Evaluation of hydrodynamic chromatography coupled to inductively coupled plasma mass spectrometry for speciation of dissolved and nanoparticulate gold and silver

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ABSTRACT

In this study, hydrodynamic chromatography coupled to inductively coupled plasma mass spectrometry has been evaluated for the simultaneous determination of dissolved and nanoparticulate species of gold and silver. Optimization of mobile phase was carried out with special attention to the column recovery of the different species and the resolution between them. Addition of 0.05 mM penicillamine to the mobile phase allowed the quantitative recovery of ionic gold and gold nanoparticles up to 50 nm, whereas 1 mM penicillamine was necessary for quantitative recovery of ionic silver and silver nanoparticles up to 40 nm. The resolution achieved between ionic gold and 10 nm gold nanoparticles was 0.7, whereas it ranged between 0.31 and 0.93 for ionic silver and 10 nm silver nanoparticles, depending on the composition of mobile phase. Mass concentration detection limits for gold and silver species were 0.05 and 0.75 μg L^{-1}, respectively. The developed methods allowed the simultaneous detection of nanoparticulate and dissolved species of gold and silver in less than 10 minutes. Size determination and quantification of gold and silver species was carried out in different dietary supplements, showing good agreement with the results obtained by electron microscopy and total and ultrafiltrated contents, respectively.

Keywords: Gold; Silver; Nanoparticles; Speciation; Hydrodynamic Chromatography, ICP-MS.

Declarations

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Authors' contributions

MSJ is the supervisor and coordinator of all experimental work and data revision. She did the experimental work regarding the analysis of gold dietary supplement. She is the responsible of the writing of manuscript.

MB is the responsible of experimental work regarding the characterization and quantification of silver nanoparticles and dissolved silver and the analysis of silver dietary supplements. She is the author of all figures.

DI did the experimental work regarding the characterization of gold nanoparticles and dissolved gold.

MTG is the coordinator of work in the group laboratory being the responsible of supplies and has collaborated in the writing and revision of the manuscript.

JPA is the responsible of FESEM and TEM measurements and data interpretation. She has collaborated in the revisión of the manuscript.

FL is the main researcher of project RTI2018-096111-B-I00 (MICINN/FEDER). He has supervised either the experimental work either the writing and revision of the manuscript.

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Introduction

The continuous advances in nanoscience involve the growing use of different engineered nanoparticles (ENPs) in an increasing number of consumer products. This fact requires the adaptation of existing techniques and methods, or the development of new ones, to monitor their occurrence, fate and transformations in different scenarios. In order to understand the environmental impact or the toxicological mechanisms of inorganic ENPs, it is critical to discriminate among dissolved and particulate forms of the element involved. Different techniques and methodological approaches for the characterization and quantification of ENPs and its derivatives in complex samples have been recently reviewed [1-5].

Asymmetric flow field flow fractionation (AF4) and hydrodynamic chromatography (HDC) are commonly coupled to inductively coupled plasma mass spectrometry (ICP-MS) as element specific detector for the separation and determination of inorganic ENPs in a variety of samples [1, 3]. Gray et al. [6] compared HDC and AF4, both coupled with ICP-MS, with respect to their capacity to detect, quantify, and characterize gold nanoparticles (AuNPs). They found that, although HDC is a robust and versatile separation technique, its resolving power is much lower than AF4, being even lower in the smallest size range. On the other hand, recoveries for HDC were better than for AF4 on average, ranging from 77 to 96% for HDC and from 4 to 89% for AF4. An additional advantage of HDC over AF4 lies in the analysis time, which can be reduced to less than 10 minutes, in comparison with 30-45 minutes of AF4, and dissolved low molecular mass species are not lost, as in AF4 due to the ultrafiltration membranes used in its separation channel. Thus, HDC-ICP-MS can provide simultaneous information about dissolved and particulate species of an element in less than about ten minutes, which is not the case of AF4.

HDC is a liquid chromatographic separation technique in which the sample is injected into a column packed with non-porous beads, building up flow channels, and separation is produced by the velocity gradient within the capillaries between beads. Thus, larger particles are transported faster than smaller ones, as they spend less time near the edges of the capillaries. In packed-columns, the beads should be inert, that is, made of a material that minimizes non-HDC enthalpic interactions between the beads and the dissolved analytes. Such non-HDC effects can be

minimized through the addition of salts and/or surfactants to the solvent/mobile phase to screen electrostatic and Van der Waals interactions, which are especially common in aqueous media [7]. Separation in HDC arises from the parabolic or Poiseuille-like flow profile that develops, under laminar flow conditions in the interstitial medium of a packed column, where the fastest streamlines of flow are in the middle of the interstitial medium and the slowest ones are near the packing particles. The first experimental paper on HDC was published in 1974 by Small [8], who employed a series of packed columns to separate and determine the particle size of various polystyrene latexes, carbon black and colloidal silica. Since the first application of HDC coupled to ICP-MS [9], HDC has become popular in environmental analysis to understand the behavior, occurrence and fate of nanomaterials (NMs) and colloids [10-14]. Due to the simplicity of the separation mechanism, the size determination and method development are straightforward [7, 9, 11]. For instance, the coupling of HDC to ICP-MS in single-particle mode (SP-ICP-MS), first described by Pergantis et al. [15], was applied to characterize AuNPs in drinking water by determining particle mass, hydrodynamic diameter, and number concentration.

The relative low size resolution of HDC compared to other size separation methods [5] is more relevant when analyzing NP mixtures [6, 9, 15, 16]. Gray et al. [6] showed the inability of HDC to separate a mixture of AuNPs, but also showed potential for separation of the Au dissolved from the AuNPs standards. They suggested it might be possible to separate Au dissolved from NPs greater than 20 nm although without reporting any recoveries for dissolved Au. To the best of our knowledge, there are very few references in which the separation and potential quantification of ENPs and their corresponding metal ions using HDC-ICP-MS have been studied. Philippe et al. [11] studied the effect of the eluent composition on the retention factors of polystyrene nanoparticle standard, Rh^{3+} and AuNPs. They reported that when using a mobile phase without phosphate ions the recovery for gold ions was 20% worse than when phosphate is used due probably to a stabilization effect of phosphate ions throughout the elution. In any case they did not report any quantitative recoveries for AuNPs neither for ionic Au. Roman et al. [17] using HDC-SP-ICP-MS determined the concentration of dissolved Ag and the distribution of silver NPs (AgNPs), in terms of hydrodynamic diameter, mass-derived diameter, number and mass

concentration, in plasma and blood of burn patients. Peak from dissolved Ag in plasma (most probably complexed by proteins) could not be chromatographically resolved from those of 20-nm particles, thus an innovative algorithm was implemented to deconvolute the signals of dissolved Ag and AgNPs and to extrapolate a multiparametric characterization of the particles in the same chromatogram. Pitkänen et al. [18] studied the quantitative characterization of AuNPs contrasting Size-Exclusion Chromatography (SEC) and HDC coupled with ICP-MS. They obtained that column recoveries for AuNPs were complete and at least matched for both alternative methods. They reported a recovery value of 99±2 % for ionic Au using HDC-ICP-MS although no chromatogram of AuNPs and ionic Au all together was shown.

The aim of this work was to develop and evaluate separation methods based on the use of hydrodynamic chromatography coupled to ICP-MS for the simultaneous speciation of dissolved species and nanoparticles of gold and silver, being scarce the references in bibliography. The aim is focused in both, nanoparticles size characterization and quantification of the different species. Optimization of the proposed HDC-ICP-MS method was carried out paying special attention on column recovery and resolution between dissolved species and nanoparticles. The methods were applied to the size characterization and quantitation of gold and silver species in dietary supplements containing colloidal and/or ionic species.

Experimental

Materials and methods

Instrumentation

The high performance chromatographic system used was a Waters 2796 Bioseparations Module (Waters Corporation, Milford, USA). HDC separations were performed in a PL-PSDA type 1 column (Agilent Technologies, Germany) with a nominal separation range of 5-300 nm, a length of 80 cm and an internal diameter of 7.5 mm. The exit of the column was connected in series to an UV-visible detector (Waters 996 Photodiode Array detector), and to an ICP mass spectrometer (ELAN DRC-e, PerkinElmer, Toronto, Canada) for element specific detection. The outflow from the system was delivered directly to the nebulizer of the spectrometer, a glass concentric Slurry

nebulizer with a cyclonic spray chamber (Glass Expansion, Melbourne, Australia) were used. Table 1 summarizes the experimental ICP-MS and HDC conditions. Origin 8 (OriginLab, Northampton, MA, USA) was used for processing the chromatograms.

A gold dietary supplement was analyzed by Transmission Electron Microscopy (TEM) with a JEOL-2000 FXII (JEOL Ltd, Tokio, Japan) at 200 kV. The sample was prepared by placing 20 µL of the supplement suspension on a carbon-coated copper grid and drying at room temperature. Silver dietary supplements were analyzed by Field-Emission Scanning Electron Microscopy (FESEM) with Energy-Dispersive Spectrometry (EDS). The observations were performed in a Merlin™ FESEM microscope equipped with a Gemini column (Carl Zeiss Nano Technology Systems, Germany), working at 5 kV with the in-Lens secondary-electron detector, in order to improve resolution. FESEM was coupled with an X-Max X-ray microanalyzer (Oxford Instruments, UK). 20 µL of the product were deposited on a copper-grid holder and, once the solvent was evaporated, coated with carbon. Image analysis was carried out using ImageJ software, and average particle diameter was determined by randomly measuring the area of a certain number of particles in the images, and calculated as equivalent circle diameter.

Chemicals

Diluted suspensions of gold and silver nanoparticles were prepared from commercially available suspensions. Suspensions of gold nanoparticles, stabilized in 2mM citrate with nominal concentrations of 50 mg L^{-1} and nominal diameters of 10, 50 and 100 nm (11.6±1, 52±6 and 97±11 nm, respectively), and suspensions of silver nanospheres (Nanoxact), stabilized in 2 mM citrate with nominal concentrations of 20 mg L^{-1} and nominal diameters of 10, 20, 40 and 60 nm (10.3±2.1, 18.6±2.7, 39±5 and 59±6 nm), were all purchased from NanoComposix (San Diego, CA, USA). Ionic gold (1001±4 mg L^{-1}) and ionic silver standards (994±3 mg L^{-1}) were provided by Fluka BioChemika (Buchs, Switzerland).

Table 2 summarizes the different mobile phases tested for HDC separations. The pH of the mobile phases was in all cases 7.5. Na_2HPO_4, DL-penicillamine (PA) and Triton X-100 were obtained from Sigma–Aldrich Chemie (Stenheim, Germany), sodium dodecyl sulphate (SDS) from Bio-

Rad Laboratories (Hercules, USA), and formaldehyde 37% (w/w) solution from Probus (Barcelona, Spain).

Ultrapure water (Milli-Q Advantage, Molsheim, France) was used for the preparation of the HDC mobile phases and dilutions.

Samples

A liquid nutritional supplement containing gold nanoparticles, with a nominal concentration of 20 mg L^{-1} and a nominal size of 3.2 nm, was purchased from a website distributor.

Three commercial colloidal silver products, purchased from different website distributors, were also analyzed. These products are recommended as health products intended for oral administration or as surface sanitizers for external use. The products were kept in a dark place at room temperature until analysis.

Determination of the total Au and Ag concentration in nanoparticle suspensions and nutritional supplements

Dissolution of gold nanoparticles was performed by adding 150 µL of nitric acid (69/70%, J.T. Baker (Phillipsburg, USA) and 310 µL of hydrochloric acid (37%, J.T. Baker (Phillipsburg, USA) to 100 µl of the suspension to be analyzed [11]. After one hour at room temperature, solutions were diluted with ultrapure water up to 10 mL. The concentrations of total gold in AuNPs and the nutritional supplement were measured by ICP-MS using rhodium as internal standard. Dissolution of AgNPs was performed by adding 750 µL of nitric acid to 750 µL of AgNPs standard and after one hour at room temperature, solutions were diluted with ultrapure water up to 5 mL. For the nutritional supplements, between 500 µL of sample and 1.5 mL of nitric acid were added to 500 µL of the supplement, after one hour at room temperature, solutions were diluted with ultrapure water up to 5 mL. The concentrations of total silver in AgNPs and nutritional supplements were measured by flame atomic absorption spectrometry (FAAS).

Determination of ionic gold and silver in nutritional supplements

The ionic species in the suspensions were isolated by removing gold and silver nanoparticles using Nanosep Pall centrifugal ultrafilter devices with cut-off membranes of 3 kDa (equivalent to 2 nm hydrodynamic diameter). Ultrafiltration devices were washed by centrifugation with 500

µL of ultrapure water twice. The second washing was kept to check for any potential contamination. Suspensions were sonicated for two minutes, 500 µL were subjected to centrifugation for 30 min at 9000 rpm and 20 ºC (in a Thermo Heraeus Multifuge X1R, equipped with a fixed angle rotor for Eppendorf tubes, Walthman, USA). For gold determination the ultrafiltrate (ca. 500 µL) was diluted up to 10 mL with 5% HCl prior to ICP-MS analysis. For silver determination the ultrafiltrate (ca. 500 µL) was diluted up to 5 mL with 1% HNO_3 prior to FAAS analysis.

Column recovery calculation

Potential loses of analytes during the chromatographic separations were evaluated through the corresponding column recoveries, which were calculated from the ratio of the ICP-MS peak areas of standards injected into the chromatographic system with and without the HDC column, under the selected operational conditions. Three replicates of each measurement were performed. 250 ng mL^{-1} of Au(III), AuNPs (10, 50 and 100 nm), Ag(I) and AgNPs (10, 20, 40 and 60 nm) solutions were used for recovery calculations. Au(III) and Ag(I) solutions were stabilized with different concentrations of PA depending on the mobile phase used.

Resolution calculation

In all cases, resolution was calculated as $2(t_2-t_1)/(w_1+w_2)$, where T_1 and T_2 are retention times, and W_1 and W_2 are peak widths of 10 nm AuNPs or AgNPs and ionic Au or Ag, respectively.

Results and discussion

Selection of mobile phases

Although different mobile phases have been reported for the separation of metallic nanoparticles by HDC, the column manufacturer recommends a mobile phase consisting of 0.5 mM Na_2HPO_4, 0.45 mM SDS, 0.05% Triton X-100, 0.05% formaldehyde, pH 7.5 (CM mobile phase), which is used in most of the published works as such or with small modifications [6, 9, 11, 12, 17]. The low ionic strength of this mobile phase reduces electrostatic interactions between the NPs and the packing material because of the increase of the double layer [20], the non-ionic surfactant Triton

X-100 is used to prevent the aggregation of nanoparticles, whereas the anionic surfactant SDS prevents the sorption of nanoparticles onto the column packing material. Formaldehyde is used for its bactericidal properties and sodium hydrogen phosphate helps to maintain the neutral pH of the mobile phase.

Whereas most of works have focused on the separation of nanoparticles, the objective of this work was the development of chromatographic methods that allowed the separation of dissolved forms of gold and silver from their nanoparticle counterparts with adequate resolution and recoveries of the different species. Figure 1a shows the separation of AuNPs using the manufacturer mobile phase at a flow rate of 1.6 mL min^{-1}. Standard AuNPs of 10, 50 and 100 nm were eluted at 8.26, 8.08 and 7.94 min, respectively. Recoveries for the AuNPs ranged from 68 to 77 % (table 3), whereas ionic gold was fully retained in the column.

By using a simpler mobile phase containing 0.45 mM SDS at pH 7.5 (based on our previous experience [21]), AuNPs were eluted at slightly different retention times (8.10, 7.90 and 7.82 min for 10, 50 and 100 nm AuNPs, respectively), as it can be shown in the chromatogram of figure 1b. Better recoveries were obtained for AuNPs of 10~~,~~ and 50 nm, except for~~and~~ 100 nm (88, 83 and 44%, respectively, see table 3), although ionic gold was still retained into the column and no peak was obtained.

Although the nature of the packing material is not available from the manufacturer, cation exchange resin beads have been reported as a typical packing in HDC [22]. Thus it seems feasible that both the nature of the packing and the instability of the cationic gold in the media contribute to its irreversible retention into the column. Different ligands (e.g., PA, thiosulfate) have been used for stabilization of cationic forms of elements subjected to separation by micellar electrokinetic [23] or reverse phase chromatography [19]. The addition of L-cysteine, methionine and PA to a mobile phase consisting of 0.45 mM SDS at pH 7.5 was studied in order to stabilize ionic gold without affecting the elution of the nanoparticles. PA was finally selected, since using L-cysteine or methionine ionic gold was not eluted or irreproducible results were obtained.

Three concentrations of PA (0.05, 0.2 and 0.5 mM) were investigated. Recoveries obtained for the AuNPs of 10, 50 and 100 nm and ionic gold at different PA concentrations are shown in Table

3. ~~Recoveries were around 100 % for ionic Au, 10 nm and 50 nm AuNPs with a concentration of 0.05 mM PA.~~ Recoveries in the range of 88-116% were obtained for ionic Au and 10 nm AuNPs at any PA concentration. For 50 nm AuNPs the recovery was around 100% with a PA concentration of 0.05 mM and decreased down to 76% at the highest PA concentration of 0.5 mM.~~At the highest PA concentration of 0.5 mM the recovery for 50 nm AuNPs decreased down to 76%.~~ Recoveries for the 100 nm AuNPs were low at any PA concentration (72- 61%), but this was also the case for the manufacturer mobile phase and 0.45 mM SDS mobile phase without PA (69% and 44%, respectively), as it is also shown in table 3). Best recoveries for ionic Au and AuNPs were obtained by using a PA concentration of 0.05 mM, being used in all further experiments.

Figure 1c shows the chromatogram obtained using a mobile phase containing 0.45 mM SDS and 0.05 mM PA. Using this mobile phase at 1.6 mL min^{-1}, an acceptable resolution of 0.70 between 10 nm AuNP and ionic Au was obtained (see Figure 1c), which could be improved by working at lower flow rates (up to 1.03 at 1.0 mL min^{-1}, as it can be seen in figure 1 of supporting information). ~~In all cases, resolution was calculated as $2(t_2-t_1)/(w_1+w_2)$, where T_1 and T_2 are retention times, and W_1 and W_2 are peak widths of 10 nm AuNPs and ionic Au, respectively.~~

Separation conditions for ionic silver and silver nanoparticles were based on the previous experience gained with gold. To evaluate the behavior of AgNPs in the HDC column, both the manufacturer mobile phase and 0.45 mM SDS were studied by using UV-visible detection. The best recoveries were obtained with the manufacturer mobile phase, ranging from 100 to 92% for 10-60 nm AgNPs (table ~~2~~ S1 of supporting information). Therefore, the manufacturer mobile phase with different PA concentrations of (0.05, 0.2, 0.5 and 1 mM) was considered for stabilizing ionic silver and studying its separation from AgNPs using ICP-MS detection. Recoveries for the different PA concentrations are shown in Table 3. As it can be observed, good recoveries for ionic Ag (106%) were achieved by using 1 mM PA, higher than for ionic gold (0.05 mM). The recoveries for AgNPs were around 80% for 10 and 40 nm and 47% for 60 nm. In spite of the good

recovery obtained by stabilizing the ionic silver with 1 mM of PA, resolution between 10 nm AgNPs and ionic Ag was too low (R=0.16) (figure 2 of supporting information). The use of a lower flow rate (1 mL min^{-1}) did not improved the resolution in this case (R= 0.17). As an alternative for improving resolution, the reduction of the retention time of the nanoparticles by decreasing the ionic strength of the mobile phase was investigated, because at high ionic strength strong Vander Waals interactions dominate over the hydrodynamic effect, increasing the retention times of nanoparticles [20]. Mobile phases with lower ionic strength than manufacturer's one, containing 1 mM PA for stabilization of ionic silver and different SDS concentrations, were studied. The resolution between 10 nm AgNPs and ionic Ag peaks increased from 0.31 up to 0.93 by decreasing SDS concentration from 0.45 down to 0.22 mM. Although the best resolution was achieved for the mobile phase containing 0.22 mM SDS and 1mM PA, recoveries were acceptable just for 10 nm AgNPs and ionic Ag (101 and 90%, respectively); whereas for 20, 40 and 60 nm AgNPs recoveries were below 20%, most probably because of the instability and sorption of the larger NPs onto the column packing material. Thus, a mobile phase containing 0.34 mM SDS and 1mM PA was selected as a compromise between resolution and recovery. The chromatograms obtained under such conditions are presented in figure 2. The resolution achieved between the 10 nm AgNPs and ionic Ag peaks was 0.63, with recoveries for Ag (I), 10 nm and 20 nm AgNPs of 103%, 102% and 72%, respectively.

Analytical performance

Both logarithmic [9,10,13] and linear [11] fittings (by plotting retention time vs. diameter, or retention time vs. square root of diameter, respectively) have been applied for size calibration in HDC. Although some improvement in linearity has been reported when using the latter approach, no significant differences were observed in this work, with good logarithmic and linear fits, showing correlation coefficient in the range of 0.986 to 1 in both cases (table ~~4~~ S2 of supporting information).

The retention times obtained for the different mobile phases studied are summarized in tables ~~1~~ S3 and ~~3~~ S4 of the supporting information for gold and silver species, respectively. In all cases,

retention time repeatability was about 0.1% (n=3). Gold and silver mass concentration calibrations were performed by using ionic standards prepared in the mobile phase selected for each element and injected in flow injection mode without column. Flow injection and chromatographic peaks were processed through their peak areas for mass concentration determination, showing good linearity in the range of 50 to 300 µg L^{-1} (R=0.999).

Limits of detection (LOD) and quantification (LOQ) were related to the detectability of the peaks according to their height, and they were calculated as 3 and 10 times the baseline standard deviation divided by the slope of the peak height calibration. Under such conditions, best-case limits of detection and quantification for gold were 0.05 and 0.16 µg L^{-1} respectively, and 0.75 and 2.5 µg L^{-1} for silver.

Analysis of gold and silver species in nutritional supplements

One gold and three silver nutritional supplements were analyzed using the mobile phases optimized in the study and the operating conditions described in the Experimental Section. Table 4 summarizes the concentrations and sizes obtained for the different species found in the nutritional supplements.

According to the manufacturer, the gold nutritional supplement contained 20 mg L^{-1} of gold as gold nanoparticles of 3.2 nm, whereas ionic gold was absent. Total content of gold was determined by ICP-MS following the procedure described in the Experimental Section, obtaining a concentration of 21.0±0.8 mg L^{-1}, in agreement with the value given by the manufacturer. The nutritional supplement was analyzed by HDC-ICP-MS using 0.45 mM SDS and 0.05 mM PA as mobile phase. Figure 3a shows the chromatogram corresponding to a 1:150 dilution of the product in mobile phase. The chromatogram consisted of a peak at 7.99 min and a small shoulder at 8.~~74~~ 47 min. The peak at 7.99 min corresponded to nanoparticles of 6.5±0.1nm (using linear fitting calibration), in agreement with the size of 6 nm found by France et al. using MECK-ICP-MS [23]. The shoulder at 8.~~74~~ 47 min corresponded to ionic gold, whose presence was also reported by France et al. [23]. The nutritional supplements were also studied by electron microscopy in order to compare NP sizes. The NP size was determined by calculating the equivalent circle diameter

~~Image analysis was carried out using ImageJ software, and average particle diameter was calculated~~ by randomly measuring 87 particles in the images. In this way, the study of the gold supplement by TEM also showed a distribution maximum in a similar size (figure 4), with 5.1±2.4 nm diameter, even though some bigger particles appeared in the TEM images. Quantification of the peak corresponding to AuNPs was ~~136.1±5.0 µg L^{-1}, which corresponded to a concentration of~~ 20.4±0.8 mg L^{-1} in the original supplement, in agreement with the total concentration found by ICP-MS (21.0±0.8 mg L^{-1}). Although the determination of Au(III) by ultrafiltration and ICP-MS was attempted, it was below the detection limit of the method (2.3 µg L^{-1}). To check the capability of the method for detection and quantification of ionic gold, the nutritional supplement was spiked with ionic gold (155 µg L^{-1}) (see figure 3b). Ionic gold was eluted in the sample at 8.78 min with a mass recovery of 88.7±4.5%.

Three different nutritional supplements containing colloidal and/or ionic silver were analyzed. According to the manufacturer, Supplement 1 contained colloidal and ionic silver with a silver concentration of 25 mg L^{-1}, Supplement 2 contained colloidal silver with a concentration of 10 mg L^{-1} and a size range between 0.6-5 nm and Supplement 3 contained colloidal silver with a concentration of 30 mg L^{-1} and a size range between 1-100 nm. The three supplements were firstly analyzed by ultrafiltration and flame atomic absorption spectroscopy (FAAS) to determine ionic and total silver, obtaining the concentrations shown in table 4. The three nutritional supplements were analyzed by HDC-ICP-MS using the mobile phase containing 0.34 mM SDS and 1 mM PA at a flow rate of 1.6 mL min^{-1} after dilution in the same mobile phase. The chromatograms obtained are shown in figure 5. The chromatogram of Supplement 1 showed two peaks at 7.99 and 8.48 min, corresponding to AgNPs of 23.7±0.8 nm and ionic Ag. The chromatogram of Supplement 2 just showed a peak at 8.46 min with a small shoulder at 8.1min which can be assigned to ionic Ag and AgNPs of 11.6±2.2 nm; whereas the chromatogram of Supplement 3 showed two peaks at 7.89 and 8.48 min, corresponding to AgNPs of 47.9±0.03 nm and ionic Ag, respectively. In all cases size linear fittings calibration have been used.

The silver supplements were studied by FESEM, due to the expected bigger AgNP sizes. Figure 6 shows FESEM micrographs from Supplements 1 and 2. AgNP size distributions were also

calculated using ImageJ software (diameters of 82 and 67 particles were randomly measured, respectively for Supplements 1 and 2), showing mean sizes of 23.4±5.8 and 14.2±6.8 nm, which were in fair agreement with the sizes obtained by HDC-ICP-MS. AgNPs in Supplement 1 were quite regular in size. However, in FESEM images, silver distribution of Supplement 2 was heterogeneous. No reliable electron images were obtained for Supplement 3, most probably due to the composition of the sample.

The concentrations of different silver species obtained for each supplement are shown in table 4, where results obtained for total and ionic silver by ultrafiltration and FAAS are also included for comparison. HDC-ICP-MS quantification for Supplements 1 and 2 were in very good agreement with the results obtained by ultrafiltration and FAAS. The small shoulder obtained for Supplement 2 by HDC-ICP-MS, corresponding to 11.6 nm AgNPs, was not quantifiable (bellow LOQ: 2.5 µg L^{-1}). Different batches from the same supplements had been previously analyzed by Anodic Stripping Voltammetry [24], obtaining similar results. For Supplement 3, the concentration obtained by HDC-ICP-MS was lower than the total silver concentration determined by AAS due to the low recoveries achieved for nanoparticles over 40 nm.

Conclusions

Although HDC-ICP-MS has been used successfully for the separation and quantification of inorganic nanoparticles, this hyphenated technique suffers from the inherent low resolution of hydrodynamic chromatography. However, the strength of HDC-ICP-MS lies in its capability for the simultaneous determination of dissolved species and nanoparticles of the same element, which had not been exploited yet. In this study, a HDC-ICP-MS methodology has been developed for the separation and determination of dissolved species and nanoparticles of gold and silver, by stabilizing the ionic forms of both elements by adding penicillamine to the mobile phases. Using a mobile phase containing penicillamine at 0.05 mM, ionic gold quantitatively eluted from the HDC column, whereas higher concentration, up to 1 mM, were required for ionic silver. Acceptable resolution (around R=0,7) between dissolved species and metallic nanoparticles was obtained with the optimized mobile phases.

Using the developed methods, it was possible the sensitive and simultaneous determination of the mass concentration as well as the size of different gold and silver species in different consumer products. The sizes of the different gold and silver NPs found by HDC-ICP-MS in the dietary supplements were in very good agreement with the sizes found by TEM or FESEM respectively. Quantitative results of different gold and silver species in the consumer products were also in good agreement with the ones obtained by ICP-MS (gold) and ultrafiltration and FAAS (silver) except for the concentration of AgNPs in one of the sample due to the low recovery of the NPs of big size found in the sample.

Simultaneous size determination and quantification of different gold and silver species was carried out by first time by HDC-ICP-MS.

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Conflicts of interest/Competing interests

No conflicts of interest

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Table 1 Instrumental operating conditions

ICP-MS	
Forward power (w)	1100
Argon gas flow rate	
Plasma (L min^{-1})	15
Auxiliar (L min^{-1})	1.2
Nebulizer gas flow rate (L min^{-1})	1.0
Sweeps per reading	1
Dwell time	50 ms
Acquisition mode	Peak hopping
Isotopes monitored	^{196}Au, ^{107}Ag, ^{109}Ag
HDC	
Column	PL-PSDA type 1
Flow rate (mL min^{-1})	1.6
Injection volume (μL)	50

Table 2 Composition of the different mobile phases used in HDC-ICP-MS separations

Gold species	
• 0.5 mM Na_2HPO_4, 0.45 mM SDS, 0.05% Triton X-100, 0.05% formaldehyde (CM)	pH 7.5
• 0.45 mM SDS	pH 7.5
• 0.45 mM SDS, 0.05-0.5 mM DL- Penicillamine	pH 7.5
Silver species	
• 0.5 mM Na2HPO4, 0.45 mM SDS, 0.05% Triton X-100, 0.05% formaldehyde, 0.05-1 mM DL-penicillamine	pH 7.5
• 0.22-0.45 mM SDS, 1 mM DL- Penicillamine	pH 7.5

Table 3 Recoveries for gold and silver species separated by HDC-ICP-MS and different mobile phases (n=3). Flow rate: 1.6 mL min^{-1}. CM: mobile phase recommended by the column manufacturer

Mobile phase	Recovery (%)			
	Au(III)	10 nm AuNPs	50 nm AuNPs	100 nm AuNPs
CM	not eluted	76.9 ± 1.4	74.3 ± 1.0	68.7 ± 3.2
0.45 mM SDS	not eluted	87.9 ± 1.4	83.3 ± 4.7	44.1 ± 2.0
0.45 mM SDS +				
0.05 mM PA	94.2 ± 2.7	103 ± 1.3	102 ± 1.6	71.5 ± 9.4
0.20 mM PA	88.4 ± 4.7	114 ± 2.3	82.1 ± 1.6	65.9 ± 4.1
0.50 mM PA	89.2 ± 6.1	116 ± 2.4	76.0 ± 8.1	61.1 ± 1.7

	Ag(I)	10 nm AgNPs	20 nm AgNPs	40 nm AgNPs	60 nm AgNPs
CM +					
0.05 mM PA	25.4 ± 4.3	65.8 ± 4.7	65.7 ± 3.5	65.7 ± 9.2	23.9 ± 0.9
0.2 mM PA	39.6 ± 0.6	100.0 ± 5.2	107.0 ± 10.7	90.9 ± 9.8	48.7 ± 2.9
0.5 mM PA	34.6 ± 6.5	76.5 ± 5.6	81.0 ± 0.9	80.3 ± 9.0	75.6 ± 2.2
1.0 mM PA	106.0 ± 5.9	89.3 ± 8.4		88.9 ± 8.6	47.3 ± 0.7
1mM PA +					
0.45 mM SDS	105.0 ± 19.5	62.9 ± 2.0		82.6 ± 4.9	56.8 ± 6.1
0.34 mM SDS	103.0 ± 10.1	102.3 ± 3.4	71.9 ± 10.7	29.5 ± 6.4	3.7 ± 0.9
0.22 mM SDS	101.0 ± 10.1	90.2 ± 9.2	21.3 ± 3.8	9.7 ± 4.7	6.0 ± 1.3

Table 4 Sizes and concentration of different gold and silver species in nutritional supplements

	HDC-ICP-MS		ICP-MS	TEM
Concentration	AuNPs ($mg\ L^{-1}$)	Au(III) ($mg\ L^{-1}$)	Total Au ($mg\ L^{-1}$)	
Gold supplement	20.4 ± 0.8	~~<##~~<LOD	21.0 ± 0.8	
Size	AuNPs (nm)			AuNPs (nm)
	6.5 ± 0.1			5.1± 2.4

	HDC-ICP-MS			AAS		FESEM
Concentration	AgNPs ($mg\ L^{-1}$)	Ag(I) ($mg\ L^{-1}$)	Total Ag ($mg\ L^{-1}$)	Ag (I) ($mg\ L^{-1}$)	Total Ag ($mg\ L^{-1}$)	
Silver supplement 1	4.3 ± 1.2	14.2 ± 0.5	18.5	16.8 ± 0.5	21.3 ± 2.1	
Silver supplement 2	<LOQ	10.9 ± 0.6	10.9	11.6 ± 1.0	12.0 ± 1.6	
Supplement 3	26.3 ± 0.3	3.7 ± 0.7	29.9	1.7 ± 0.6	37.5 ± 0.8	
Size	AgNPs (nm)					AgNPs (nm)
Silver supplement 1	23.7 ± 0.8					23.4± 5.8
Silver supplement 2	11.6 ± 2.2					14.2± 6.7
Silver supplement 3	49.7 ± 0.03					-

List of figures and captions

Fig. 1 HDC-ICP-MS chromatograms corresponding to ionic gold and 10 nm, 50 nm and 100 nm AuNPs (250 μg L^{-1}) using different mobile phases. (a) Column manufacturer mobile phase, (b) 0.45 mM SDS, (c) 0.45 mM SDS and 0.05 mM penicillamine. Concentration: 250 μg L^{-1}. Flow rate: 1.6 mL min^{-1}

Fig. 2 HDC-ICP-MS chromatograms corresponding to ionic silver and 10 nm, 20 nm, 40 nm and 60 nm AgNPs using mobile phase containing 0.34 mM SDS and 1 mM penicillamine Concentration: 250 μg L^{-1}. Flow rate: 1.6 mL min^{-1}

Fig. 3 HDC-ICP-MS chromatograms of (a) gold nutritional supplement and (b) spiked with 155 μg L^{-1} of ionic gold. Mobile phase: 0.45 mM SDS and 0.05 mM penicillamine. Flow rate: 1.6 mL min^{-1}

Fig. 4 TEM image and size distribution of the gold nutritional supplement

Fig. 5 HDC-ICP-MS chromatograms of silver nutritional supplements. (a) supplement 1, (b) supplement 2, (c) supplement 3. Mobile phase: 0.34 mM SDS and 1 mM penicillamine. Flowrate: 1.6 mL min^{-1} flow

Fig. 6 FESEM images and size distributions of silver nutritional supplement 1 (a) and 2 (b)

Fig. 1

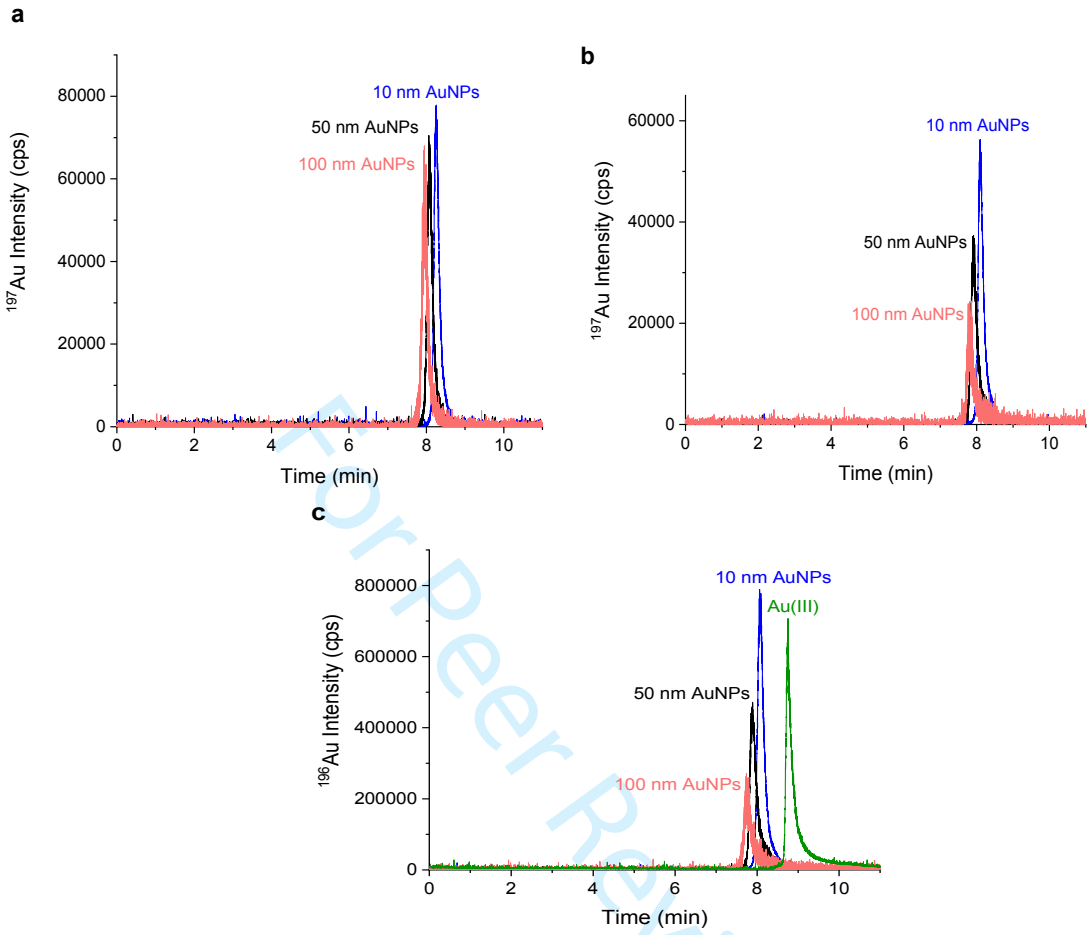


Fig. 2

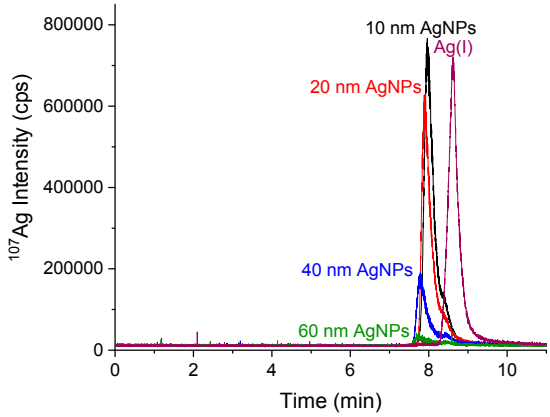


Fig. 3

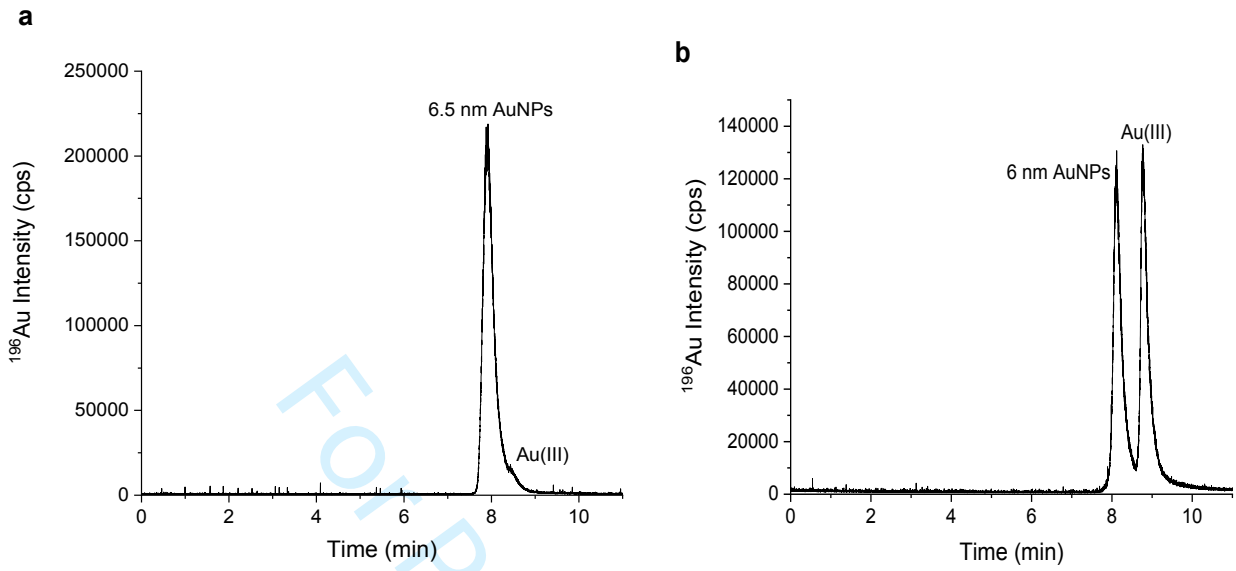


Fig. 4

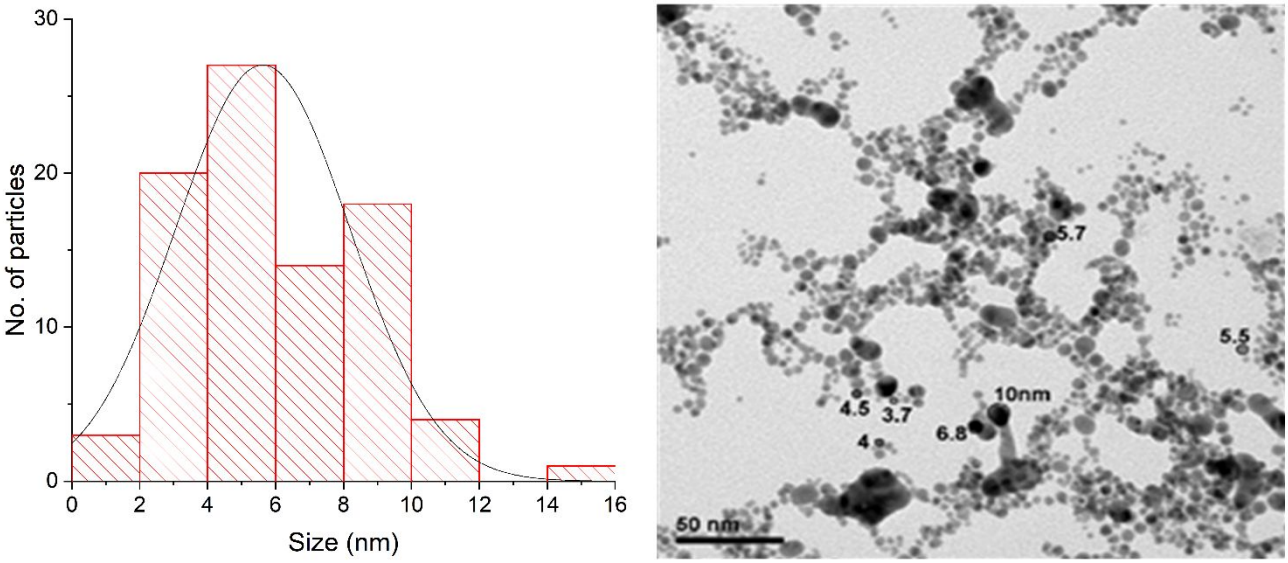


Fig. 5

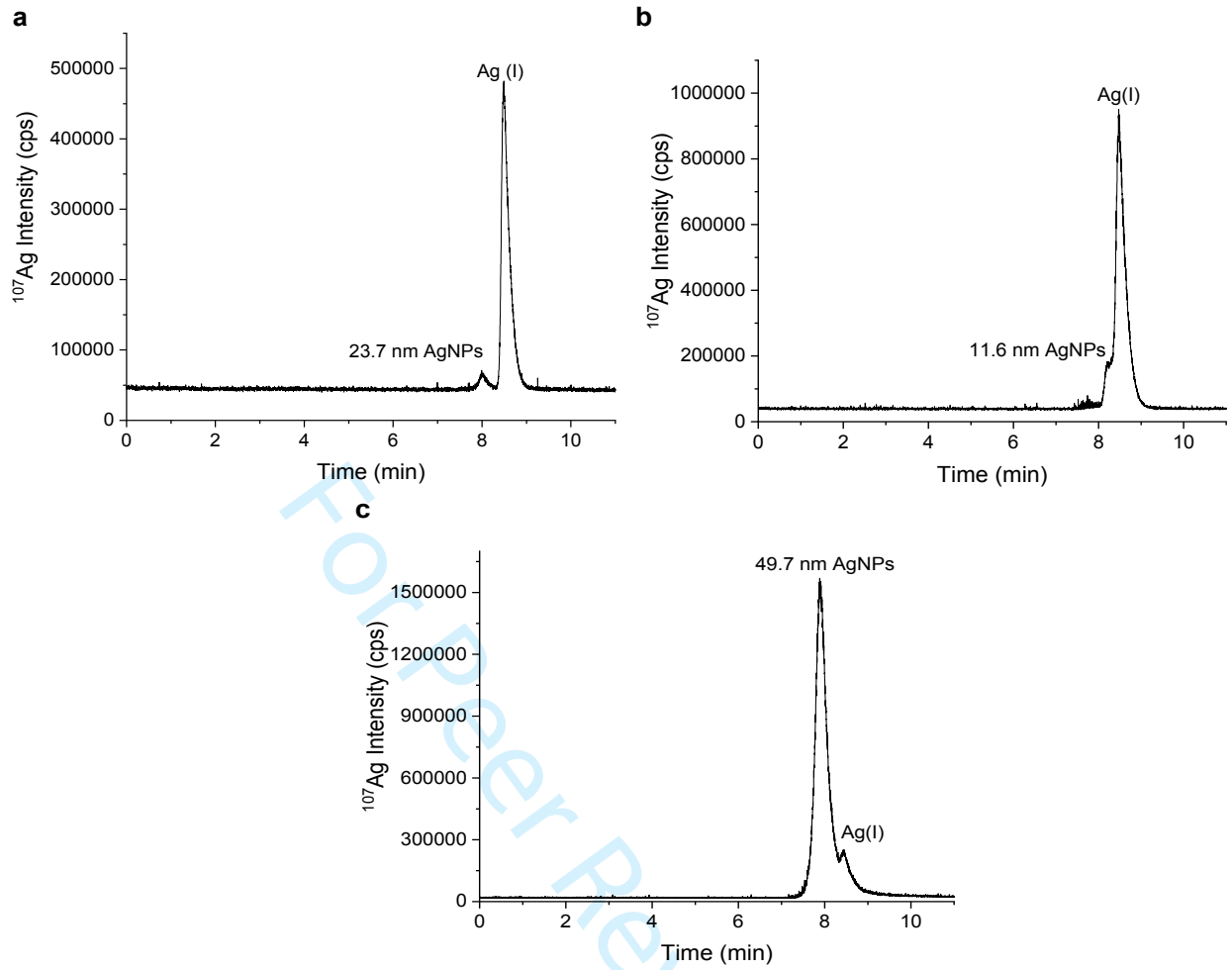
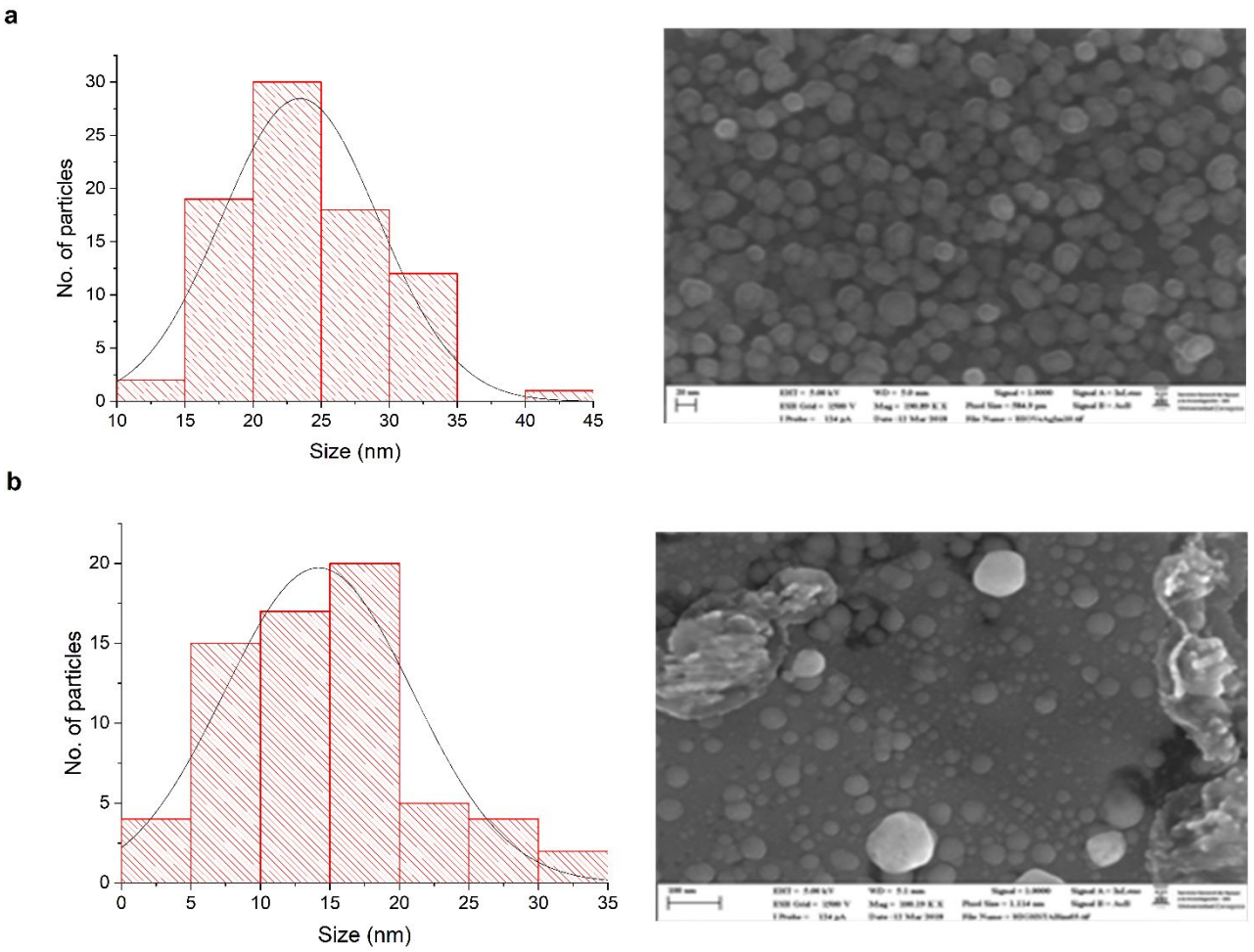


Fig. 6



Electronic Supplementary Material

Evaluation of hydrodynamic chromatography coupled to inductively coupled plasma mass spectrometry for speciation of dissolved and nanoparticulate gold and silver

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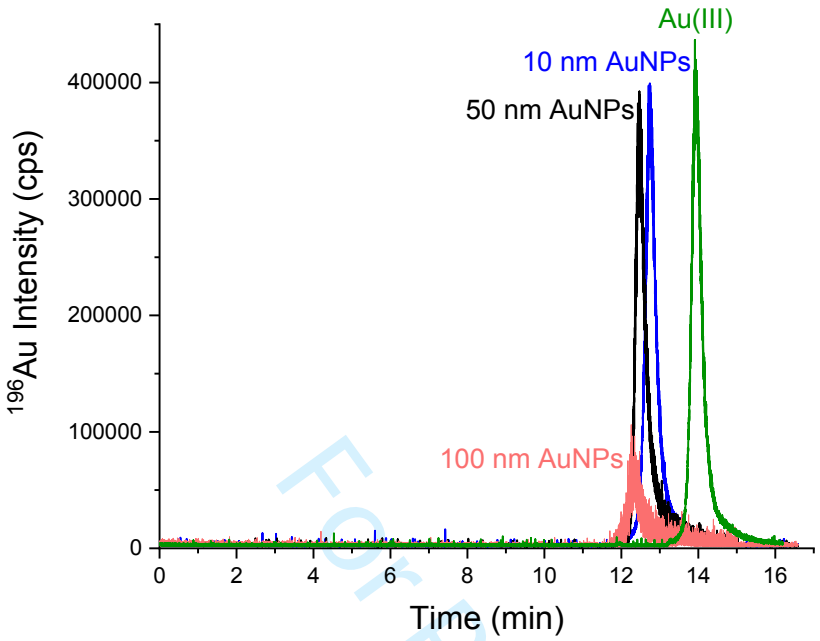

Fig. S1 HDC-ICP-MS Chromatograms corresponding to ionic gold and 10 nm, 50 nm and 100 nm AuNPs using mobile phase containing 0.34 mM SDS and 0.05 mM PA. Flow: 1 mL min^{-1}

Table S1 Recoveries (%) (peak area, 3 replicates) for different mobile phases in the separation of AgNPs by HDC (Flow: 1.6 mL min^{-1})

	10 nm AgNPs	20 nm AuNPs	40nm AuNPs	60 nm AgNPs
CM Mobile phase	100 ± 15	92. ± 17	102 ± 1	93 ± 9
0.45 mM SDS	68± 6	80 ± 4	29 ± 1	16 ± 2

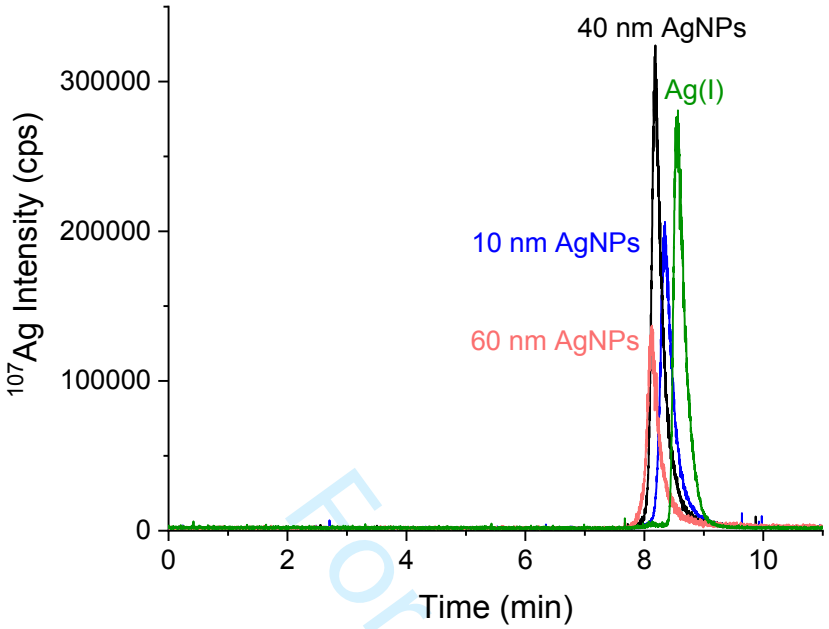

Fig. S2 HDC-ICP-MS chromatograms corresponding to ionic silver and 10 nm, 20 nm, 40 nm and 60 nm AgNPs using mobile phase containing 0.5 mM Na_2HPO_4, 0.45 mM SDS, 0.05% Triton X-100, 0.05% formaldehyde, 1 mM PA. Flow: 1.6 mL min^{-1}

Table S2 Calibration functions of retention times versus nominal diameters/nominal diameters square roots for the different mobile phases obtained in the separation of gold and silver species by HDC-ICP-MS (Flow rate: 1.6 mL min^{-1})

	Time/Size		Time/√Size	
	Calibration curve	R	Calibration curve	R
AuNPs				
CM Mobile phase	y=-0.131lnx±8.56	R_{ln}=0.991	y = -0.046x±8.41	R_{linear}=0.9999
0.45 mM SDS	y=-0.121lnx±8.37	R_{ln}=0.9999	y = -0.041x±8.23	R_{linear}=0.998
0.45 mM SDS +				
0.05mM PA	y=-0.136lnx±8.38	R_{ln}=0.9998	y = -0.048x±8.22	R_{linear}=0.9995
0.20mM PA	y=-0.131lnx±8.31	R_{ln}=0.9992	y = -0.039x±8.14	R_{linear}=0.994
0.50mM PA	y=-0.132lnx±8.40	R_{ln}=0.988	y = -0.046x±8.24	R_{linear}=0.998
AgNPs				
CM mobile phase				
+ 0.05 mM PA	y=-0.135lnx±8.58	R_{ln}=0.9993	y=-0.001x±2.88	R_{linear}=0.987
+ 0.2 mM PA	y=-0.126lnx±8.56	R_{ln}=0.9999	y=-0.001x±2.88	R_{linear}=0.988
+ 0.5 mM PA	y=-0.133lnx±8.56	R_{ln}=0.9997	y=-0.001x±2.88	R_{linear}=0.986
+ 1 mM PA	y=-0.129lnx±8.64	R_{ln}=1	y=-0.001x±2.90	R_{linear}=0.983
1 mM PA				
+ 0.45 mM SDS	y=-0.134lnx±8.59	R_{ln}=1	y=-0.001x±2.88	R_{linear}=0.981
+ 0.34 mM SDS	y=-0.135lnx±8.23	R_{ln}=0.988	y=-0.009x±2.83	R_{linear}=0.991
+ 0.22 mM SDS	y=-0.100lnx±8.06	R_{ln}=0.9997	y=-0.001x±2.80	R_{linear}=0.986

Table S3 Retention times for the different mobile phases studied in the separation of gold species (Flow rate: 1.6 mL min^{-1})

T (min)	Au(III)	10 nm AuNPs	50 nm AuNPs	100 nm AuNPs
CM Mobile phase		8.26 ± 0.01	8.08 ± 0.01	7.94 ± 0.01
0.45 mM SDS		8.10 ± 0.01	7.90 ± 0.01	7.82 ± 0.01
0.45 mM SDS				
+0.05 mM PA	8.76 ± 0.02	8.07 ± 0.01	7.89 ± 0.01	7.74 ± 0.01
+0.20 mM PA	8.81 ± 0.03	8.01 ± 0.01	7.88 ± 0.07	7.74 ± 0.09
+0.50 mM PA	8.77 ± 0.01	8.09 ± 0.01	7.92 ± 0.01	7.77 ± 0.02

Table S4 Retention times for the different mobile phases studied in the separation of silver species (Flow rate: 1.6 mL min^{-1})

T (min)	Ag(I)	10 nm AgNPs	20 nm AgNPs	40 nm AgNPs	60 nm AgNPs
CM mobile phase					
+ 0.05 mM PA	8.53±0.01	8.26±0.01	8.19±0.01	8.08±0.01	8.02±0.02
+ 0.20 mM PA	8.60±0.01	8.27±0.02	8.19±0.01	8.10±0.01	8.05±0.02
+ 0.50 mM PA	8.60±0.01	8.25±0.01	8.19±0.01	8.07±0.01	8.01±0.02
+ 1 mM PA	8.54±0.02	8.35±0.02	-	8.17±0.02	8.12±0.03
1 mM PA					
+ 0.45 mM SDS	8.65±0.05	8.29±0.01	-	8.10±0.01	8.05±0.01
+ 0.34 mM SDS	8.63±0.01	7.96±0.03	7.91±0.01	7.79±0.03	7.73±0.02
+ 0.22 mM SDS	8.64±0.01	7.80±0.03	7.79±0.02	7.65±0.03	7.61±0.01