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Hydrodynamic chromatography - inductively coupled plasma mass spectrometry, with post-column injection capability for simultaneous determination of nanoparticle size, mass concentration and particle number concentration (HDC-PCi-ICP-MS).

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Hydrodynamic chromatography - inductively coupled plasma mass spectrometry (HDC-ICP-MS) is a technique that is widely used in the size-characterisation of nanoparticles. In this work, a system was modified to facilitate the injection of NIST-traceable standards into the post-column effluent, which then allowed the response from an eluting nanoparticle to be quantified against the response from the post-column standards. Combining the simultaneously acquired particle sizing data and mass concentration data allowed accurate quantification of the particle number concentration to be made in a single analytical run. This unique single-method approach was successfully validated against a nanoparticle system which had previously been characterised in a number of recent peer-reviewed publications. In addition to this, its robustness was assessed using extracts from a study investigating the fate of nanoparticles in sewage sludge, and found to provide much improved data compared to what might have been achieved using an external calibration approach.

With in-vial limits of detection of 2 and 10 ng ml^{-1} for titanium and silver respectively, it is insufficient for use with environmental waters, but is foreseen as being useful in screening nanoparticle production processes, or in the characterisation of higher concentration materials. As this instrumental configuration is likely to be of use to researchers involved in the general area of quantitative trace element speciation, detailed description of construction of the interface is given as Electronic Supplementary Information.

Introduction

Nanotechnology has been described as "the next Industrial Revolution".[1] However, when taking up a new technology, consideration also needs to be given to any accompanying risk.[2] Unfortunately for nanotechnology, understanding the potential risk is particularly complex because of the unique properties of materials when produced in the 'nano' form, compared to their 'bulk' counterparts. Specific 'nano' issues include; the appropriateness of existing toxicological assessment methodologies[3] and the accuracy and robustness of current analytical characterisation techniques, as well as the relative importance of the various parameters being measured.[4] It is this latter 'analytical' aspect that this paper seeks to address.

The literature contains a number of excellent reviews covering the analytical methodologies which have been developed over the last 10 to 15 years to specifically address the characterisation of nanoparticulate materials.[5&6] From these reviews, the key parameters that require measurement include particle size (and its distribution),

mass concentration, particle number concentration, surface potential, surface area, particle morphology and intended/extraneous[7] chemical composition. Unfortunately, there is a degree of uncertainty regarding the relative priority of these parameters when, for instance, one is establishing a toxicological assessment protocol,[8-10] or characterising a drug delivery[11] or medical diagnostic system, or even certificating a new nanomaterial itself.[12]

In this paper, the development of a simple approach for the simultaneous quantification of particle size, mass concentration, particle number concentration (PNC), and intended/extraneous elemental composition (all in a single analytical run) is described. The analytical system was based on the HDC-ICP-MS arrangement first applied to nano-characterisation by Tiede *et al,*[13&14], and further developed to include analysis in 'single particle' mode, by Pergantis and co-workers.[15] An additional HPLC pump and injection port has been incorporated into the HDC-ICP-MS system, allowing aliquots of NIST-traceable ionic standards (and sample) to be added directly into the post-column flow, just prior to the detector. From this simple configuration, it will be shown that:

a) particle mass concentration can be accurately quantified against a calibration curve constructed 'per sample' from the response obtained from the aliquots of standards (this approach could also be used to quantify the concentration of extraneous elements, if needed);

b) particle size data can be obtained by direct comparison of sample retention times against that of reference standards;

c) particle number concentration can be calculated using data from a & b;

d) on-column losses can be determined by comparison of on-column and post-column injected aliquots of sample.

The system was validated using a silver nanoparticle test material which had been used in other published research.[16&17] In this paper, the revised configuration will be referred to as hydrodynamic chromatography-post-column injection-inductively coupled plasma mass spectrometry (HDC-PCi-ICP-MS).

Experimental

Reagents and chemicals

Ultrapure water (18.2 mΩ cm^{-1}) was obtained from a Milli-Q system (Millipore Co., Bedford, USA). HDC mobile phase concentrate was purchased from Agilent (Agilent, Cheadle, UK), but was a modified version of that described by McGowan and Langhorst, [18] consisting of ultrapure water, 0.5 mM Na_2HPO_4, 0.05% non-ionic surfactant (Triton-X100, $C_{14}H_{22}O(C_2H_4O)n$), 0.013% $C_{12}H_{25}SO_4Na$ (SDS), and 0.05% formaldehyde, adjusted to pH 7.2. Gold particle standards (citrate stabilized - 5, 20, 40, 100 and 150 nm) were purchased from BBI Solutions (BBI Solutions, Cardiff, UK). PVP-stabilized Ag-NP dispersion was obtained from Nanogap (Nanogap, Milladoirio, Spain), with a nominal size stated as being 42 ± 10 nm, and a mass concentration ~0.02 wt %. The mass concentration was accurately determined at Fera (using their standard 'totals' operating procedure), and was reported as 186.4 ± 1.4 µg g^{-1}. All ionic standards were NIST-traceable (Sigma-Aldrich, Poole, UK). Fresh dilutions were made-up each day, in mobile phase.

Instrumentation

Size separation was achieved using a PL-PSDA Type-1 HDC column (separation range 5-300 nm) (Agilent, Shropshire, UK) attached to a Gilson GX-271 Liquid Handler/Autosampler with Dual Auto-injection capability to facilitate the automatic On-Column (OC) and Post-Column (PC) injections. The pump system comprised of 2 x 307 Gilson Pumps (Gilson Ltd, Luton, UK). Detection was achieved using an Agilent 7500 ICP-MS (Agilent, Cheadle, UK). The overall system was controlled using the 'Trilution' software (Gilson Ltd, Luton, UK) which operated the autosampler and LC modules, and also initiated ICP-MS data acquisition. A schematic of the complete system is presented in figure 1. Detailed description of the development and optimisation of the HDC-PCi interface is presented in the electronic supplementary information (ES1).

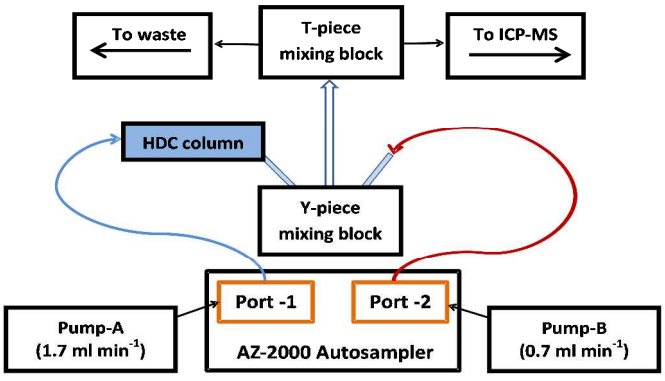

Figure 1: Schematic of the HDC-PCi-ICP-MS system.

Following injection of an aliquot of sample onto the HDC column *via* Port-1, aliquots of NIST-traceable ionic standards (and/or

sample) were injected, via Port-2, into a flow of mobile phase (0.7 ml min^{-1}) which then mixed with the post-column effluent *via* a Y-piece 'low swept volume' mixing block, (U-466) (Crawford Scientific Ltd, Strathaven, UK). The mixed flow then passed into a T-piece, where it was sampled, under natural aspiration, by a SeaSpray nebuliser (ARG-1-USS04X) (Glass Expansion, W. Melbourne, Australia) into an Agilent CX7500 ICP-MS, operating in 'helium' mode. Excess solution flowed to waste, under gravity. The ICP-MS set-up and operating parameters are given in ES-2.

To ensure that the OC and PC peaks were due to the element of interest, and not interfering species, additional isotopes were monitored so that the relevant ratios could be compared against literature values. In addition to this, the acquisition menu included other elements, so that extraneous material within the nanoparticles could also be quantified, if required.

Calculation of particle number concentration.

Two parameters were needed to calculate particle number concentration (PNC); the mass concentration of particles within a sample, and their size. Mass concentration was calculated using a calibration curve constructed using the responses obtained from PC-injected ionic standards which accompanied every OC injection of nanoparticle samples. Particle size was extrapolated from an external calibration curve constructed from OC-injected gold standards (BBI Solutions, Cardiff, UK).

Using these two pieces of data (obtained from a single chromatogram – see Results and Discussion) the following formula could be used to calculate the PNC:

$$\text{PNC} = \frac{\text{Mass concentration}}{\text{Mass per nanoparticle}}$$

where the mass per nanoparticle is calculated using the particle volume multiplied by particle density: $4/3\pi r^3$ x density = g NP^{-1}

Results and discussion

System stability and limit of detection

The stability of the system was assessed over a period of 15 hours, using samples collected during an investigation into the fate and behaviour of nanoparticles (Ag and TiO_2) in sewage sludge (to be reported elsewhere [19]). The solid samples were extracted into either EDTA (0.05 mol l^{-1}) or deionised water, and the resulting extracts analysed unfiltered. Each analytical run was comprised of an OC injection of sample, immediately followed by PC injections of ionic standards (at two analyte concentrations), from which a 'per-sample' calibration curve was constructed. Each analytical run took 12.5 minutes, including loop-flushing and filling.

Because the fate/behaviour study protocol required that the sewage sludge samples should be extracted in a single block, it was decided that a number of sample extract vials would be reanalysed at intervals during the 15 hour analytical run (6 replicates per sample). This data would allow any within-run changes to be monitored, even though preliminary work had suggested that the extracts were relatively stable. In addition, in this paper, the data has been used to show the effectiveness of the per-sample calibration procedure for correcting any instrument-based variability occurring during the run due to the dirtying of the ICP-MS interface (cones, quartz-ware, *etc*). The data collected was as follows, and is presented in table 1;

a) the area response obtained from one of the PC ionic calibration standards injected during the analysis of each sample, and;

b) the concentration of analyte in the sample, calculated using the per-sample calibration curve.

	Area response from a PC 100 ng ml^{-1} ionic standard		Analyte concentration in OC extract solution (ng ml^{-1})	
Replicates = 6	Mean	**RSD (%)**	Mean	**RSD (%)**
Ag in EDTA extracts	44244	**23.4**	491.9	**5.2**
Ag in water extracts	40595	**18.5**	359.2	**3.5**
Ti in EDTA extracts	11550	**11.3**	17,023	**7.0**
Ti in water extracts	11772	**8.9**	1,643	**3.6**

Table 1. Effectiveness of the per-sample calibration approach to correcting ICP-MS instrumental drift

The data presented in Table 1 shows;

a) the ICP-MS response varied significantly over the duration of the analytical batch (~15 hours), as indicated by large RSD% values quoted for the PC injections of an ionic standard (column 3). This time-based variability results from changes in the condition of the ICP-MS cones and glassware due to the continuous nebulisation of HDC mobile phase (high organic content), and injections of 'dirty' extract solutions (high dissolved solids). It should be noted that, when operating an ICP-MS under standard conditions, *i.e.,* direct nebulisation or

flow-injection, it is common practise to include an internal standard in each sample and standard vial, the response from which is used to correct any time-based instrumental drift. However, this was not an option when performing chromatographic analyses.

b) the use of the per-sample calibration approach has successfully corrected the time-based drift, as is indicated by the significantly smaller RSD% values quoted in column 5, obtained from the calculated analyte concentration data.

c) the variability observed within the area response data for the ionic titanium standard is less than that for the ionic silver (23.4% and 18.5% *vs* 11.3% and 8.9% respectively), even though both analytes were introduced into the PC flow in the same injection slug. This is likely to be due to the significant peak-tailing and elevated baseline observed for the PC injected peaks, in the silver chromatograms.

Figure 2 shows the difference in the peak shape obtained from a flow-injected solution containing ionic titanium and silver, and would suggest that the latter element is interacting fairly strongly with component(s) within the system.

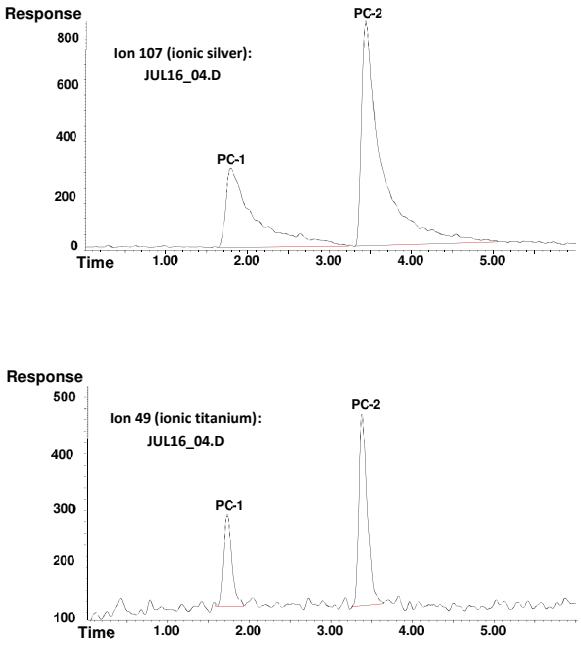

Figure 2. Chromatograms obtained from flow-injected (PC) aqueous standards of ionic silver and ionic titanium, showing differences in peak symmetry and baseline recovery between the two elements.

Attempts were made to counteract this phenomenon because it affected the accurate quantification of the PC standards at lower concentration levels. The chemical composition and internal diameter of the tubing was changed, (PEEK, PTFE and HD-PE, and 0.12 to 0.48 mm i.d., respectively), but had little or no effect on the quality of the silver peak. Consideration was given to modifying the composition of the PC mobile phase to improve the peak shape, *i.e.,* by altering the pH or organic content, but was not pursued further because the study for which this system was being developed didn't require very low limits of detection.

The in-vial LoD values for silver and titanium were 10 and 2 ng/ml respectively. It is likely that these values could be improved by increasing the injection volume (currently 20 µl) and investigating different nebulisation options. An evaluation of on-column recoveries indicated that nanoparticulate silver did not behave in the same way as its ionic counterpart, giving good peak symmetry, and a recovery value of 97.74% ± 0.28 (calculated from the chromatogram presented in figure 3).

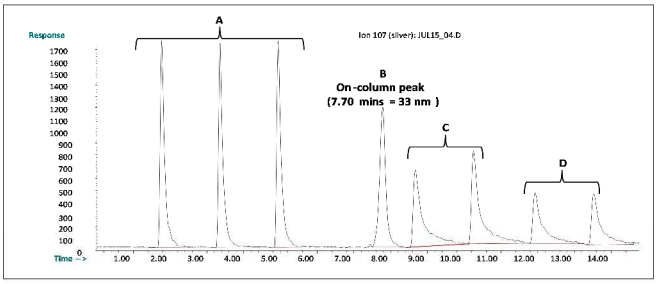

Figure 3. Typical HDC-PCi-ICP-MS chromatogram, showing OC and PC injection peaks: A = triplicate PC injections of an Ag-NP dispersion; B = OC injection of the same dispersion (eluting at RT = 7.70 mins); C = PC injections of 250 ng ml^{-1} Ag-ionic; D = PC injections of 150 ng ml^{-1} Ag-ionic

Validation of the PNC quantification approach

In order to validate the above approach, a PVP-stabilised silver nanoparticle (Ag-NP) system was used as the test material because it had been characterized and reported in the peer-reviewed literature.[16 & 17] Following sonication (see ES-3 for details), an aliquot of the Ag-NP was diluted in mobile phase, and injected onto the HDC column. While the Ag-NP was passing down the column, the autosampler made triplicate injections, into the post-column effluent, from the same vial. Once the chromatographed peak had eluted from the column, aliquots of two ionic silver standards (at 250 and 150 ng ml^{-}

[1]) were injected into the PC flow (in duplicate). Figure 4 shows a representative chromatogram, with the peaks annotated.

From this single chromatogram it was possible to obtain the following information:

i) Accurate quantification of the in-vial analyte concentration;

Using the response area data from the PC ionic standards, a 'per-sample' calibration curve was constructed, from which the mass concentration of analyte in the eluting OC peak was extrapolated, and was found to be 184.0 ng ml^{-1} (as total silver)

ii) Accurate assessment of on-column losses;

Simple comparison of the OC injection *vs* the PC injection response areas provided quantitative information regarding on-column losses. The response from the triplicated PC injections of the Ag-NP dispersion was 147960 (RSD% = 2.8%), and the eluted Ag-NP was 144616, giving a column recovery value of 97.74%. This value could be used (if needed) to correct the in-vial analyte mass concentration.

iii) Particle sizing data:

The theory of HDC allows the size of the eluting particle to be extrapolated from a retention time calibration curve, produced using appropriate sizing standards (5 to 150 nm). The calibration curve produced in this study, comprised of HDC-ICP-MS data from 5 gold nanoparticle standards, is presented in ES-4, and was used to calculate that the hydrodynamic radius of the Ag-NP was 33 nm. This was confirmed by triplicated analysis of the particles using disc centrifugation analysis (see ES-3). NOTE: although the absolute resolving power of HDC is currently insufficient to baseline-separate particles of ~65nm and below, it is possible to obtain reproducible differences in retention times for single standards, and to then construct a valid size-based calibration curve. It might also be worth researchers investigating the use of commercially-available peak deconvolution software to asist in generating a 'virtual' improvement in the low-end size separation, for use with particle mixtures.

iv) Particle number concentration (PNC):

Using the above data, from a single chromatographic run, and the following formula, it was possible to calculate the PNC as follows:

$$\text{PNC} = \frac{\text{Mass concentration}}{\text{Mass per nanoparticle}}$$

For the Ag-NP system in this study; the density of silver was 10.5 g cm^{-3}, and the radius of the particle (r) = 16.5 nm, giving a mass per particle of 1.97 $x10^{-16}$ g NP^{-1}

From the chromatogram, the mass concentration was calculated thus:

Vial concentration = 184.0 ng ml^{-1} (as total Ag)

Dilution factor from stock Ag-2103 = 1,000 fold

Therefore: mass concentration in original material = 1.84 x 10^{-4} g g^{-1} (assuming the density of the dispersion is 1.00);

making the PNC:

$$= \frac{1.84 \times 10^{-4} \text{ g g}^{-1}}{1.97 \times 10^{-16} \text{ g NP}^{-1}}$$

$$= \mathbf{9.34 \times 10^{11} \text{ NPs g}^{-1}}$$

which is in very good agreement with the value calculated using the manufacturer's data specification, *i.e.*, (42 ± 10 nm and ~200 µg g^{-1} - cited in [17], giving a calculated range of PNC values between 2.59 x 10^{11} to 11.1 x 10^{11} NPs g^{-1} (based on the quoted particle size range).

Conclusions

This study has shown that, through the simple reconfiguration of existing hardware, it is possible to generate high quality data pertaining to a number of key nano-metric parameters. The approach was validated against one of the few reference nanomaterials available that has been reported in the peer-reviewed literature; and the robustness of the methodology was successfully proven against real samples arising from a challenging environmental fate/behaviour investigation. It is hoped that further work can be carried out regarding the resolving power of the separation method, and on increasing the sensitivity of the overall technique. In addition to its use within the area of nano-metrics, PCi-ICP-MS may also prove to be useful for those involved in the development of quantitative trace element speciation generally, as it appears to offer a robust platform for performing compound-independent concentration measurements.

Unfortunately, HDC-PCi-ICP-MS is still constrained by the limits of detection of the basic HDC-ICP-MS technique, which, as outlined by Gray *et al*,[20] is insufficient for direct analysis of the ultra-low levels of ENPs, such as those predicted to be present in environmental waters. However, the data presented in this paper has

shown that the methodology was sufficient for use in a study looking at the fate/behaviour of silver and titanium dioxide nanoparticles in sewage sludge, where the predicted concentrations[21] are much higher than those in environmental waters. We therefore expect the modified system to be of great use to those researchers wanting to characterise ENP solutions at higher levels, *e.g.*, when screening within-batch performance of the nanoparticle production process, or prior to preparing spike solutions for toxicity testing, or calculating dose/response functions for nano-pharma formulations.

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Notes and references

Food and Environment Research Agency, Sand Hutton, York, United Kingdom. YO41 1LZ

Electronic Supplementary Information (ESI) available: formatting of various HDC / ICP-MS interface configurations; HDC-PCi-ICP-MS operating conditions; Ag-NP preparation conditions; disc centrifugation data and HDC particle size calibration curve. See DOI: 10.1039/b000000x/

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ES-1 Configuration of HDC-PCi-ICP-MS interface components

Although the major components used in the HDC-PCi-ICP-MS were off-the-shelf items, their configuration into a working system hinged on two things; i) the ability to establish a reproducible and robust zone-of-mixing for the OC eluent and the PC injection solution; and ii) a stable, noise-free interface between the zone-of-mixing and the ICP-MS. Once these challenges had been addressed, it was hoped that it would be relatively straight forward to apply the system to the measurement of the various parameters.

Optimisation of OC and PC flow mixing.

In order to get efficient and reproducible mixing of the OC and PC liquids, a number of different designs of mixing block were investigated. The simplest were T-pieces made of PTFE, which were standard items from the ICP-MS consumable list but, where necessary, were adapted in-house. Figure ES-1a presents the T-piece configured so as to have the confluence of the OC (blue tubing) and PC (red tubing) flows occurring head-on, with the mixed flow passing at right angles through the block, through to the ICP-MS *via* the white tubing. Figure ES-1b shows the configuration where the OC and PC flows meet at right angles to each other, before passing on to the ICP-MS. In addition to this, the diameters of the various inlet and outlet ports were further adapted, in an attempt to either constrict or increase the relative flows and volumes of the liquids encountering each other. Unfortunately, none of the above configurations resulted in a consistent mixing of the two flows, as indicated by the increased levels of noise observed in the chromatographic baseline.

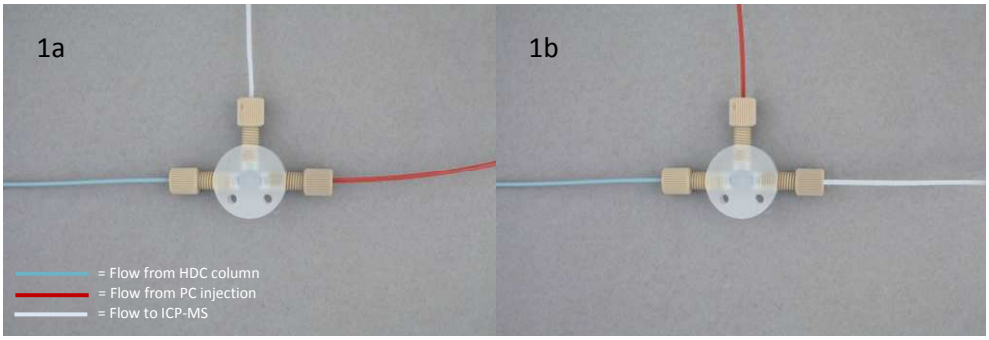


Figure ES-1. Configuration of the PTFE T-piece mixing block

In order to try and improve this situation, two Y-piece units were investigated, one from a Perkin Elmer FIAS system (Part number *B0507962, Perkin-Elmer, Beaconsfield, UK*), and the other taken from a UPLC-MS system (Part number U-466, Crawford Scientific Ltd, Strathaven, UK).

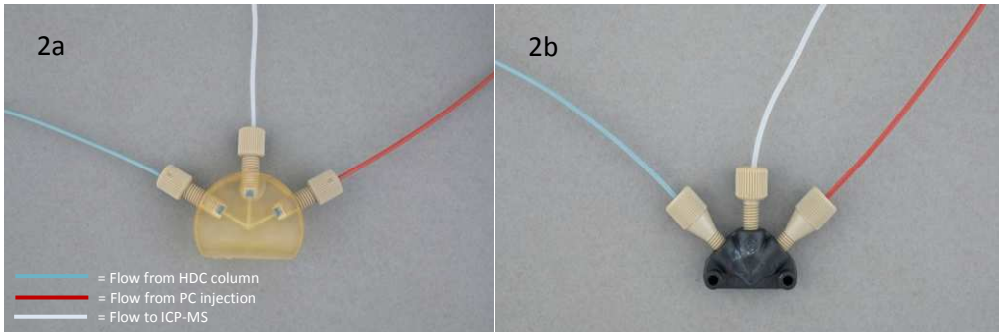


Figure ES-2. Configuration of the two Y-piece mixing blocks.

Perkin Elmer mixing block: Figure ES-2a shows the two incoming flows meeting at approximately 90° before turning virtually back on themselves to pass through the outlet port. In addition to this, it was hoped that a void volume at the point where the two fluids meet, which had been specifically designed to efficiently mix reagents in the FIAS system, might allow time for them to properly mix before passing on

to the detector. Unfortunately, although allowing effective mixing of the liquids, the void volume was such that it had a negative impact on the quality of the chromatographic peak eluting from the column, and gave a slightly noisier signal.

Upchurch Scientific 'low swept volume' mixing block: Figure ES-2b shows that this unit has a similar inlet/outlet configuration, but with its low swept volume (2.2 µl), had a virtually indiscernible impact on the quality of the chromatographic peak. **For this reason, all data in the main report was obtained using this item to mix the OC and PC flows.**

Optimisation of the OC / PC flow rates:

As the OC flow rate was fixed at 1.7 ml min^{-1}, based on the manufacturer guidelines for optimal HDC separation, various PC flow rates were investigated. If too low (<0.2 ml min^{-1}), the OC flow dominated access to the mixing area within the Y-piece, resulting in a relatively noisy signal being obtained from the flow-injected analyte. Flow rates greater than 1 ml min^{-1} resulted in a significant reduction in the response obtained from the eluting nanoparticle peak due to the diluting effect of the increased volume of liquid passing through the system. An optimal PC flow rate was found to be 0.7 ml min^{-1}, based on the quality of the PC-standard peak (in terms of both peak shape and signal noise), and was used for all experiments discussed in the main paper.

Interfacing the HDC-PCi flow to the ICP-MS:

A PTFE T-piece was placed immediately after the Y-piece mixing block, from which the nebuliser of the ICP-MS was able to take up solution by natural aspiration. Excess liquid flowed to waste by gravity, *via* tubing with a much larger internal diameter. It was found that the ICP-MS signal was much quieter when neither of these flows was pumped. The length of the tubing between the Y-piece mixing block, the T-piece and the nebuliser was kept as short as was practicably possible so as to keep peak-broadening to a minimum. It also helped the performance of the nebuliser by reducing in-tubing drag during natural aspiration. Figure ES-3 shows the component parts of the HDC-PCi-ICP-MS interface.

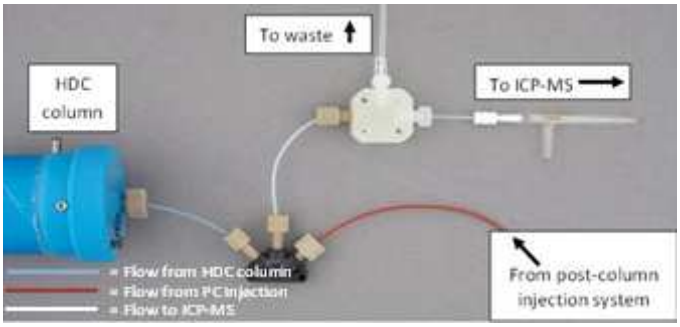


Figure ES-3. Configuration of the post-column interface components for HDC-PCi-ICP-MS.

Optimisation of the nebuliser operation settings:

As the transfer of the eluent mixture to the ICP-MS occurred as a result of the nebuliser's natural aspiration, the gas flow settings were very different to those used if operating the instrument under normal 'pumped' conditions. Optimisation of the gas flows was achieved by naturally aspirating a tune solution (containing the elements of interest, at about 10 ng ml^{-1}, made up in mobile phase) into the plasma, and then tuning the nebuliser and make-up gases so that the back-ground signal (48 m/z) was minimised, and the analyte signal maximised. 48 m/z was chosen because it had been observed to be particularly prone to the production of polyatomic interferences (based on S, N and O), so was useful as an indicator of the conditions within the plasma. As best-practise, it was helpful to also monitor at least two isotopes for each of the elements of interest, *e.g.,* for silver, monitor Ag^{107} and Ag^{109}, when altering the plasma gas flows. A constant ratio of the two isotopes confirmed that increases in the monitored signal was most likely to be due to improved analyte ionisation or transfer rather than the production of a polyatomic interference within the changing plasma. This approach was also used when performing analyses, because monitoring at least two isotopes allowed the identity of eluting peaks to be confirmed post-run.

ES-2 ICP-MS operating parameters

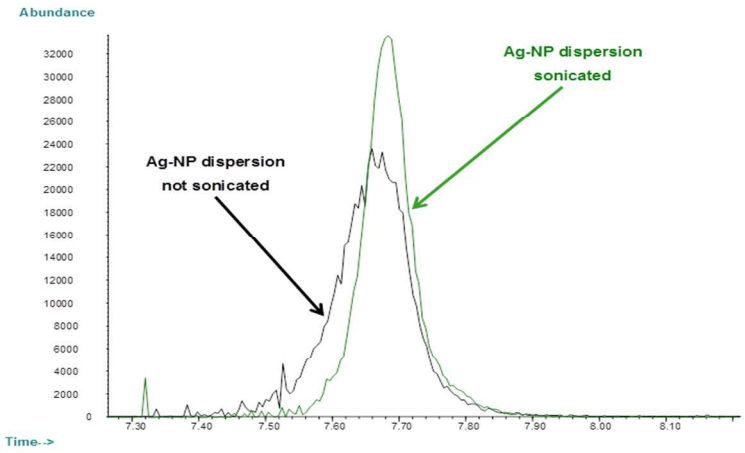
Table ES-1 Instrument parameters for the ICP-MS

Parameter	Setting
RF power	1600W
Reaction cell mode	On: He gas: 4.9 mL/min
Nebulizer pump	Natural aspiration
Nebulizer gas flow:	
Carrier gas	1.09 L/min
Make-up gas	0.26 L/min
Sample depth	10 mm
S/C temperature	2 ℃
Extract 1	0 V
Extract 2	-100 V
Omega Bias-ce	-18 V
Omega Lens-ce	0.8 V
Cell entrance	-36 V
OctP RF	190 V
OctP Bias	-7 V
Isotopes monitored	^{46}Ti, ^{47}Ti, ^{48}Ti, ^{49}Ti, ^{107}Ag, ^{109}Ag and ^{197}Au
HDC column flow	1.7 ml min^{-1}
Post-column column flow	0.7 ml min^{-1}

ES-3 Preparation of PVP-Ag nanoparticle material.

The improved peak symmetry, following sonication, indicates that the added energy has caused disruption of larger-sized structures within the original dispersion, resulting in a smaller, more mono-disperse system.

Figure ES-4. Chromatograms showing the effect of sonication on the silver nanoparticle dispersion



This was supported by disc centrifugation data obtained on the same samples, with the polydispersity index (PDI) value being reduced to nearly 1 (where '1' indicates a totally monodisperse system), and is in line with the findings of Loeschner et al.[17] when they described the particle distribution as not being monodisperse, *i.e.,* exhibiting a bimodal size distribution.

Table ES-2. Disc centrifugation data showing effect of sonication on the silver nanoparticle dispersion

Sample replicates = 3	Average size (nm)	PDI
Unsonicated dispersion	39.2	1.98
Sonicated dispersion	33.0	1.14

Figure ES-5 a to c: disc centrifugation data showing:

Figure ES-5a. Ag-NP before sonication (bimodal distribution @ ~33 and ~40 nm)

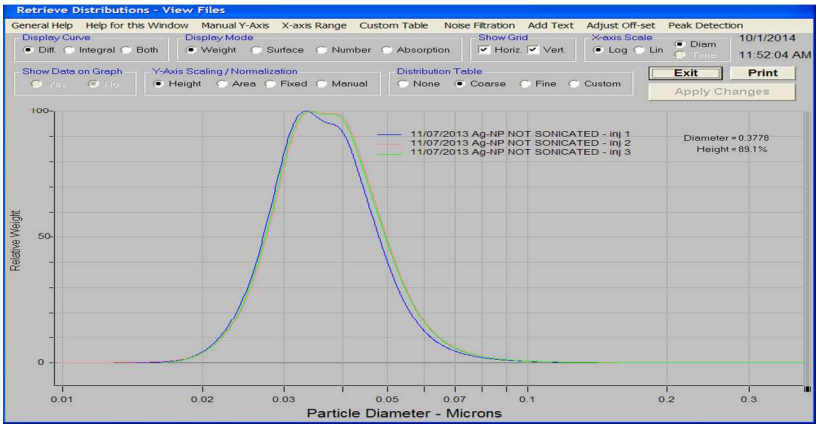


Figure ES-5b. Ag-NP after sonication (highly monodisperse @ ~33 nm)

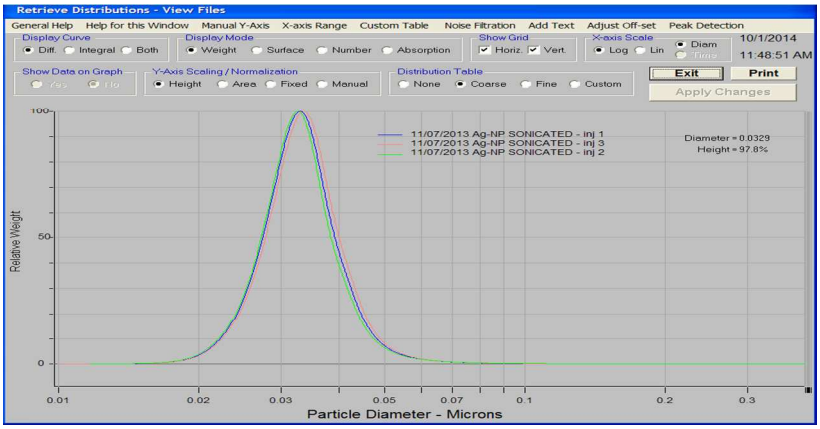
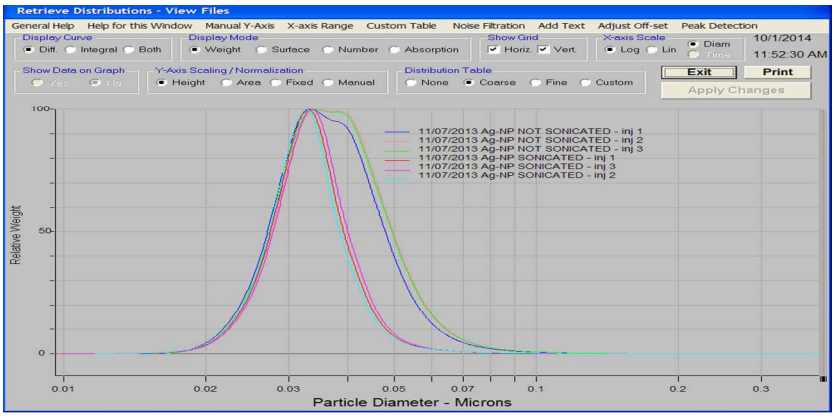


Figure ES-5c. Overlay of plots



ES-4 Construction of a size-based calibration curve, using HDC-ICP-MS data

The theory of HDC allows the size of the eluting particle to be extrapolated from a retention time calibration curve, produced using appropriate sizing standards (5 to 150 nm hydrodynamic diameter). The calibration curve produced in this study, comprised of HDC-ICP-MS data from five gold nanoparticle standards, is presented in Figure ES-6, and was used to calculate that the hydrodynamic radius of the Ag-NP used in the fate/behaviour study. This was confirmed by triplicated analysis of the particles using disc centrifugation analysis.

Figure ES-6 Size (hydrodynamic diameter) calibration curve constructed using HDC-ICP-MS data from five gold nanaoparticle standards (citrate-stabilised), analysed in triplicate