Capillary Electrophoresis Coupled with Inductively Coupled Mass Spectrometry as an Alternative to Cloud Point Extraction Based Methods for Rapid Quantification of Silver Ions and Surface Coated Silver Nanoparticles

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Abstract: Speciation and accurate quantification of ionic silver and metallic silver nanoparticles are critical to investigate silver toxicity and to determine the shelf-life of products that contain nano silver under various storage conditions. We developed a rapid method for quantification of silver ions and silver nanoparticles using capillary electrophoresis (CE) interfaced with inductively-coupled plasma mass spectrometry (ICPMS). The addition of 2-mercaptopropionylglycine (tiopronin) to the background electrolyte was used to facilitate the chromatographic separation of ionic silver and maintain the oxidation state of silver. The obtained limits of detection were $0.05 \mu\text{g kg}^{-1}$ of silver nanoparticles and $0.03 \mu\text{g kg}^{-1}$ of ionic silver. Nanoparticles of varied sizes (10 nm to 110 nm) with different surface coating, including citrate acid, lipoic acid, polyvinylpyrrolidone and bovine serum albumin (BSA) were successfully analyzed. Particularly good recoveries (>93%) were obtained for both ionic silver and silver nanoparticle in the presence of excess amount of BSA. The method was further tested with six commercially available dietary supplements which varied in concentration and matrix components. The summed values of silver ions and silver nanoparticles correlated well with the total silver concentration determined by ICPMS after acid digestion. This method can serve as an alternative to cloud point extraction technique when the extraction efficiency for protein coated nanoparticles is low.

Keyword: Silver nanoparticles, Ionic silver, Speciation and quantification, Capillary electrophoresis, Inductively Coupled Mass Spectrometry

1. Introduction

In recent years the application of nanotechnologies in consumer products has increased at a rapid pace. Among all material categories, nanoscale silver (Ag) represents one of the greatest market share, which is attributed to its antimicrobial properties. Many nano-Ag containing products are designed for health or medical purposes, such as in dietary supplements and wound dressing.[1] Despite the potential application of adding Ag nanoparticles (NPs) to the products, there is an increasing concern of the safe use of such materials. Human exposure to Ag is growing as the availability of Ag NPs containing products continuously expands. Recent studies have suggested that both Ag NPs and Ag ions can induce cytotoxicity through different mechanism.[2-4] Additionally, numerous studies have suggested that Ag NPs are prone to oxidation and can release Ag ions to the surrounding environment, and that reverse formation to NPs from Ag ions is also a possibility.[5-8] The determination of the amount of both Ag NPs and Ag ions, instead of total Ag concentration in a sample is important to allow a sound correlation to be established between each Ag species and any toxicity effects. Additionally, such speciation analysis is important for an accurate exposure assessment of consumer products that contain Ag NPs.

Several liquid chromatographic methods have been developed for the speciation of nano-scale silver.[9, 10] By using ICPMS as the detection method, LODs can easily reach a sub $\mu\text{g kg}^{-1}$ level. However, online speciation and quantification of Ag NPs and ionic Ag remain a challenge because of the relatively low recovery due to irreversible binding of analyte to the stationary phase.[9, 10] Asymmetric flow field flow fractionation (AF4) is another popular technique in characterizing metallic and metal oxide NPs.[11, 12] We previously reported a robust method using AF4 coupled with ICPMS for the online enrichment, speciation, and quantification of Ag^+ and NPs.[13] A detection limit as low as $0.004 \mu\text{g kg}^{-1}$ was achieved and the method also demonstrated its ability in analyzing bovine serum albumin (BSA) coated Ag NPs. A common limitation for AF4 methods is the membrane fouling effect caused by the particle sticking to the membrane, which leads to sample loss and reduced recovery.[11]

Currently, cloud point extraction (CPE) has been considered as a powerful technique for the quantification of Ag NPs in consumer products and environmental samples due to simplicity of the technique which requires only a thermostat water bath and centrifuge.[14, 15] In this method, NPs are encapsulated in the micelle after the addition of surfactant and concentrated to a small volume with the assistance of centrifugation. The NP concentration in the enriched phase is determined by ICPMS after acid digestion. Because ionic Ag is not extractable when certain chelating agents are added, the speciation of Ag NPs and ions can be achieved by measuring both total Ag and Ag NP concentration.[15] The reported values of limit of detection (LOD) for CPE are generally in the sub $\mu\text{g kg}^{-1}$ range, although a LOD as low as 0.6 ng kg^{-1} has been reported for waste water sample by using electrothermal atomic absorption spectrometry.[14-16] However, studies have shown that the extraction efficiency was significantly reduced when Ag NPs were coated with proteins, which limits its application in biological samples.[17]

Capillary electrophoresis has been a widely used separation technique for a variety of samples.[18-20] Previously, we have demonstrated that when interfaced with ICPMS, CE has shown strong potential for directly analyzing nanomaterials in consumer products without extraction and extensive sample preparation. Here, we report an analytical method based on CE-ICPMS to perform rapid online speciation and quantification of Ag NPs and Ag^+ . The nanoparticle size and surface coating effect on the performance of this method was evaluated. Six commercially available Ag NP based dietary supplements in a variety of matrices were analyzed. This method serves as an excellent alternative to CPE for the speciation and quantification of Ag species by offering a much faster analysis and improved separation efficiency with nearly perfect sample recoveries.

2. Experimental Section

2.1. Materials and Chemicals. Cyclohexylaminoethane sulfonic acid (CHES, 99.9%) was purchased from MP Biomedical (Carlsbad, CA, USA). Penicillamine and sodium dodecyl sulfate (SDS, 99%) were

86 purchased from Acros Organics (Waltham, MA, USA). Potassium hydroxide pellets (>85%), nitric acid
87 (68%-70%, OPTIMA ultra-pure grade), BSA lyophilized powder, sodium citrate dehydrate,
88 electrophoresis grade of Triton X-100 (TX-100) and ethylenediaminetetraacetic acid disodium (EDTA)
89 were acquired from Fisher Scientific (Waltham, MA, USA). 2-Mercaptopropionylglycine (tiopronin),
90 sodium hydroxide monohydrate (TraceSELECT, $\geq 99.9995\%$ metal basis), 1,2-
91 diaminocyclohexanetetraacetic acid monohydrate (CDTA, 98.5%) and 2-propanol (TraceSELECT,
92 $\geq 99.9\%$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Single element ICPMS standards
93 Ag (10 mg kg^{-1}), In (10 mg kg^{-1}), and Ag 109 isotope (^{109}Ag , 10 mg kg^{-1}) were purchased from Inorganic
94 Venture (Christiansburg, VA, USA). Ag single element ICPMS standard (1000 mg kg^{-1}) was purchased
95 from Spex Certiprep (Metuchen, NJ, USA). All chemicals were used as received without further
96 purification. Ag NP suspensions (10, 20, 30, 60, 110 nm) stabilized by citrate acid (CA),
97 polyvinylpyrrolidone (PVP) or lipoic acid (LA) were acquired from Nanocomposix (San Diego, CA,
98 USA) and stored at 4°C in the dark. NPs were purified using spinning dialysis (Amicon Ultra Centrifugal
99 Filter with a nominal molecular weight limit of 100K) prior to the analysis. A portion of the purified NPs
100 were further digested with nitric acid in a CEM MARS 5 microwave digestion system (Matthews, NC,
101 USA) and diluted 10 times with 2% nitric acid. This portion was analyzed by ICPMS to determine the Ag
102 concentration of the purified sample. Calibration standards were prepared by diluting Ag standards with
103 2% nitric acid. A second Ag standard from a different source was used to prepare independent calibration
104 verification. In was added to the samples as internal standard. The analysis was performed using an
105 Agilent 7700x ICPMS (Santa Clara, CA, USA) under no-gas mode. BSA coated Ag NPs were prepared
106 according the literature method with modifications.[21] Specifically, Ag NPs were mixed with 5 mg mL^{-1}
107 of BSA solution to reach a final concentration of $2 \text{ } \mu\text{g mL}^{-1}$ of Ag and incubated overnight. Then BSA
108 coated Ag NP solutions were diluted with background electrolyte (BGE) to the desired concentrations and
109 allowed to equilibrate for 30 min before injection. A total of six commercially available dietary
110 supplements (labeled as DS 1-6) that declare colloidal Ag were purchased from internet sources. Each of
111 the dietary supplements was an aqueous suspension and was used for the analysis without further

purification. The total Ag concentrations of the dietary supplements were determined by ICPMS after acid digestion with nitric acid. Details of these products, including ingredients and labeled concentration are listed in Table 3. Both commercial Ag NPs and Ag based DS were diluted with BGE to the desired concentration before analysis. Deionized water ($>18\text{M}\Omega\text{cm}^{-1}$) from a Milli-Q reference system (Millipore, Billerica, MA, USA) was used throughout the experiments.

2.2. Instrumentation. Dietary supplements were studied on a JEOL 1400 TEM (Peabody, MA, USA) operated at 80 kV. The images were acquired by a TVIPS TemCam F416 camera and ImageJ software was used to process the images and to obtain size statistics of Ag NPs. A few drops of samples were placed directly on a 300 mesh copper grid and air dried overnight. Energy-dispersive X-ray spectroscopy (EDS) study was performed on JEOL 2100 TEM (Peabody, MA, USA) operated at 200 kV equipped with an EDAX Genesis 2000 detector (EDAX Inc., Mahwah, NJ, USA). The absorption spectra of DS 1-6 were collected on a PerkinElmer LAMBDA 45 UV/Vis System (Waltham, MA, USA) in the range of 300 nm to 700 nm with bandwidth of 1 nm.

Capillary electrophoresis separation was performed on a 7100 Capillary Electrophoresis system (Agilent Technology, Santa Clara, CA, USA). Coated fused-silica capillaries (i.d. 100 μm ; o.d. 360 μm ; length 60 cm) were obtained from Molex (Phoenix, AZ, USA). A new capillary was initialized by flushing with 0.1 N NaOH for 30 min and BGE for 30 min, followed by rinsing with water for 5 min. The capillary was conditioned each day before use with 0.1 N NaOH for 15 min, BGE for 15 min and water for 3 min. Between each run the capillary was rinsed with water for 60 sec and then equilibrated with 0.1 N NaOH and BGE for 3 min respectively. Samples were hydrodynamically injected at 30 mbar for 15 sec followed with an injection of BGE at 15 mbar for 10 sec. The temperature of the cartridge was set at 23 $^{\circ}\text{C}$ (ambient temperature), and the applied voltage for the separation was 25 kV. The interface between CE and ICPMS (Agilent 7700x) was setup accordingly to our previously reported method.[18] Briefly, the outlet of the capillary was directly connected to a Mira Mist CE nebulizer (Burgener Research Inc. Mississauga, Ontario, Canada). A solution containing 1% HNO_3 (v/v), 10% 2-propanol and 5 $\mu\text{g kg}^{-1}$ of In was used as the makeup solution and was introduced to the nebulizer by a syringe pump at an infusion

rate of 13 $\mu\text{L min}^{-1}$. ICPMS was operated under no gas mode and mass isotope ^{107}Ag , ^{109}Ag , and ^{115}In were monitored. Details of the operation conditions are listed in Table 1. The pH value of the BGE was adjusted by adding 1 N NaOH or 0.1 N HCl solution and measured with an Orion Star A214 pH meter (Thermo Scientific. Waltham, MA, USA).

Table 1. CE/ICPMS operating parameters

CE Parameters	
capillary	polymer coated fused silica capillary, I.D. 100 μm , O.D. 360 μm , length 60 cm
BGE	CHES 10 mM, TX-100 30 mM, pH=9.5
make up solution	1% HNO_3 and 10% 2-propanol in water
voltage	25 kV
temperature	23 $^{\circ}\text{C}$
sample injection	hydrodynamic, 30 mbar, 15s
ICPMS Parameters	
RF Power	1500 W
sample depth	8.0 mm
plasma gas	15.0 L min^{-1}
carrier gas flow	1.05 L min^{-1}
makeup gas flow	0.50 L min^{-1}
monitored isotope	^{107}Ag , ^{109}Ag , ^{115}In
nebulizer	Mira Mist CE

3. Results and Discussion

Separation of analytes in CE is based on the difference in electrophoretic mobility, which is determined by the charge-to-size ratio. For speciation of Ag^+ and Ag NPs, alkaline condition is preferred over acidic or neutral condition due to the dissolution and aggregation of Ag NPs at low pH and increased run-to-run variation at pH value close to 7.[21, 22] From our previous study, CHES with a pKa value of 9.3 was demonstrated suitable for this purpose.[18] A pH value of 9.5 was selected to take advantage of the strong electroosmotic flow (EOF) at this condition, which can increase the net migration speed of analytes and effectively reduce the analysis time. Several types of surfactants were found to be helpful in stabilizing nanoparticles in buffer solution and assist the separation.[10, 18, 23] In this study, we focused on separating Ag^+ from Ag NPs and quantitatively determine the concentration of each species through a

rapid analysis. Therefore, non-ionic surfactant TX-100 was chosen over anionic SDS or SDBS in order to avoid prolonged elution for large particles which leads to significant peak broadening due to particle distribution and longitude diffusion.[18] Using chelating agents is necessary to capture and stabilize Ag^+ in the solution so they can be separated from Ag NPs. Tiopronin, a thio-containing compound, was used for this purpose. It has been used to treat cystinuria and rheumatoid arthritis and as capping ligand for the synthesis of gold nanocluster.[24, 25] We found that adding tiopronin to concentration of 1 mM in BGE was optimal for the analysis. We studied several factors, including surfactant concentration and applied voltage in order to optimize the separation conditions. The results suggested that a surfactant concentration of 30 mM is sufficient to obtain optimal peak shape and repeatability (Figure 1). Both lower and higher concentration of TX-100 resulted in distorted peak and increased run-to-run variation of the migration time of the analytes. The effect of applied voltage on the analysis is similar to our previous studies (Figure 1).[18] While no significant change in the separation efficiency was observed, the total analysis time was reduced due to faster migration speed induced by enhanced EOF under higher voltage.

Figure 1. Optimization of speciation conditions in CE-ICPMS; Sample solution is a mixture of $10 \mu\text{g kg}^{-1}$ of Ag^+ and $20 \mu\text{g kg}^{-1}$ of 30 nm CA coated Ag NPs. a) Separation at different concentrations of TX-100 surfactant (CHES, 10 mM; pH 9.5; tiopronin, 1 mM; voltage, 30 kV). b) Separation at different applied voltages (TX-100, 30 mM; CHES, 10 mM; tiopronin 1 mM, pH 9.5;).

175

176 **Figure 2.** a) Analysis of CA coated 30 nm Ag NP, Ag^+ and a mixture of Ag NP and Ag^+ ; signal from
 177 ^{107}Ag channel was plotted. b) Analysis of a mixture of 30 nm Ag NP synthesized with naturally occurring
 178 Ag source (^{107}Ag and ^{109}Ag) and Ag^+ prepared from isotope of ^{109}Ag ; signal from both ^{107}Ag and ^{109}Ag
 179 channels were plotted. (Separation conditions: TX-100, 30 mM; CHES, 10 mM; pH 9.5; tiopronin, 1 mM;
 180 voltage, 30 kV)

181 Sample solutions containing only Ag NPs or Ag^+ were analyzed separately to determine the
 182 identity of each individual peak (Figure 2a). Because Ag NPs were stabilized by non-ionic surfactant,
 183 their surface charges were suppressed and eluted first. Ionic Ag eluted at a later time, which can be
 184 attributed to its chelation with tiopronin. In order to further verify the elution order of Ag species, a
 185 solution containing a mixture of 30 nm Ag NPs synthesized from a naturally occurring Ag source (51.839
 186 % ^{107}Ag and 48.161 % ^{109}Ag) and Ag^+ prepared solely with isotope ^{109}Ag was analyzed. As shown in
 187 Figure 2b, in both ^{107}Ag and ^{109}Ag channels two peaks with the same migration time of 3.5 min were
 188 observed. The ratio of these two peak areas were the same as the natural abundance of Ag isotopes,
 189 suggesting that the peaks corresponded to the elution of Ag NPs. An additional peak with a migration
 190 time of 5 min was observed in ^{109}Ag channel but not in ^{107}Ag channel. Because ionic Ag was prepared
 191 with only Ag isotope 109, the additional peak ^{109}Ag channel corresponded to Ag^+ , verifying Ag^+ eluted at
 192 a later time than NPs. It has been reported that reversible transformation between Ag^+ and Ag NPs exists
 193 under certain conditions, which can be detrimental to quantitative characterization.[5-7] Therefore, it is

important to examine the stability of both Ag^+ and NPs in this method to confirm that there is no inter-species conversion during the course of analysis. As shown in Figure S1, two single species samples were analyzed by CE-ICPMS on each of three consecutive days, and no conversion was observed for either Ag^+ or Ag NPs, demonstrating that samples are stable under the designed buffer condition. For the separation in CE, the surface coating can potentially affect the interaction between NPs and buffer composition, resulting in an altered electrophoretic behavior. We have studied Ag NPs with four types of common coatings including CA, PVP, LA and BSA in CE-ICPMS. The results (Figure S2) suggested that no significant difference existed in the migration behavior, which can be largely attributed to the use of non-ionic surfactant.

Under optimized conditions, speciation of Ag NPs and Ag can be achieved in six minutes (Figure 2a). With seven consecutive injections, the relative standard deviations (RSD%) for NPs and ions were 2.85% and 1.52% on migration time, and 2.59% and 1.91% on peak area respectively. For quantitative analysis, a mixture containing CA coated 30 nm Ag NPs and Ag^+ in the range of 0.5—50 $\mu\text{g kg}^{-1}$ were analyzed and a linear relationship between the concentration and the peak area was found with a R^2 of 0.999. The LOD (3σ criteria, $n=7$) was determined to be 0.05 $\mu\text{g kg}^{-1}$ for Ag NPs and 0.03 $\mu\text{g kg}^{-1}$ for Ag. The quantification process using CE-ICPMS was first evaluated on multiple types of Ag NPs. Several previous studies have reported size-dependent signal response of nanoparticles in ICPMS, which can be explained by the variation in the nebulization efficiency.[9, 26] We first spiked BGE with 30 $\mu\text{g kg}^{-1}$ of CA coated Ag nanoparticles in different sizes to investigate their signal response. As shown in Figure 3, CA coated NPs of 10, 20, 60 and 110 nm showed similar recoveries which were in the range of 95% to 105%. This result can be explained by the configuration of nebulization of CE-ICPMS. At a makeup flow rate of 13 $\mu\text{L min}^{-1}$, the spray chamber was dry and no visible droplets were formed, suggesting nearly 100% transport efficiency. The effect of surface coating on the particles recoveries was also studied (Figure 3), and the results show that the recoveries are all within 90% to 110% range, suggesting no significant difference among CA, LA, PVP and BSA coatings.

Figure 3. The effect of nanoparticle size and surface coating on the Ag recovery in CE-ICPMS analysis. The Ag NP concentration was $30 \mu\text{g kg}^{-1}$ in all samples. Three consecutive measurements were collected to calculate average recoveries and deviations. The red dot line indicates a 10% variation range of the sample recovery.

To evaluate the capability of the developed CE-ICPMS system in quantifying mixtures of Ag^+ and Ag NPs, we prepared a series of mixtures with relative concentrations of ions and NPs. The results suggested good recovery values for all concentration levels. It has been reported that free ionic Ag^+ can be adsorbed onto NPs surface, especially for NPs coated with BSA, which can capture Ag^+ through its exposed thiol group in cysteine residues. In previous studies, EDTA or CDTA was added as chelating agent for the speciation of ionic and NPs form of gold.[18] However, due to the strong interaction of BSA with Ag^+ , CDTA was unable to chelate with all Ag^+ in the presence of BSA (data not shown), resulting in a significant underestimation of the amount of Ag^+ and overestimation of the amount of Ag NPs. Adding 1 mM tiopronin has proven to be an effective way to speciate Ag^+ and Ag NPs even in the presence of large excess amount of BSA molecules. From the results (Table 2) CE-ICPMS was able to determine the amount of both particulate and ionic forms of Ag with satisfactory recovery.

Table 2. Speciation and quantification of CA and BSA coated Ag NPs and Ag⁺ at different concentration

size (nm)	coating	spiked ($\mu\text{g kg}^{-1}$)		detected ($\mu\text{g kg}^{-1}$) ^a		Recovery, %	
		Ag NP	Ag ⁺	Ag NP	Ag ⁺	Ag NP	Ag ⁺
10	CA	30	0	32.81 $\pm$ 1.24	ND ^b	109	ND
		15	15	14.29 $\pm$ 0.26	14.63 $\pm$ 0.26	95.3	97.5
		10	20	10.83 $\pm$ 1.27	22.96 $\pm$ 1.09	108	115
20	CA	30	0	32.48 $\pm$ 0.65	ND	108	ND
		15	15	14.28 $\pm$ 1.46	16.40 $\pm$ 0.32	95.3	109
		10	20	9.81 $\pm$ 0.82	20.19 $\pm$ 1.01	98.1	101
60	CA	30	0	33.37 $\pm$ 2.74	ND	111	ND
		15	15	15.42 $\pm$ 0.99	14.82 $\pm$ 0.49	103	98.8
		10	20	11.03 $\pm$ 1.12	19.77 $\pm$ 1.20	110	98.9
10	BSA	15	15	15.26 $\pm$ 0.08	14.42 $\pm$ 0.17	102	96.1
30	BSA	15	15	15.24 $\pm$ 0.53	16.04 $\pm$ 0.97	102	107
60	BSA	15	15	16.49 $\pm$ 0.37	14.98 $\pm$ 0.32	110	99.9

^an=3^bbelow the LOD

In real life situations, NPs are rarely dispersed in purified water. The matrix components can potentially interact with the separation system and negatively affect the speciation and quantification. In order to demonstrate the robustness of our CE-ICPMS system, we applied our analysis procedure to six commercially available Ag based dietary supplements. As shown in Table 3, all six supplements containing different ingredients and matrix components. UV-Vis spectroscopy was first used to examine the products (Figure S3), and all samples showed strong absorption around 400 nm, indicating the possible presence of Ag NPs in the products. TEM was used to further characterize the supplements (Figure S4). NPs were found in all six products and DS 2 to DS 6 contained significant amount of Ag NPs, of which elemental composition was confirmed by EDS (Figure S5). Due to the relative low concentration of Ag NPs and large amount of silica nanospheres in DS 1, EDS was unable to capture a detectable level of Ag signal. The total Ag amounts were determined by ICPMS after nitric acid assisted microwave digestion. From CE-ICPMS analysis, all the products contain a considerable amount of Ag⁺ in solution and the sum of ionic and nanoparticle forms of Ag exhibited a good match with the total Ag concentration determined by the ICPMS after acid digestion. These results demonstrate the robustness of

our method in handling interference from complex matrices by providing accurate quantification of Ag nanoparticles and ions in consumer products.

Table 3. CE-ICPMS analysis of six Ag based dietary supplements (n=3).

sample	matrix ^a	labeled Ag concentration (mg kg ⁻¹) ^b	CE-ICPMS(mg kg ⁻¹)			total Ag by ICPMS (mg kg ⁻¹) ^d	recovery ^e
			Ag NP	Ag ⁺	sum ^c		
DS 1	Au (4.5 mg kg ⁻¹) and SiO ₂ (500 mg kg ⁻¹) nanoparticles, H ₂ O	4.5	5.40±0.12	0.05±0.01	5.45±0.13	5.21±0.32	105%
DS 2	0.1% casein, H ₂ O	250	259.28±3.75	16.30±1.17	275.56±4.56	276.30±2.62	99.7%
DS 3	<0.1% casein, H ₂ O	500	486.90±7.42	42.02±0.70	528.92±6.73	514.94±1.25	103%
DS 4	H ₂ O	500	151.99±4.57	386.42±8.65	538.41±7.61	565.79±0.06	95.2%
DS 5	polysorbate 20, sodium bicarbonate, H ₂ O	45	42.50±1.86	21.20±1.18	63.69±0.83	69.75±0.52	91.3%
DS 6	Echinacea purpurea, olive leaf, H ₂ O	30	28.18±0.36	0.28±0.01	28.46±0.36	29.85±0.93	95.3%

^a additional ingredients on the label

^b manufacture claimed total silver concentration

^c summed values of silver nanoparticles and silver ions concentration

^d total silver concentration measured by ICPMS after acid digestion

^e recovery=sum/total Ag by ICPMS

4. Conclusion

We developed a fast and reliable analytical method using CE-ICPMS for the speciation and quantification of Ag NPs and Ag⁺ in consumer products. The results have demonstrated that our method is capable to offer an alternative way to cloud point extraction technique in analyzing variable sized Ag NPs. The method delivered a detection limit of 0.05 µg kg⁻¹ for Ag NP and 0.03 µg kg⁻¹ for Ag⁺, which can be used for trace-level analysis of Ag containing samples. Notably, the successful speciation and accurate quantification of Ag NPs and Ag⁺ in the high concentration of BSA and in six dietary supplements have demonstrated the robustness of our method in handling various surface coatings and complex matrices. Under current method design, we intended to separate Ag ions from Ag NPs and quantify each fraction. Therefore, Ag NPs of different sizes are almost co-eluted with very limited

separation between each fraction. If a mixture of Ag NPs in variable sizes is analyzed, the quantification process gives the total concentration of NPs, which is similar to cloud point extraction method. Further investigation for simultaneous speciation and size based separation of Ag⁺ and Ag NPs is underway.

Conflict of interest

The authors declare no competing financial interest.

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