Rapid Chromatographic Separation of Dissoluble Ag(I) and Silver-Containing Nanoparticles of 1–100 Nanometer in Antibacterial Products and Environmental Waters

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S Supporting Information

ABSTRACT: Sensitive and rapid methods for speciation analysis of nanoparticulate Ag (NAg) and Ag(I) in complex matrices are urgently needed for understanding the environmental effects and biological toxicity of silver nanoparticles (AgNPs). Herein we report the development of a universal liquid chromatography (LC) method for rapid and high resolution separation of dissoluble Ag(I) from nanoparticles covering the entire range of 1–100 nm in 5 min. By using a 500 Å poresize amino column, and an aqueous mobile phase containing 0.1% (v/v) FL-70 (a surfactant) and 2 mM Na₂S₂O₃ at a flow rate of 0.7 mL/min, all the nanoparticles of various species such as Ag and Ag₂S were eluted in one fraction, while dissoluble Ag(I) was eluted as a baseline separated peak. The dissoluble Ag(I) was quantified by the online coupled ICP-MS with a detection limit of 0.019 µg/L. The NAg was quantified by subtracting the dissoluble Ag(I) from the total Ag content, which was determined by ICP-MS after digestion of the sample without LC separation. While the addition of FL-70 and Na₂S₂O₃ into the mobile phase is essential to elute NAg and Ag(I) from the column, the use of 500 Å poresize column is the key to baseline separation of Ag(I) from ~1 nm AgNPs. The feasibility of the proposed method was demonstrated in speciation analysis of dissoluble Ag(I) and NAg in antibacterial products and environmental waters, with very good chromatographic repeatability (relative standard deviations) in both peak area (<2%) and retention time (<0.6%), excellent spiked recoveries in the range of 84.7–102.7% for Ag(I) and 81.3–106.3% for NAg. Our work offers a novel approach to rapid and baseline separation of dissoluble metal ions from their nanoparticulate counterparts covering the whole range of 1–100 nm.

INTRODUCTION

Due to their excellent antibacterial activity, silver nanoparticles (AgNPs) are used in a wide spectrum of consumer products including fabrics, sporting goods, personal care products, food supplements, and paints.^{1–6} The mass production and widespread use of AgNP-containing products lead to the unavoidable release of silver into the environment intentionally or accidentally,^{7–9} and therefore a considerably increased potential risk of silver to organisms^{5,10–12} and general population.^{13,14}

Although the environmental effects and biological toxicity of AgNPs have been intensively studied, the mechanisms of the toxicity of AgNPs to organisms are not quite clear yet.^{12–14} This can be partly ascribed to the coexistence of AgNPs and Ag(I) resulted from the medium redox potential of silver ($\varphi^0(\text{Ag}^+/\text{Ag}^0) = 0.80 \text{ V}$),^{15,16} which makes it easy for the dissolution of AgNPs to release Ag(I),^{15–20} and the reduction of released Ag(I) to AgNPs by humic acid and microbiological activities at room temperature.^{21–24} In addition, AgNPs are inclined to occur morphology transformation,¹⁶ and chemical transformation such as sulfuration and chlorination to form nanoparticulate Ag₂S and AgCl, respectively.¹⁷ Both physical

and chemical transformation would in turn alter their bioavailability and toxicity.²⁰ Furthermore, Ag(I) is present in various species including free Ag⁺, complexes of Ag⁺, AgNP-adsorbed Ag⁺, and undissolved Ag(I) salts like AgCl, which show different bioavailability and complicate the understanding of the toxicology mechanism of AgNPs. To assess the risk of exposure and further understand the mechanisms of the toxicity of AgNPs to organisms, rapid, high resolution and cost-effective methods for accurate speciation analysis of Ag(I) and nanoparticulate Ag (NAg) in various matrices are required.

A variety of methods have been developed to determine the nanoparticulate and ionic silver species.²⁵ Ion selective electrode (ISE)^{26,27} is sensitive, selective and inexpensive in direct measurement of free Ag⁺, but it is not suitable for analysis of samples with complex matrices. While single particle ICP-MS was proposed for quantification of AgNPs and dissolved Ag(I), it only allows for the quantification of NPs with sizes

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≥ 20 nm, as small NPs are hardly distinguished from the metal ions.^{28,29} Although filtration,³⁰ centrifugation,⁷ centrifugal ultrafiltration,¹¹ and diffusion gradient thin film (DGT)³¹ were off-line coupled with ICP-MS, inductively coupled plasma optical emission spectrometer (ICP-OES) or atomic absorption spectroscopy (AAS) for the analysis of AgNPs and Ag(I), these separation techniques are unable to distinguish the small sized AgNP clusters from Ag(I). While this limitation can be overcome by separation with dialysis,¹⁸ cloud point extraction (CPE),^{32–37} and ion-exchange resin method,^{35,38} these techniques required a relatively long separation time that is between 40 min and 45 h. To overcome this restriction, ICP-MS was online coupled with asymmetric flow field-flow fractionation^{39–41} for selective determination of AgNPs in the presence of Ag(I), and with liquid chromatography (LC)⁴² or capillary electrophoresis (CE)⁴³ for speciation test of AgNPs and Ag(I). Although the authors of the latter two methods^{42,43} did not define the specific species of the determined Ag(I), it can be defined as dissolvable Ag(I). This is because strong complex agent $\text{Na}_2\text{S}_2\text{O}_3$ or penicillamine were added into the systems, and part of the originally undissolved Ag(I) such as AgCl and AgNP-adsorbed Ag^+ were dissolved as complexes of $\text{S}_2\text{O}_3^{2-}$ or penicillamine. These two LC and CE methods are very successful in separation of >7 nm AgNPs⁴² and 6 nm AuNPs⁴³ from their dissolvable ionic counterparts, respectively, but it remains a great challenge to discriminate small sized AgNPs (e.g., <5 nm) from Ag(I). Moreover, their applicability in analysis of real samples with complex matrices has not been verified yet.

The objective of this study is to develop a rapid and sensitive method for speciation analysis of dissolvable Ag(I) and NAg in complex matrices, and therefore to offer a robust analytical method for studying the environmental behavior and toxicology of AgNPs. The dissolvable Ag(I) was defined as Ag(I) species including the free Ag^+ , soluble complexes of Ag^+ , as well as AgNP-adsorbed Ag^+ and originally undissolved Ag(I) salts that can be dissolved in the presence of $\text{Na}_2\text{S}_2\text{O}_3$. We at first established a novel LC separation procedure in which the dissolvable Ag(I) was baseline separated from all the NPs (1–100 nm) following a size-exclusion mechanism. The dissolvable Ag(I) was quantified by the online coupled ICP-MS, while the NAg was obtained by subtracting the dissolvable Ag(I) from the total Ag content, which was determined by ICP-MS after digestion of the sample without LC separation. Then this developed method was applied to determine NAg and dissolvable Ag(I) in antibacterial products and environmental waters.

MATERIALS AND METHODS

Chemical and Materials. Aqueous dispersions of citrate-stabilized AgNPs with nominal particle sizes of 10, 20, 40, 60, and 100 nm were purchased from Sigma-Aldrich (St. Louis, MO) and used as obtained. Their hydrodynamic diameter (nm) and polydispersity index (PDI, shown in the parentheses) measured by dynamic light scattering (DLS) were 12.4 (0.299), 25.2 (0.122), 46.4 (0.221), 68.0 (0.196), and 102.2 (0.076), respectively. Another commercial AgNP dispersion with unknown coating (18.7 ± 4.4 nm) was purchased from Shanghai Huzheng Nanotechnology Co., Ltd. (Shanghai, China). Gold NPs (AuNPs, 3–5 nm) were obtained from Jieyi Biotechnology Co., Ltd. (Shanghai, China). FL-70, a mixture of nonionic and anionic surfactants, was purchased from Fisher Scientific (Fair Lawn, NJ). Triton X-114 (TX-114)

was obtained from Acros Organics (Geel, Belgium). Brij-010 was purchased from Sigma-Aldrich (St. Louis, MO). Ag^+ standard used for ICP-MS (1000 mg/L) determination was from National Institute of Metrology (Beijing, China). Sodium dodecyl sulfate (SDS, 98.5%) and sodium thiosulfate of guaranteed reagent grade were obtained from Sinopharm Chemical Reagent Co. (Beijing, China). High purity nitric acid (65%) was obtained from Merck (Darmstadt, Germany). The other reagents were purchased from Beijing Chemicals (Beijing, China). All the reagents were used as obtained without further purification. Ultrapure water (18.3 M Ω) produced with a Milli-Q Gradient system (Millipore, Billerica, MA) was used throughout the experiments. Both of the 500 and 1000 Å poresize amino column (Venusil Durashell-NH₂, 5 μm particle size, 250×4.6 mm) were purchased from Bonna-Agela Technologies Inc. (Tianjin, China).

Glutathione (GSH)-protected Ag nanoclusters (~ 1 nm), PVP-capped AgNPs (30.9 ± 4.9 nm), PVA-capped AgNPs (46.7 ± 9.0 nm), and Ag_2S NPs (4 and 18 nm) were prepared according to literature. Details of their synthesis and characterization were described in the SI.

LC-ICP-MS Analysis System. The Agilent 1200 series LC system (Agilent Technologies, Palo Alto, CA) was coupled to the ICP-MS instrument (Agilent 7700cs) by directly connecting the column outlet to the cross-flow nebulizer of ICP-MS through a commercial polytetrafluoroethylene (PTFE) connector. All the interfaces and injection loop were of polyetheretherketone (PEEK). LC separation of samples (20 μL) was conducted with an amino column (Venusil Durashell-NH₂, 5 μm particle size, 500 Å pore size, 250×4.6 mm) and a mobile phase of 0.1% (v/v) FL-70 and 2 mM $\text{Na}_2\text{S}_2\text{O}_3$ at a flow rate of 0.7 mL/min. At this flow rate, the relevant parameters of ICP-MS were daily optimized by introducing a multielement tuning solution. Typical experimental parameters of the LC-ICP-MS setup are summarized in Table 1.

Table 1. Instrumental Parameters of LC-ICP-MS System

ICP-MS parameters	
Instrument	Agilent 7700 ICP-MS
RF power	1500 W
sampling depth	8 mm
carrier gas	1.0 L/min
integration time	0.5 s
monitored isotope (m/z)	$^{107}\text{Ag}^+$, $^{109}\text{Ag}^+$, $^{197}\text{Au}^+$
LC parameters	
column	Venusil Durashell-NH ₂ , 500 Å poresize, 250×4.6 mm
column temperature	25 °C
flow rate	0.7 mL/min
injection volume	20 μL
mobile phase	0.1% FL-70, 2 mM sodium thiosulfate

Quantification of Dissolvable Ag(I) by LC-ICP-MS. Ag(I) in samples was separated from AgNPs and quantified by the online coupled LC-ICP-MS system. The calibration curve was prepared by plotting the peak area against the concentration of a series of standards of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 $\mu\text{g/L}$ Ag^+ prepared in 2 mM $\text{Na}_2\text{S}_2\text{O}_3$. Just before injection into the LC-ICP-MS system, into the environmental water and antibacterial product samples were added 2 mM $\text{Na}_2\text{S}_2\text{O}_3$. Each standard and sample solution was injected in triplicate into the LC-ICP-MS system, and the mean peak area of Ag(I) was adopted to calculate the concentration of dissolvable Ag(I). The

Table 2. Concentrations of Dissoluble Ag(I), Total Ag, NAg and the Spiked Recovery of Dissoluble Ag(I) determined by the Proposed LC-ICP-MS Method, and the Concentration of NAg Determined by the CPE-ICP-MS Method in the Antibacterial Products (mean $\pm$ s, $n = 3$)

sample	function and appearance	spiked NAg ($\mu\text{g/g}$)	spiked Ag(I) ($\mu\text{g/g}$)	detected Ag(I) ($\mu\text{g/g}$)	recovery of Ag(I) (%)	total Ag ($\mu\text{g/g}$)	calculated NAg ($\mu\text{g/g}$)	detected NAg by CPE ($\mu\text{g/g}$)
S1	female antibacterial hydrogel lotion, snuff liquid	0	0	34.3 $\pm$ 0.5		104 $\pm$ 1	69.7 $\pm$ 1.1	59.8 $\pm$ 0.7
		0	102	136 $\pm$ 1	99.7 $\pm$ 1.1			
		0	1012	1065 $\pm$ 5	101.8 $\pm$ 0.5			
		113.4 ^a	11.1 ^a	45.7 $\pm$ 0.4	102.7 $\pm$ 5.4			
S2	antibacterial nasal spray, yellow liquid	0	0	1.16 $\pm$ 0.05		18.0 $\pm$ 1.0	16.8 $\pm$ 1.0	12.3 $\pm$ 0.3
		0	27.5	29.0 $\pm$ 0.1	101.2 $\pm$ 0.4			
		0	240	236 $\pm$ 4	97.8 $\pm$ 1.7			
		30.8 ^a	3.03 ^a	4.13 $\pm$ 0.02	98.0 $\pm$ 1.6			
S3	antibacterial hydrogel, snuff sticky slime	0	0	3.09 $\pm$ 0.11		55.0 $\pm$ 2.0	51.9 $\pm$ 2.0	50.8 $\pm$ 2.8
		0	60.3	64.1 $\pm$ 0.3	101.1 $\pm$ 0.5			
		0	534	546 $\pm$ 6	101.7 $\pm$ 1.1			
		66.4 ^a	6.5 ^a	9.56 $\pm$ 0.08	99.5 $\pm$ 2.2			
S4	acne serum, snuff liquid	0	0	9.3 $\pm$ 0.2		34.5 $\pm$ 0.3	25.2 $\pm$ 0.4	22.2 $\pm$ 0.5
		0	35.2	44.3 $\pm$ 0.6	99.4 $\pm$ 1.7			
		0	352	358 $\pm$ 2	99.1 $\pm$ 0.6			
		39.4 ^a	3.87 ^a	11.3 $\pm$ 0.1	ND ^b			
S5	female antibacterial hydrogel lotion, sticky gel	0	0	1355 $\pm$ 14		1446 $\pm$ 36	91.0 $\pm$ 38.6	267 $\pm$ 12
		0	1253	2568 $\pm$ 44	96.8 $\pm$ 3.7			
		471.5 ^a	46.2 ^a	1348 $\pm$ 25	ND ^b			
		2357 ^a	231 ^a	1574 $\pm$ 1.3	94.8 $\pm$ 6.1			

^aSpiked with a commercial AgNPs containing 8.9% Ag⁺, and the corresponding spiked amounts of NAg and Ag(I) were determined by spiking the same amount of AgNPs into purewater and determined after diluting the sample to containing 20 $\mu\text{g/L}$ of total Ag. ^bThe spiking level is far lower than the initial value.

concentration of NAg was obtained by subtracting the dissoluble Ag(I) from the total Ag content determined as follows.

Digestion and Determination of Total Ag. To determine the total Ag content, antibacterial products were digested using a microwave digestion system (CEM Mars 5 Xpress, Matthews, NC) with a procedure modified from our previous studies.^{32,33} Briefly, into the antibacterial products (0.02–0.2 g) were added 3 mL of concentrated HNO₃ and 2 mL of concentrated H₂O₂. After predigested for at least 2 h, the mixture was microwave digested using a program as follows: ramp to 120 °C (800 W) in 12 min and hold for 10 min, followed ramp to 180 °C (1600 W) within 5 min and hold for 30 min. The total Ag content in the digested solution was determined by ICP-MS after diluted with water to 50–100 mL. As for environmental waters, 9.5 mL of aqueous sample was mixed with 0.5 mL of concentrated HNO₃ and mechanically shaken (250 rpm) for 2 h at room temperature instead of microwave digestion prior to total Ag quantification.⁴⁴ In comparison to the time-consuming and labor-intensive microwave digestion procedure, this method is much simpler and offers similar results for samples with simple matrix.

Cloud Point Extraction and ICP-MS Determination of AgNPs. For comparison, the AgNP concentration in the antibacterial products was also determined by CPE modified from our previously reported procedure.^{32,33} Briefly, into a 15 mL polypropylene centrifuge tube was added $\sim$ 8 mL of water sample or appropriate amounts of antibacterial product sample (0.02–0.2 g) dissolved in $\sim$ 8 mL water. The solution was

adjusted to pH 3.0 by dropwise addition of diluted HNO₃, and then 0.1 mL of 1 M Na₂S₂O₃ and 0.2 mL of 10% (w/v) TX-114 were added. The mixture was diluted to 10 mL and incubated in a 40 °C water bath for 30 min, and then centrifuged at 2000 rpm ($\sim$ 640 g) at room temperature for 5 min to enhance the phase separation. The TX-114-rich phase with the extracted AgNPs was collected for quantification with ICP-MS after microwave digestion as described above.

Characterization of AgNPs. The hydrodynamic diameters of citrate-stabilized AgNPs were measured by dynamic light scattering (DLS) with a Malvern Nano ZS (Malvern Instruments, UK). The UV–vis spectra of AgNPs in the antibacterial products was directly recorded in 300–800 nm with a Shimadzu UV-3600 (Kyoto, Japan). Transmission electron microscopy (TEM) and energy dispersive spectrometer (EDS) analysis were performed with an H-7500 (Hitachi, Japan) at 80 kV or a TECANI G20 (FEI, USA) at 200 kV. TEM samples were prepared by placing 5 μL aliquots of the AgNP aqueous sample or antibacterial product dilutions with 1:1 water and methanol onto a carbon-coated grid and drying at room temperature under vacuum.

Sample Collection. Five commercial AgNP-containing antibacterial products were obtained from local drugstores, and details of them are listed in Table 2. Lake water was collected from a lake located in Peking University (Beijing, China). Municipal sewage influent and effluent were taken from Qinghe wastewater treatment plant (WWTP) in the northwest part of Beijing (China). All samples were collected in the glass bottles, which were rinsed with the sample for three times in

Figure 1. Chromatograms of AgNPs and dissoluble Ag(I) at different separation conditions. (A) 10 nm AgNPs (20 $\mu\text{g/L}$); amino column, 500 Å poresizes; mobile phase, different concentrations of FL-70 without $\text{Na}_2\text{S}_2\text{O}_3$; (B) 10 nm AgNPs (200 $\mu\text{g/L}$); amino column, 500 Å poresizes; mobile phase, mixture of 0.1% (v/v) FL-70 and different concentrations of $\text{Na}_2\text{S}_2\text{O}_3$; (C) amino column, 500 Å poresizes; mobile phase, 0.1% (v/v) FL-70 and 2 mM $\text{Na}_2\text{S}_2\text{O}_3$; (D) amino column, 1000 Å poresizes; mobile phase, 0.1% (v/v) FL-70 and 2 mM $\text{Na}_2\text{S}_2\text{O}_3$.

advance, and filtered through 0.45 μm membrane syringe filter before use.

RESULTS AND DISCUSSION

Optimization of LC Separation Conditions. While C_8 and C_{18} columns were adopted to separate NPs in previous studies,^{42,45–48} amino column was adopted directly in this work as it was expected that the association of amino group with Ag^+ should be helpful for the separation of ionic and nanoparticulate Ag.

Surfactants play a key role in decreasing the irreversible adsorption of particles onto the column packing materials in LC separation of NPs.^{42,45–48} In this work, we at first optimized the surfactant species and concentrations in mobile phase for separation of different sized AgNPs and Ag(I), using an amino column with 500 Å pore size. No peak was observed by using mobile phases with various pHs and different concentrations of individual or mixed surfactants of SDS and Brij-010. Although SDS and Brij-010 were reported to efficiently reduce the adsorption of NPs to C_8 and C_{18} columns,⁴⁸ the addition of SDS/Brij-010 into the mobile phase had no improvement in elution of AgNPs and Ag(I) in this work, probably due to the stronger adsorption of AgNPs and Ag(I) to the packing materials with $-\text{NH}_2$ group in comparison to C_8 and C_{18} columns. The addition of surfactant FL-70 into the mobile phase resulted in the detection of AgNPs, but no signal of Ag(I) was observed (see Figure S1 in SI). We further optimized the concentration of FL-70 in the range of 0.01%–0.2% (v/v). Results clearly showed that while both the signal intensity and retention time of AgNPs increased with FL-70 concentration,

the signal of Ag(I) keeps absence (Figure 1A). In the following studies, 0.1% (v/v) FL-70 was adopted as optimum.

To elute Ag(I) from the column, into the 0.1% (v/v) FL-70 mobile phase was added various concentrations of $\text{Na}_2\text{S}_2\text{O}_3$ (0–5 mM), which was reported to efficiently desorb Ag(I) from AgNPs and other matrices in our previous studies.^{32–34} As shown in Figure 1B, with the increase of $\text{Na}_2\text{S}_2\text{O}_3$ concentration, both the Ag(I) signal intensity and the resolution between AgNPs and Ag(I) increased markedly, whereas the signal intensity of AgNPs decreased significantly. In addition, we found that the addition of $\text{Na}_2\text{S}_2\text{O}_3$ into samples just before LC-ICP-MS analysis markedly enhanced the Ag(I) response, and it was demonstrated that the highest recovery of Ag(I) ($87.4 \pm 5.2\%$) was obtained when into both the sample solution and mobile phase was added 2 mM $\text{Na}_2\text{S}_2\text{O}_3$, which was therefore adopted in the following studies.

The flow rate of mobile phase was found to have limited impact on the resolution between AgNPs and Ag(I), thus 0.7 mL/min, the highest permissible flow rate of the concentric nebulizer (0.38–0.76 mL/min) for the ICP-MS analysis, was adopted to shorten the analysis time. With this flow rate, the separation was finished in 5 min, corresponding to a sampling throughput of 12 h^{-1} .

The effect of poresizes of column packing material on the separation of AgNPs and Ag(I) was evaluated by testing the retention time of Ag(I) and different sized AgNPs. Results shown in Figure 1C and D indicate that the column poresizes showed no significant effect on the retention of Ag(I), whereas the retention of AgNPs in 1000 Å column was much more size-dependent than that in the 500 Å column. Consequently, the 500 Å column provided much higher resolution between Ag(I)

Figure 2. Separation of dissolvable Ag(I) from different nanoparticles with amino column (500 Å poresize) and mobile phase containing 0.1% (v/v) FL-70 and 2 mM $\text{Na}_2\text{S}_2\text{O}_3$. (A) PVP-AgNPs (200 $\mu\text{g/L}$), PVA-AgNPs (100 $\mu\text{g/L}$), Ag_2S NPs (50 $\mu\text{g/L}$), AuNPs (10 $\mu\text{g/L}$); (B) mixtures of different combinations of ~ 1 nm (20 $\mu\text{g/L}$), 10 nm (200 $\mu\text{g/L}$), and 100 nm (200 $\mu\text{g/L}$).

and AgNPs covering the range of 1–100 nm, especially for the glutathione (GSH)-protected Ag nanoclusters with a size of ~ 1 nm. Further experiments showed that the 500 Å column also provided baseline separation of Ag(I) from AgNPs with sizes of 20, 40, and 60 nm (SI Figure S2). Therefore, from Figure 1C and SI Figure S2, it is evident that AgNPs with sizes ranged from 1 to 100 nm were baseline separated from the Ag(I) peak by using this 500 Å column. This fulfills the purpose of this study, which is to separate AgNPs as a whole from Ag(I) rather than to fractionize different sized AgNPs. It should be highlighted that this is the first report of evidenced baseline separation of Ag(I) from ~ 1 nm Ag nanoclusters, indicating this developed method is very powerful for rapid and low-cost separation of metal nanoclusters from the counterpart metal ions.

We further verify the applicability of this method in separation of Ag(I) from various NPs, including AgNPs with different capping agents, NPs of different species such as AuNPs and Ag_2S NPs, and mixtures of AgNPs with different sizes. Results showed that PVP-capped AgNPs (~ 30 nm) and PVA-capped AgNPs (~ 45 nm) exhibited almost the same retention time (Figure 2A), which indicated that the capping agents had limited influence on the retention time as the separation was based on the size-exclusion mechanism. In addition, both the AuNPs (~ 3 nm) and Ag_2S NPs (mixture of 4 and 18 nm) showed baseline separation from Ag(I) (Figure 2A), suggesting that NPs other than AgNPs including composite nanomaterials containing Ag could be distinguished from Ag(I) by this procedure. For all the mixtures of different combinations of ~ 1 nm, 10 and 100 nm AgNPs, although the AgNP fraction showed split peaks, the Ag(I) was still baseline separated from the AgNP fraction (Figure 2B). Based on the above results, it is safe to conclude that our method is able to rapidly separate Ag(I) from various NPs ranged from ~ 1 to 100 nm.

Quantification of Ag(I) and NAg. The dissolvable Ag(I) content in samples was quantified directly by the online coupled LC-ICP-MS system. The external calibration curve, prepared by 10 AgNO_3 standard solutions containing 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 $\mu\text{g/L}$ Ag(I) , respectively, showed a good linear correlation coefficient (R^2) 0.9979 in the range of 0.1–5 $\mu\text{g/L}$ and 0.9999 in the entire range of 0.1–100 $\mu\text{g/L}$. The precision of the method, evaluated by the relative standard deviation (RSD) of analyzing 6 AgNO_3 standard solutions containing 10 $\mu\text{g/L}$ Ag(I) , was 3.9% for $^{107}\text{Ag}^+$ peak

area and 0.4% for retention time. The detection limit (LOD) of Ag(I) was 0.019 $\mu\text{g/L}$ ($S/N = 3$), which was about 1 order of magnitude lower than that of the previous reported LC-ICP-MS method (0.12 $\mu\text{g/L}$)⁴² and the CE-ICP-MS method (0.29 $\mu\text{g/L}$).⁴³

In this proposed online coupled LC-ICP-MS system, the NAg can not be quantified directly due to the size-dependent signal response of Ag in LC-ICP-MS determination, which is probably caused by the size-dependent vaporization and diffusion effects of NPs.^{43,49} Therefore, we quantify the NAg by subtracting the Ag(I) from the total Ag content, which includes various species of Ag (e.g., Ag^+ , AgNPs, Ag_2S NPs) and can be determined by ICP-MS after microwave digestion (for antibacterial products)³³ or room temperature HNO_3 digestion (for environmental waters).⁴⁴ Given the LOD of total Ag is lower than that of Ag(I) , the LOD of NAg can be regarded as same as that of Ag(I) (0.019 $\mu\text{g/L}$).

To evaluate the mass loss and sample recovery in this LC-ICP-MS procedure, 200 $\mu\text{g/L}$ each of 10 nm AgNPs, 100 nm AgNPs and Ag(I) were respectively injected into the LC separation system, and the specific specie fractions were offline collected and analyzed by ICP-MS by external calibration. Results revealed that the recovery was 87.4% for Ag(I) , while it decreased from 82.7% for 10 nm AgNPs to 64.5% for 100 nm AgNPs. The high recovery of Ag(I) suggests that its quantification can also be conducted by using a calibration curve prepared by direct ICP-MS determination of AgNO_3 standards without LC separation. Although the AgNP recovery was slightly lower than the Ag(I) , it has no influence on the AgNP quantification, which was performed by subtracting the Ag(I) from the total Ag content determined by ICP-MS after microwave digestion. The low recovery of AgNPs suggests that part of the AgNPs might be retained in the column, and therefore clog the column after a long-term usage. However, our experience showed that the column works well even after analysis of 500 samples, which is possibly due to the low sample concentration (medium $\mu\text{g/L}$ level) and small injection volume (20 μL).

Analysis of Ag(I) and NAg in Antibacterial Products.

Five antibacterial products claimed containing AgNPs were analyzed by the proposed method. Figure 3 shows the chromatograms of three consecutive injections of each sample, indicating the excellent repeatability with relative standard deviations of <2% in the peak area and <0.6% in retention time. The measured dissolvable Ag(I) , total Ag, and NAg contents are

Figure 3. Chromatograms, TEM images, EDS and UV-vis spectra of the antibacterial products. The white scale bar in each TEM image is representative of 200 nm. NPs were observed in four of the five antibacterial products (S1–S4), no NPs were observed in sample S5. Note, chromatograms of three consecutive injections of each sample were shown to indicate the excellent repeatability.

listed in Table 2. For samples S1–S4, the NAg contents were higher than Ag(I), indicating that NAg was the main silver species in these samples. However, no NAg was observed in sample S5. To verify the reliability of the proposed method, the spiked recovery of Ag(I) was tested by spiking AgNO_3 standard solution, and AgNPs with known content of NAg and Ag(I),

respectively. As shown in Table 2, the Ag(I) recoveries were in the range of 94.8–102.7% either by spiking AgNO_3 at levels of ~ 1 –178 times of the initial Ag(I), or by spiking AgNPs at levels of ~ 1.3 –25 times of the initial NAg together with ~ 0.3 –2 times of the initial Ag(I). The excellent recoveries revealed that the sample matrix and spiked AgNPs have no influence on

Table 3. Concentrations of Dissoluble Ag(I), Total Ag, NAg and the Spiked Recovery of Dissoluble Ag(I) and NAg Determined by the Proposed LC-ICP-MS Method

sample	spiked NAg ($\mu\text{g/L}$)	spiked Ag(I) ($\mu\text{g/L}$)	detected total Ag ($\mu\text{g/L}$)	detected Ag(I) ($\mu\text{g/L}$)	recovery of Ag(I) (%)	calculated NAg ($\mu\text{g/L}$)	recovery of NAg (%)
influent of WWTP	0	0	0.065	ND ^b		ND	
	0	1.00	0.83 ± 0.02	0.85 ± 0.05	84.7 ± 5.0	ND	
	18.2 ^a	1.79 ^a	16.4 ± 0.4	1.60 ± 0.10	89.4 ± 5.6	14.8 ± 0.4	81.3 ± 2.2
	91.0 ^a	8.94 ^a	104.8 ± 10.1	8.10 ± 0.18	90.6 ± 2.0	96.7 ± 10.1	106.3 ± 11.1
effluent of WWTP	0	0	0.045	ND		ND	
	0	1.00	0.87 ± 0.12	0.87 ± 0.05	87.0 ± 5.0	ND	
	18.2 ^a	1.79 ^a	16.6 ± 0.7	1.70 ± 0.13	95.0 ± 7.3	14.9 ± 0.8	81.9 ± 4.4
	91.0 ^a	8.94 ^a	90.9 ± 7.5	7.81 ± 0.20	87.4 ± 2.2	83.1 ± 7.5	91.3 ± 8.2
lake water	0	0	ND	ND		ND	
	0	1.00	0.85 ± 0.02	0.96 ± 0.02	96.4 ± 2.0	ND	
	18.2 ^a	1.79 ^a	18.8 ± 0.4	1.70 ± 0.06	95.0 ± 3.4	17.1 ± 0.4	94.0 ± 2.2
	91.0 ^a	8.94 ^a	98.4 ± 7.7	8.23 ± 0.18	92.1 ± 2.0	90.2 ± 7.7	99.1 ± 8.5

^aSpiked with a commercial AgNPs containing 8.9% Ag⁺, and the corresponding spiked amounts of NAg and Ag(I) were determined by spiking the same amount of AgNPs into purewater and determined after diluting the sample to containing 20 $\mu\text{g/L}$ of total Ag. ^bBelow the limit of quantification.

the determination of Ag(I), which was mainly attributed to the strong association of $\text{S}_2\text{O}_3^{2-}$ with Ag(I) and the high resolution between NAg and Ag(I) of the proposed method.

The applicability of the proposed approach for quantification of NAg was validated by another independent measurement method, the CPE-based method reported in our previous studies.^{32–34} Results shown in Table 2 indicated that the NAg concentration determined by the two methods agreed very well, except for sample S5, for which the concentration of NAg determined by the proposed LC-ICP-MS (91.0 $\mu\text{g/g}$) was much lower than that by the CPE method (267 $\mu\text{g/g}$). This difference was attributed to the method error of the CPE procedure, in which the coextraction of Ag(I) with AgNPs into TX-114-rich phase gives rise to a large positive error if the Ag(I) concentration is over 2-fold of the AgNP concentration.^{32,33} The results also demonstrated that for samples with higher concentration of Ag(I) than that of NAg like in the case of S5, this proposed LC-ICP-MS method is superior than the CPE-based method in quantification of NAg and Ag(I).

Although the proposed LC-ICP-MS method is able to differentiate silver-containing NPs from Ag(I), it is incapable of distinguishing AgNPs from other silver-containing NPs (e.g., Ag_2O , AgCl , Ag_2S) coexisted in the samples.¹⁷ Thus, further characterization experiments were performed to identify AgNPs in the samples. TEM observation showed that NPs were detected in all the samples except for sample S5, which was in good agreement with the chromatograms obtained by LC-ICP-MS (Figure 3). Moreover, the slight split of the NAg peak in the chromatogram of S3 was supported by the coexistence of large and small AgNPs in the TEM image. Although among UV–vis spectra of the five antibacterial products only samples S1 and S2 exhibited the characteristic maximum absorbance of AgNPs (~ 410 nm), EDS analysis confirmed that the NPs detected in samples S1–S4 were AgNPs (Figure 3).

Analysis of Ag(I) and NAg in Environmental Waters.

To further evaluate the feasibility of the proposed LC-ICP-MS method, three real environmental water samples including lake water, influent and effluent of a WWTP were analyzed, and the recoveries were determined by spiking 1.00–8.94 $\mu\text{g/L}$ Ag(I) and 18.2–91.0 $\mu\text{g/L}$ NAg. As shown in Table 3, the respective

Ag(I) and NAg recoveries of the three real water samples were in the range of 84.7–96.4% and 81.3–106.3%, which were excellent regarding to the low spiking levels and the complex matrices. The results suggest that the real water sample matrices showed no interference on the determination of both Ag(I) and NAg. In the future, sample preconcentration methods could be developed for coupling with the suggested LC-ICP-MS to improve the analytical performance.

In summary, we have developed an efficient approach for rapid and baseline separation of dissoluble Ag(I) from NAg covering the whole range of ~ 1 to 100 nm, using size exclusion chromatography with aqueous solution of surfactant FL-70/ $\text{Na}_2\text{S}_2\text{O}_3$ as mobile phase and 500 Å pore sized amino column as separation column. This presented separation technique is easily online coupled to ICP-MS for direct and rapid detection of dissoluble Ag(I), while the NAg can be quantified by subtracting the dissoluble Ag(I) from the total Ag content determined by ICP-MS after digestion of the sample without chromatographic separation. Our method has been verified in speciation analysis of NAg and dissoluble Ag(I) in antibacterial products and environmental waters, suggesting it is a robust analytical approach for studying the environmental behavior and toxicology of AgNPs. The dissoluble Ag(I) determined in this present study includes species of free Ag⁺, dissoluble complexes of Ag⁺, AgNP-adsorbed Ag⁺, and Ag(I) salts that are originally undissolved but can form dissoluble complexes with $\text{S}_2\text{O}_3^{2-}$. The relevance of these species of Ag(I) to the toxicity of AgNPs remains further study.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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