM and ISE Method

sample	size	coating	free Ag ⁺ (μ g/L)	labile Ag(I) (μ g/L)	total Ag(I)		
					spiked (μ g/L)	detected (μ g/L)	recovery ^g (%)
AgNP-1 ^a	37.5 $\pm$ 6.0	PVP	18	26.6 $\pm$ 1.4	0	36.2 $\pm$ 2.4	
					10	46.0 $\pm$ 2.5	98.0 $\pm$ 10.9
					20	54.8 $\pm$ 2.8	93.2 $\pm$ 7.5
AgNP-2 ^b	16.2 $\pm$ 5.4	PVP	25	38.8 $\pm$ 4.0	0	57.2 $\pm$ 1.0	
AgNP-3 ^c	48.9 $\pm$ 12.1	PVP	20	31.4 $\pm$ 1.0	0	47.8 $\pm$ 8.2	
AgNP-4 ^d	22.2 $\pm$ 5.9	unknown	17	16.2 $\pm$ 0.4	0	23.2 $\pm$ 2.4	
					10	33.0 $\pm$ 1.0	98.1 $\pm$ 6.1
					20	40.3 $\pm$ 2.2	85.3 $\pm$ 8.5
AgNP-5 ^e	9.12 $\pm$ 2.22	PVP		12.3 $\pm$ 0.8	0	15.9 $\pm$ 0.6	

^aTotal silver concentration of $\sim$ 1.0 mg/L. ^{b,c}Before analysis, the samples were diluted 250 times to have a total silver concentration of $\sim$ 0.5 mg/L. ^d0.0960 g of wound dressing was soaked with water 20 h and diluted 500 times to have a total silver concentration of $\sim$ 30.4 μ g/L. ^eTotal silver concentration of 30 μ g/L. ^fDetermined by ISE as described in literature²⁴. ^gThe recovery was defined as $100 \cdot (C_d - C_0) / C_s$, in which C_d is the concentration determined in donor phase after spiking, C_0 is the initial concentration in donor phase, C_s is the spiked concentration.

Table 3. Labile and Total Ag(I) (Mean $\pm$ s, n = 3) in Environmental Waters Determined by the Proposed HFSLM Method

sample	pH	DOC ^a (mg/L)	spiked AgNP-5 (μ g/L) ^b	detected labile Ag(I) (μ g/L)	detected total Ag(I) (μ g/L)	extraction efficiency of total Ag (I) ^c (%)
Lake water	8.5	13.6	0	ND ^d	ND	
			30	6.01 $\pm$ 0.05	14.8 $\pm$ 0.6	93.1
River water	8.3	37.8	0	ND	ND	
			30	6.92 $\pm$ 0.33	11.8 $\pm$ 0.7	74.2
Effluent of WWTP	8.1	6.67	0	ND	ND	
			30	5.66 $\pm$ 0.26	13.8 $\pm$ 1.2	86.8

^aDissolved organic carbon. ^bAs total silver. ^cCalculated based on the difference between the determined total Ag(I) in real water sample (C_{rw}) and in pure water (C_{pw}) at the same spiked level of AgNP-5, by $100 \cdot (C_{pw} - C_{pw}) / C_{pw}$. ^dBelow the detection limit.

HFSLM with ICP-MS for the first time. While AgNPs were completely excluded by the liquid membrane, the Ag(I) in the sample were extracted into the liquid membrane with the help of TOPO carrier, and trapped in the acceptor through the exchange reaction with CuS₂O₃. The labile Ag(I) was sensed by using the negligible depletion extraction with large volume ratio of sample to acceptor, whereas the total Ag(I) was determined through exhaustive extraction with very small volume ratio of sample to acceptor. Given labile metal ions are more relevant to bioavailability and toxicity in comparison to total metal ions, this proposed method is expected to contribute in evaluating the bioavailability and toxicity of metal/metal oxide nanoparticles in aqueous systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b02917.

Additional information as noted in the text (PDF)

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Notes

The authors declare no competing financial interest.

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