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Detection and characterization of silver nanoparticles in aqueous matrices using asymmetric-flow field flow fractionation with inductively coupled plasma mass spectrometry

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ABSTRACT

Engineered nanomaterials (EN) may be released into the environment as a result of their use in various consumer products. Silver nanoparticles (nAg) are widely used as an antimicrobial agent in personal care and household products, and in textiles. Since there is high potential for nAg to be released into municipal wastewater and then discharged into the aquatic environment, there is a need to develop methods for the analysis of these materials in aqueous matrices. Asymmetric-flow field flow fractionation (AF4) with on-line detection by ultra violet–visible (UV–Vis) spectroscopy or inductively coupled plasma mass spectrometry (ICP-MS) was used to detect and characterize nAg in aqueous matrices. Analysis of a mixture of 20, 40 and 60 nm nAg standards suspended in water resulted in a well resolved fractogram. Retention times of nAg separated by AF4 were correlated with the particle sizes of the standards. The limit of detection (LOD) for analysis of nAg using the on-line AF4/ICP-MS method was 0.80 ng mL^{-1}. Two calibration approaches (i.e., external calibration and standard addition) were used to quantify nAg concentrations, and both methods gave similar results. Using the on-line AF4/ICP-MS analytical method, nano-sized Ag was detected and quantified in untreated wastewater (i.e., influent) collected from a wastewater treatment plant. The concentration and the modal size of nAg in the influent were 1.90 ng mL^{-1} and 9.3 nm respectively.

1. Introduction

Nanomaterials are substances with at least one dimension between 1 and 100 nm [1,2]. These include engineered, incidental and naturally occurring nanomaterials. Engineered nanomaterials (EN) are increasingly being used in consumer products; for example textiles, electronics, photovoltaics, pharmaceuticals, cosmetics and products for environmental remediation. Due to their antibacterial properties, silver nanomaterials (nAg) are currently the most widely used EN, and are found in a variety of consumer products, including socks, underwear and other clothing, shoe liners, adhesive bandages, antibacterial sprays, food storage containers, laundry additives, home appliances and paints [2–4]. According to a 2011 report from 'The Project on Emerging Nanotechnologies', there are 313 listed consumer products containing nAg [2]. Recent investigations have shown that nAg and dissolved silver (dAg) are being released into domestic wastewater though laundering of fabrics containing nAg (e.g., socks and undergarments) and from there, nAg may enter the municipal sewage stream of wastewater treatment plants (WWTPs) [5–7]. Therefore, it is inevitable that nAg will enter the aquatic environment from discharges of domestic and industrial wastewater.

To date, there are no published data on the concentrations of nAg in municipal wastewater or in surface waters impacted by wastewater discharge. A recent study using a predictive model indicated that nAg may enter surface water at part per billion (ppb; ng mL^{-1}) concentrations through discharges from WWTPs [8], but these estimates have not be verified by the measurement of nAg in wastewater or in surface waters. In a recent study of nAg spiked into a pilot scale WWTP, freely dispersed nAg was only observed in the treated effluent shortly after initial spiking and much of the material was sorbed to sludge or converted by anaerobic processes to Ag_2S [9]. Once released into surface waters, nAg may induce toxic effects on aquatic organisms. [10,11] Our recent studies have shown that exposure to nAg at ppb concentrations can inhibit the growth of natural bacterial communities in water collected from ponds, streams and lakes [12]. The toxicity of nAg may also be related to the release of Ag^+ from the surface of nAg [13].

In order to assess the environmental impacts of nAg, analytical methods are needed to determine the concentrations and particle sizes of this material in aquatic matrices. Analytical approaches

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should be able to analyze the samples with minimal or no sample preparation, provide information on size and composition and detect concentrations in the low ppb range.

Microscopy techniques for the characterization of nanomaterials, including scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) require extensive sample preparation and cannot readily provide information on concentrations [14,15]. Particle sizing techniques, such as small-angle X-ray scattering (SAXS) and dynamic light scattering (DLS) have limitations for analysis in complex heterogeneous matrices and cannot be used to characterize suspensions at low concentrations. Ultrafiltration and centrifugation are also not suitable for complex, heterogeneous samples (e.g., wastewater) and do not provide direct information on size distribution. Separation techniques, such as size exclusion chromatography (SEC), capillary electrophoresis (CE), hydrodynamic chromatography (HDC) and field-flow fractionation (FFF) are promising tools to characterize nAg in aqueous matrices [14,15]. Furthermore, the interfacing of these techniques with an element selective detector, such as ICP-MS, confers a high degree of selectivity. Size exclusion chromatography has a limited size separation range and surface adsorption on the stationary phase can cause unwanted interactions between the column and the analyte [14,16,17]. Capillary electrophoresis is a powerful separation tool, but interpretation of migration times can be cumbersome [17]. Recently, HDC has been shown to be a robust method for the analysis of nanomaterials in environmental samples [17,18]. Unlike SEC, the use of non-porous beads as the stationary phase in HDC considerably reduces interactions with particles [15].

Size exclusion chromatography, CE and HDC all have merits for the analysis of EN and natural colloids. However, FFF is the most versatile analytical technique in terms of separation range, selectivity and resolution [19]. Asymmetric-flow FFF (AF4) is currently the most widely used technique, and is typically coupled with on-line detection by UV–Vis spectroscopy. While UV–Vis has the advantage of ease of operation and low cost, it suffers from low selectivity and sensitivity for nanomaterial analysis. Inductively coupled plasma-mass spectrometry (ICP-MS) has previously been used as an on-line detector coupled to AF4, due to its high sensitivity and selectivity, large dynamic range and capacity for multi-element monitoring [20]. Very recently, Baalousha et al. [21] published a review article on FFF applications using different detection schemes for different sample matrices. In a recent article, Poda et al. [22] described a method using symmetrical FFF coupled to ICP-MS to characterize nAg in aqueous matrices. This article described nAg analysis in terms of size, but concentration data and calibration approaches for quantitative measurements were not included. Bolea et al. [23] described AF4 with ICP-MS detection for the analysis of nAg in consumer products, including the AF4 separation conditions that affect the stability, recovery and resolution of the analyte. Delay et al. [24] utilized an AF4 method with UV–Vis and ICP-MS detection to characterize the composition and size of nAg in aqueous systems that were varied in terms of natural organic matter and ionic strength. Cumberland et al. [25] employed AF4/UV–Vis along with DLS and TEM to study the particle size distribution of nAg under environmentally relevant conditions of pH, dissolved organic carbon and calcium concentration. Collectively, these articles show the utility of using FFF with ICP-MS, but there are no published data on the analysis of nAg using this technique in real environmental samples.

In this study, we describe methods for determining both the size and the concentration of nAg in aqueous matrices using on-line and off-line AF4/ICP-MS, and apply this analytical technique to the analysis of nAg in samples of surface water and in a sample of municipal wastewater. The objective of this study was to develop an analytical method using AF4 with on-line UV–Vis and/or ICP-MS detection for determination of both the size range and the concentration of nAg in aqueous samples. Because all laboratories may not have the capacity for on-line AF4/ICP-MS, off-line ICP-MS analysis after AF4 separation was also evaluated. The method developed was then used to determine the concentration and size of nAg in surface waters collected from lakes and a river, and in untreated wastewater collected from a municipal wastewater treatment plant (WWTP).

2. Experimental

2.1. Materials and solutions

Standard suspensions were prepared from nAg capped with carboxy-functionalized polyacrylate (ViveNano Inc., Toronto, Ontario, Canada) supplied as a colloid in water at a concentration of 1500 $\mu g\,mL^{-1}$. According to the manufacturer, 90% of the material in this colloid is in the size range of 10 nm ± 2 nm. Our previous analysis [12] showed that this material has an Ag content of ~20% by weight, and the remainder of the material is presumably made up of capping agent. Colloidal standards of uncapped nAg with average sizes of 20, 40 and 60 nm suspended in water (5 $\mu g\,mL^{-1}$, Ag content) were purchased from B.B. International (Cardiff, South Wales, UK).

Standard solutions (1000 $\mu g\,mL^{-1}$) of dAg and indium were obtained from SCP Science (Baie-Durfe, Quebec, Canada). High purity water (18.2 MΩ; Milli-Q Element System, Miilipore Corp., Billerica, MA, USA) and trace metal grade HNO_3 (VWR International, Mississauga, Ontario, Canada) was used for the preparation of all solutions. Untreated wastewater (i.e., influent) was collected as a 24 h composite sample from the municipal WWTP that serves a population of approximately 75,000 people in the city of Peterborough, Ontario, Canada. Wastewater was collected in August 2010 in a clean polypropylene bottle and stored in a refrigerator until the analysis. Surface water samples were collected in July 2010 from Otonabee River upstream of the Peterborough WWTP, and from Plastic Lake and Chemong Lake in central Ontario, Canada in clean polypropylene bottles of 1 L and stored in a refrigerator until the analysis. These two lakes are susceptible to point source releases of nanomaterials from sewage discharges [12].

To calculate the modal size of nanoparticles, a simplified form of the FFF equation (Eq. (1)) and the Stokes–Einstein equation (Eq. (2)), [26] were used.

$$t_r = \frac{W^2}{6D\ln(1 + F_c/F_{out})} \quad (1)$$

$$D = \frac{k_B T}{6\pi\eta r} \quad (2)$$

where t_r, W, D, F_c, F_{out}, k_B, T, η and r are retention time, channel height (thickness of spacer), diffusion coefficient of the particles, cross flow, channel flow, Boltzman's constant, temperature (K), dynamic viscosity of fluid (e.g., 0.01 $g\,cm^{-1}\,s^{-1}$ for water) and radius of particle, respectively.

2.2. Sample preparation

Working standards of dAg for ICP-MS analysis were prepared daily by serial dilution of the 1000 $\mu g\,mL^{-1}$ stock solution with 2% (v/v) nitric acid. Working standards of nAg for AF4 analysis were prepared from ViveNano colloidal nAg standards by diluting with high purity Milli-Q water. Prior to AF4/ICP-MS analysis, the wastewater sample (i.e., influent) was filtered using a 0.45 μm syringe filter (25 mm CA; Canadian Life Sciences, Peterborough, Ontario, Canada). For off-line ICP-MS analysis, collected fractions

Table 1
ICP-MS operating conditions and data acquisition parameters.

Instrument parameters	
RF power (kW)	1.40
Nebulizer flow rate (L min^{-1})	0.92
Plasma gas flow rate (L min^{-1})	18.0
Auxiliary gas flow rate (L min^{-1})	2.0
Sheath gas flow rate (L min^{-1})	0.21
Sample cone	C series Ni, 1.1 mm ø orifice
Skimmer cone	C series Ni, 0.5 mm ø orifice
Data acquisition parameters	
Steady state mode	
Isotopes monitored	^{107}Ag, ^{115}In
Dwell time (μs)	10,000
Scan mode	Peak hopping
TRA mode	
Isotopes monitored	^{107}Ag, ^{115}In
Dwell time (μs)	200,000
Sampling time (s)	1200
Scan mode	Peak hoping

were acidified with HNO_3 to 2% before the analysis. DLS measurements of nAg colloid standards were performed in an aqueous matrix. For TEM measurements, droplets of nAg colloid were placed on a carbon coated copper TEM grid and allowed to dry for the analysis.

2.3. Instrumentation

The AF4 system used was an AF2000 Focus model purchased from Postnova Analytics Inc. (Salt Lake City, UT, USA), with an on-line UV–Vis detector operated at a wavelength of 420 nm for the analysis of Ag. Data from the UV–Vis detector was processed using the AF2000 Focus software (Postnova Analytics Inc.). The channel thickness (i.e., spacer thickness) in the AF4 cartridge was 350 μm. The semi-permeable membrane was composed of regenerated cellulose with a 10 kDa molecular weight cut-off (MWCO). High purity Milli-Q water was used as the carrier liquid. Samples were injected via a manual injector valve. For off-line collection of AF4 eluate, a fraction collector purchased from Postnova Analytics Inc. was connected with the system.

A Bruker (formerly Varian) 820 ICP-MS (Bruker Optics Ltd., Mississauga, Ontario, Canada) was used for quantification of Ag, either off-line analysis of collected AF4 fractions, or interfaced to the AF4 system for on-line analyses. The ICP-MS operating conditions and data acquisition parameters are given in Table 1. For off-line analyses, data were acquired in steady state mode and processed using the ICP-MS Expert software. For on-line analyses, data were acquired in time resolved analysis (TRA) mode and the resultant fractogram was then exported to the Galaxie chromatography software for peak integration.

A schematic of the AF4/ICP-MS interface is shown in Fig. 1. The details of the AF4 technique can be found elsewhere [26]. For on-line detection, the channel flow from the AF4 system was directed to the UV–Vis detector and then to the ICP-MS through a mixing-T. An injection valve (Rheodyne switching valve; two-position and six-port) was placed between the UV–Vis detector and the ICP-MS for on-line flow injection of dAg, used as a calibration standard. A 5 ng mL^{-1} (2% (v/v) HNO_3) solution of indium, introduced via the mixing-T, was used as an internal standard (IS) to monitor the flow stability and any changes in sensitivity during data acquisition. The eluent and IS were introduced into the ICP-MS system using a Conikal U-series nebulizer, with a nominal uptake rate of 1 mL min^{-1}. For off-line analyses, a glass concentric MicroMist nebulizer, with a nominal uptake rate of 200 μL min^{-1} was used.

DLS analysis was conducted with a Nicomp 380 DLS purchased from Particle Sizing Systems (Port Richey, FL, USA) to measure the size of the particles in the nAg colloidal standards. TEM on nAg

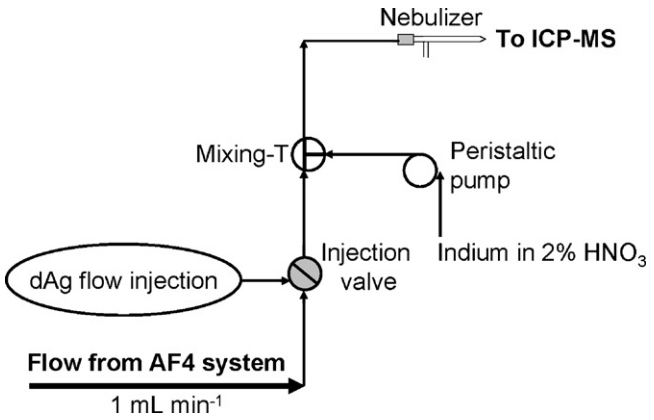

Fig. 1. Schematic of AF4/ICP-MS interface.

colloidal standards was performed by the Canadian Centre for Electron Microscopy at McMaster University in Hamilton, Ontario, Canada using a Titan 80 – 300 microscope purchased from FEI (Hillsboro, OR, USA) and operated at 300 keV.

2.4. Calibration approaches

Three different calibration approaches were evaluated for quantification of the AF4/ICP-MS measurements. For the external standard approach, nAg standards prepared from the ViveNano colloidal material were injected into the AF4 system to generate a calibration curve (response vs. concentration). With the standard addition method, multiple sample aliquots were spiked with varying amounts of the ViveNano nAg and injected into the AF4 system, and the concentration was then determined by extrapolation. The third approach used flow injection (FI) analysis of a dAg standard (0.1 mL injection volume) introduced into the eluent flow after the AF4 system via an on-line injector, as described above. Data were acquired in TRA mode and the integrated peak areas used to generate a calibration curve.

3. Results and discussion

3.1. AF4 operating conditions

The optimized parameters for AF4 separation are given in Table 2, and these conditions were used for all subsequent analyses. It is well known that ICP-MS is prone to matrix effects, defined as changes in the measured concentration related to the composition of the sample matrix. Matrix effects are often associated with suppression of ionization and a concomitant decrease in analytical sensitivity. Therefore, the Milli-Q water was used as the carrier liquid for AF4 separation.

3.2. Retention time versus particle size

Using the AF4 separation parameters given in Table 2, there was good resolution of the 20, 40 and 60 nm standards and poor

Table 2
AF4 operating parameters.

Tip flow (mL min^{-1})	3.5
Cross flow (mL min^{-1})	2.5
Injection flow (mL min^{-1})	0.2
Focus flow (mL min^{-1})	3.3
Carrier liquid	Milli-Q water
Membrane type	Regenerated cellulose with 10 kDa MWCO
Detector flow (mL min^{-1})	1
Channel thickness (μm)	350

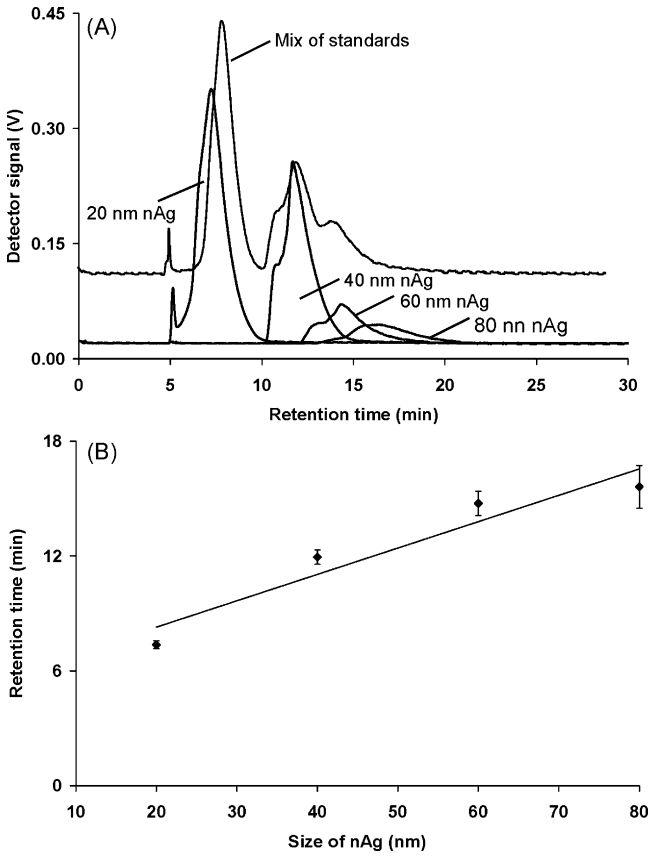

Fig. 2. AF4 separation of nAg colloid standards in water. (A) Fractograms. (B) Relationship between size of nanomaterials and its retention time. Conditions: nAg colloid standards of different sizes from B.B. International, AF4 separation with on-line UV–Vis detection of nAg at 420 nm and operating parameters as in Table 2.

resolution of the 60 and 80 nm colloidal nAg standards (B.B. International). Larger particle sizes were not included in this study since <100 nm represents the maximum size range of interest for the analysis of EN in environmental samples. Fig. 2 shows the fractograms for the standards injected either individually or in a mixture (Fig. 2A), and the linear plot ($R^2 = 0.981$, when $n = 3$ and $R^2 = 0.917$ when $n = 4$) of nAg size versus the AF4 retention time (Fig. 2B). The peak for 80 nm nAg in the fractogram of the mixture (1:1:1:1 by volume) was not observed because of the dilution effect on the sensitivity of UV–Vis detection. The resolution of different sized nAg from B.B. International was found to be 0.95 for the 20 and 40 nm standards, 0.56 for the 40 and 60 nm standards and 0.25 for the 60 and 80 nm standards. For the 10 nm nAg (ViveNano) colloidal standard, the calculated average particle size, using the linear regression equation (Fig. 2B) and the observed retention time of 5.7 min corresponded to a modal size of 9.3 nm. Variations in retention times for AF4 separations were within 5–10% RSD. For example, when the ViveNano nAg standard with a mean size of 10 nm was analyzed repeatedly by AF4 (UV–Vis detection), the RSD for the retention time was <5% ($n = 5$) for both intra-day and inter-day variability. For the 20 nm standard from B.B. International, the retention time in individual and mixture peaks varied within 10% RSD.

The presence of shouldering in the later eluting part of the fractogram peaks (Fig. 2A) could be a consequence of the particle distribution. To check the manufactures' data on the nAg standards, DLS and TEM measurements were performed. These data listed in Table 3 indicate that the standards purchased from B.B. International had a broad particle size distribution, which may have contributed to the peak shouldering. There was a reasonable

Table 3
Size measurements for nAg standards using TEM and DLS.

	10 nm[a]	20 nm[b]	40 nm[b]	60 nm[b]	80 nm[b]
DLS[c]	7.6 ± 0.7	37.5 ± 13.5	51.5 ± 31.4	92.1 ± 43.9	114.9 ± 46.8
TEM	5.1 ± 3.4	36.3 ± 17.0	75.3 ± 30.7	94.3 ± 47.3	157.2 ± 72.0

[a] ViveNano Inc.
[b] B.B. International.
[c] Volume mean values.

agreement between the DLS and TEM data. The mean sizes generated by DLS and TEM were larger than those provided by the manufacturer. It should be noted that DLS measures the hydrodynamic size of the particles rather than the physical size. The high standard deviation observed for the DLS data could be due to agglomeration of nanoparticles in the sample cell during the 10 min run time. TEM measurements may be biased to larger sizes due to the agglomeration of particles as an artefact of sample preparation. The analysis by TEM and DLS of the 10 nm standard purchased from ViveNano Inc. essentially confirmed the information provided by the manufacturer (Table 3).

3.3. Injection volume

The effect of the sample injection volume on the analytical sensitivity was investigated using the 10 nm ViveNano nAg standard (1 μg mL^{-1}) in order to maximize the sensitivity of the AF4 method. The use of a long sample loop enabled large sample volumes to be injected into the AF4 system. Fig. 3 shows fractograms for injected sample volumes of 0.5 mL, 0.75 mL and 1.5 mL. The linearity of response was good, with an R^2 value of 0.999. As shown in Fig. 3, no peak distortion or broadening was observed with increased injection volumes. Therefore, the choice of sample volume can be made based on the sensitivity required for a particular analysis. The capability of using large sample volumes will make the AF4 with UV–Vis detection more useful for routine analysis, and will attenuate the need for more sensitive (and expensive) detectors (e.g., ICP-MS).

3.4. Off-line and on-line analysis

The feasibility of off-line analysis of AF4 fractions was investigated to extend the range of applications of the analytical technique. Off-line analysis may be necessary when a lab facility does not have direct access to an ICP-MS instrument. In this circumstance, the collected fractions from AF4 system can be analyzed by ICP-MS using the instrumentation at another facility. In

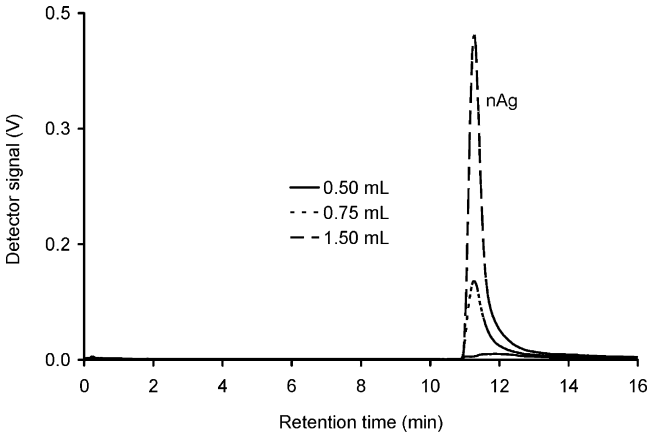

Fig. 3. Effect of injection volume on detector response of nAg colloid. Conditions: nAg (10 nm) colloid from ViveNano Inc., AF4 separation with on-line UV–Vis detection of nAg at 420 nm, operating parameters as in Table 2, injected same concentration (1 μg mL^{-1} nAg) using different loop.

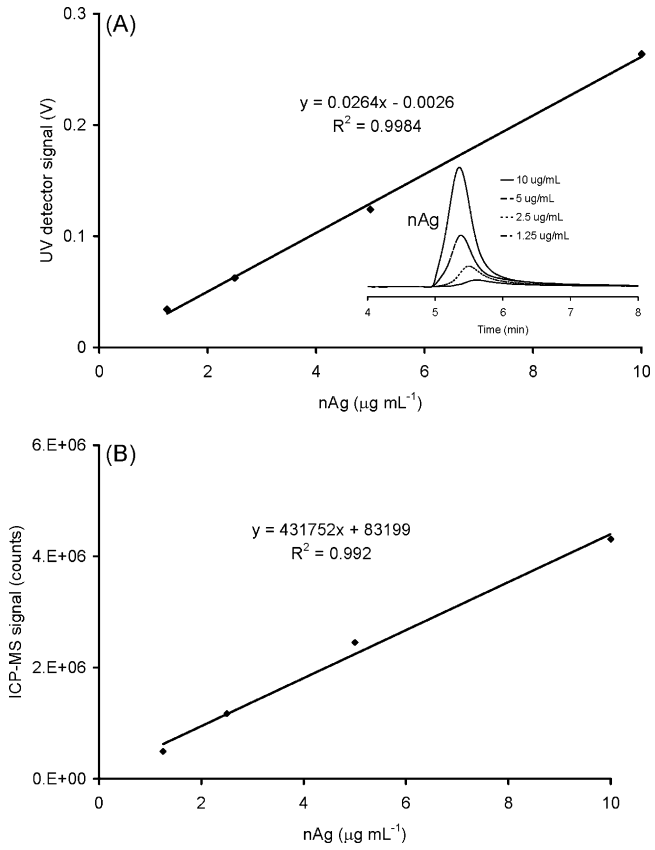

Fig. 4. Linearity of (A) on-line (UV–Vis) and (B) off-line (ICP-MS) analysis of AF4 fractions. Conditions: nAg (10 nm) colloid from ViveNano Inc., 5.0–6.5 min fractions collected, AF4 separation with on-line UV–Vis detection of nAg at 420 nm and operating parameters as in Tables 1 and 2.

addition, fractions can be collected so that other analytical methods (e.g., TEM) can be used to characterize the size and composition of the nAg in a sample. Using a 10 nm nAg standard (i.e., ViveNano), fractograms were obtained at varying concentrations (1.25–10 μg mL^{-1}) using on-line UV–Vis detection. Based upon the average retention time of 5.8 min, fractions were then collected over retention times between 5.0 and 6.5 min, followed by off-line analysis using ICP-MS. For both detection schemes, the responses were linear, with R^2 values of 0.998 and 0.992 for on-line AF4/UV–Vis and for AF4 with off-line ICP-MS detection, respectively (Fig. 4). Both showed a similar range of responses (Fig. 4). Therefore, off-line analysis of collected fractions should provide similar concentration data to on-line analysis for a colloidal system that has been characterized for AF4 retention time.

A fractogram for on-line AF4/ICP-MS analysis of the ViveNano standard (500 ng mL^{-1} nAg and 0.1 mL injection) is illustrated in Fig. 5. Monitoring of the indium internal standard ion intensity (5 ng mL^{-1} in 2% HNO_3) showed that the flow from the AF4 system was stable and no signal fluctuation was observed during the analysis. Hence, the use of an internal standard added through the mixing-T may be considered as optional. The internal standard signal monitoring can be useful to correct for signal drift (if any), which may be significant over the course of several hours. If an internal standard is not used, the flow (1 mL min^{-1}) from the AF4 system can pass directly to the nebulizer; eliminating the dilution effect and increasing the sensitivity. Also, it was found that addition of 2% HNO_3, through the mixing-T was not necessary to dissolve nAg prior to ICP-MS analysis of nAg standards. The ICP-MS nebulizer appears to efficiently ionize the nAg without need of dissolution in dilute acid prior to analysis.

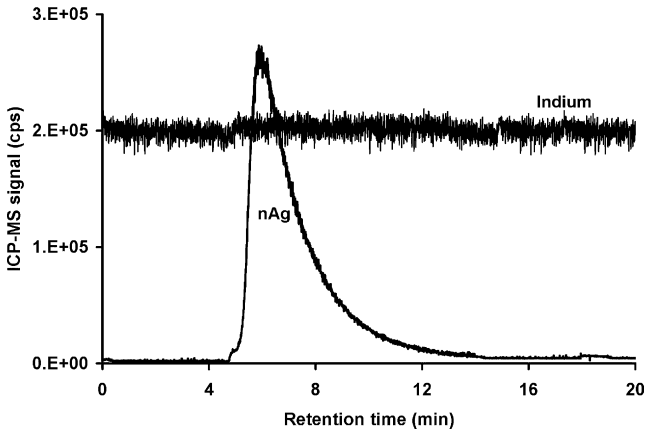

Fig. 5. A representative fractogram for on-line AF4/ICP-MS analysis of nAg. Conditions: nAg colloid (10 nm) from ViveNano Inc., Indium used as an internal standard, AF4 separation with on-line ICP-MS detection and operating parameters as in Tables 1 and 2.

3.5. AF4/ICP-MS performance

For analysis by AF4/ICP-MS, the limit of quantitation (LOQ) for nAg was calculated to be 1.4 ng mL^{-1} using a 0.10 mL injection volume. The LOQ was calculated as 10 times the standard deviation (SD) of the mean ICP-MS noise level. The limit of detection (LOD) was 0.80 ng mL^{-1} (i.e., 3 times SD of noise level). Further increases in sensitivity may be realized with larger injection volumes (see Section 3.3), as the estimated LOQ with a 1.5 mL injection volume was ~0.14 ng mL^{-1}. The linearity of response for AF4/ICP-MS was evaluated using a 10 nm ViveNano nAg standard of varying concentrations (10, 25 and 50 ng mL^{-1}), and an R^2 value of 0.992 was obtained. The recovery of nAg after AF4 separation with on-line ICP-MS detection relative to direct flow injection through the valve illustrated in Fig. 1 of an equivalent amount of nAg into the ICP-MS was 79.66 ± 7.15 (n = 4), when calculated according to Eq. (3):

$$\text{Recovery}\,(\%) = \frac{S}{S_0} \times 100 \quad (3)$$

where S is the signal (peak area) obtained from AF4 separation with ICP-MS detection, and S_0 is the signal (peak) obtained with flow injection into ICP-MS system. Thus, there is relatively little loss of nAg during AF4 separation before the eluent is directed to the detector.

3.6. Calibration

An external standard calibration approach involving the injection of a series of nAg standards prepared from the ViveNano material into the AF4 system followed by ICP-MS detection was used to generate an external calibration graph. With this approach, the calculated and actual concentrations of nAg were found to match in aqueous samples spiked with nAg. The injection of dAg (ionic silver) using the FI protocol (i.e., injection into the eluent flow after the AF4 separation) verified the Ag content in the same samples containing nAg. Hence, the external calibration approach with nAg is suitable for routine analysis of nAg concentration in aqueous samples. The FI approach requires addition of hardware, including an injection valve and a T-mixing manifold. However, the FI calibration approach only provides information on analyte concentration, whereas the external calibration approach provides information on both particle size and concentration. Calibration by standard additions, where an unknown sample is spiked with known concentrations, can also be a useful approach, as illustrated below.

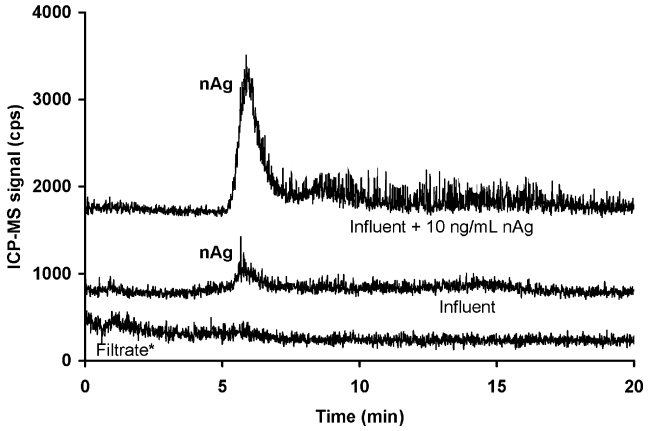

Fig. 6. Fractograms showing AF4/ICP-MS detection of nAg in wastewater influent. Conditions: AF4 separation with on-line ICP-MS detection and operating parameters as given in Tables 1 and 2, influent spiked with nAg (10 nm) from ViveNano Inc. *Filtrate of influent from ultra centrifugation (Amicon® Ultra centrifugal filter of 3 kDa MWCO).

3.7. Characterization of nAg in surface waters and wastewater

Samples of surface water were collected from Plastic Lake, Chemong Lake and the Otonabee River in central Ontario, Canada and a sample of untreated wastewater (i.e., influent) was collected from the WWTP for the city of Peterborough in Ontario, Canada. Ag was not detected by ICP-MS analysis in the three samples of surface water. For the filtered sample of WWTP influent, Fig. 6 illustrates the fractogram generated by AF4/ICP-MS analysis using a 0.10 mL injection volume and a new membrane in the channel. For these analyses, the sequence of injections into the AF4 system was (i) control, (ii) sample, (iii) spiked samples and (iv) calibration standards. The appearance of a peak in the fractograms from samples of both unspiked and spiked (10 ng mL^{-1}) influent confirms the presence of nano-sized particles composed of Ag (Fig. 6). As a control, a sample of filtrate prepared by ultrafiltration of the influent with Amicon® Ultra 3 kDa MWCO centrifugal filters according to methods described previously [12] were analyzed, and it did not contain detectable amounts of nAg (Fig. 6). This indicates that the filtrate did not contain nAg. In addition to the filtrate control, Milli-Q water was injected as a control, and no peaks were observed. The modal size of the nAg in influent sample calculated from the retention time at the peak maximum was 9.3 nm. This was calculated from the linear relationship between size and the retention time of the nAg colloid standards. Using Eqs. (1) and (2) (Section 2.1), the calculated modal size was 3.2 nm. The AF4 operation was performed at room temperature, and viscosity may vary with temperature. The discrepancy in the observed (9.3 nm) and calculated (3.2 nm) modal sizes could be the result of the use of an inappropriate value for viscosity in the Stokes–Einstein equation.

To estimate the concentration of nAg in the wastewater, the ViveNano nAg colloid of 10 nm size was used for the external calibration and standard addition methods, since the retention time of the nAg in the influent had a similar retention time. Using external calibration and standard additions methods, the nAg concentrations in the influent of the WWTP were found to be 2.16 and 1.90 ng mL^{-1}, respectively. Using the FI calibration approach, the measured Ag content was 0.38 ng mL^{-1}. The discrepancy in Ag concentrations generated using different calibration approaches indicates that the detected nAg in influent may not be composed of Ag alone. It cannot be ruled out that the detected materials could be colloid suspensions composed of insoluble forms of silver (e.g., Ag_2S and AgCl) rather than EN. A recent study indicated that nAg can be transformed into Ag_2S during anaerobic wastewater treatment processes in WWTPs [9]. It also cannot be ruled out that dAg associated with dissolved organic matter can be present as nano-sized colloidal particles, although this is speculative [27]. Currently, there is only one published report on the levels of nAg in wastewater from WWTPs. In a pilot study, Mitrano et al. [27] detected nAg at a concentration of 0.2 ng mL^{-1} in treated wastewater (i.e., effluent) from a WWTP located in Boulder, CO, USA. Gottschalk et al. [8] estimated from a predictive model that the concentrations of nAg in influents from WWTPs in Europe, USA and Switzerland would be in the range of 0.033–0.127 ng mL^{-1}. According to a report published by Swedish Environmental Research Institute, influents to municipal sewage wastewater treatment plants contained from 9 to 280 ng mL^{-1} of dAg [28]. The concentrations of nAg and dAg in wastewaters will probably vary over time and with location, depending on the usage of consumer products containing nAg, the population size, the sewage collection system (i.e., combined vs. separated) and the composition of the wastewaters (i.e., municipal vs. industrial). Further work is needed to evaluate the concentrations and composition of Ag in municipal wastewaters, and in surface waters downstream of WWTP discharges.

4. Conclusions

An analytical method to determine the size and concentration of nAg in aqueous matrices using AF4 with on-line UV–Vis and ICP-MS detection was developed and applied to the analysis of nAg standards and wastewater samples. The method is robust and does not require any sample pre-treatment prior to the analysis. For the analysis of nAg with a size range of approximately 1–80 nm, the run time per sample is ~20 min, so with washing steps the sample throughput is 3 samples per hour. Using the method presented here, the concentration and size range of nano-sized Ag was determined in influents of a wastewater treatment plant. This method shows promise for studies of the environmental fate of nAg in the aquatic environment, although it appears that the lower limit of quantification for the method will be in the high pg mL^{-1} (i.e., parts per trillion) or low ng mL^{-1} (i.e., parts per billion) range. This study provides a significant advance in the development of AF4/ICP-MS as a practical solution for determining the concentration and the range of nanoparticles suspended in aqueous matrices.

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