Feasibility study on the quantitative differentiation between nanosilver and ionic silver in aqueous suspension using single particle ICP-MS

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Feasibility study on the quantitative differentiation between nanosilver and ionic silver in aqueous suspension using single particle ICP-MS

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The issue

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particle within the range of 1-100 nm in at least one of the dimensions[1] . Now-a-days nanoproducts are widely used in various fields. Among them, silver nanoparticles (NPs) are playing a major role in the field of nanotechnology since the beginning of the 21st century. These NPs have been widely used as antibacterial agents in many devices of everyday usage, e.g. textile coating or various cosmetic or disinfectant products[2]. However, with the accelerating production and introduction of AgNPs into commercial products, there is likelihood of release into the environment, which raises health and environmental concerns. However, toxicity of silver NPs against living organisms and/or environment still remains an unsolved question, due to the fact that silver nanoparticle suspensions are mixtures of silver ions, nanoparticles and aggregates[3], which makes difficult to determine what portion of the toxicity is from the nano-form and what is from the ionic form. Therefore, there is a clear need to be able to carry out quantitative assessments of exposure to silver NPs which are capable of distinguishing between ionic silver and the silver nanoparticle/aggregate fractions to get a deeper knowledge about the fate and behaviour of these NPs in the environment and at the same time give us a better understanding about the toxicity they can cause. To achieve this, fit-for-purpose but robust methodology is now required.

On the other hand, the EU recommendation (2011/696/EU)[1] stated that, for regulatory purposes, the size distribution of a nanomaterial should be presented based on the number concentration (i.e. the number of objects within a given size range divided by the number of objects in total) rather than the mass fraction of nanoscale particles, since a small mass fraction may contain the largest number of particles. Consequently, not only is the development of techniques that allow the discrimination between ionic silver and silver NPs important, but instrumentation capable of counting particles needs to be available to be able to enforce the proposed EU regulation.

The solution

Common techniques for nanoparticle characterisation include microscopic techniques (Atomic Force Microscopy, Scanning Tunnelling Microscopy, Scanning and Transmission Electron Microscopy…)[4] which are excellent techniques for determining the size of the particles, but they do not provide information about the number-based concentration of the nanoparticle directly in suspensions. They are also not able to differentiate between the ionic silver and nanosilver.

Alternatively, other techniques that are able to count and size nanoparticles directly in suspension such as nanoparticle tracking analysis (NTA)[5] or electrical current-based platforms such as tunable resistive pulse sensing (TRPS)[6]. However, these techniques may suffer from matrix-induced interferences, which could provoke a bias in the results, and, again they are not able to distinguish between the ionic silver and the nanosilver.

The alternative presented here, funded by the Government Chemist Programme, is the use of the single particle analysis by ICP-MS (spICP-MS). SpICP-MS has been proposed as a powerful technique for the characterisation of both the size distribution and the number-based concentration of metallic NPs at the ultratrace level. Moreover this technique allows, in a single run, the simultaneous determination of ionic silver and AgNPs. The main advantage of this technique is that the analysis is relatively fast (one minute run, normally) since no matrix separation by ICP-MS hyphenation techniques is needed. Also, the sample treatment needed is minimal (usually sample dilution), which reduces the possibility of artefact formation and/or contamination, although nanoparticle aggregation/agglomeration is still an associated risk that has to be kept under control and studied during method development and validation.

Method

Chemicals and standards

Silver (Ag) standard solution (SRM 3151), polyvinylpyrrolidone coated silver nanoparticles with a nominal diameter of 75 nm (RM 8017) and citrate-stabilized gold nanoparticles with a nominal diameter of 60 nm (RM 8013) were supplied by NIST (NIST, Gaithersburg, USA). De-ionized water (18.2 MΩ cm^{-1}) was obtained from an Elga water purification unit (Marlow, UK).

Instrumentation

All measurements were carried out using an Agilent 8800 QQQ ICP-MS equipped with a standard sample introduction system consisting of a MicroMist nebulizer and Scott type double pass spray chamber. Samples were introduced directly into the ICP-MS using an ASX‑520 autosampler with the standard peristaltic pump and tubing (i.d. 1.02 mm). Analyses were performed in TRA mode using an integration time of 3 ms. Total acquisition time was fixed at 60s for all analyses. The general settings of the ICP-MS are given in Table 1.

Table 1. ICP-MS settings

Parameter	Value
RF Power	1550 W
Sampling depth	8.0 mm
Carrier gas	1.09 L/min

Sample/skimmer cones	Ni/Ni
Sample uptake rate	0.35 mL/min
Spray chamber temperature	$2^{o}C$
Dwell time	3 ms
Acquisition time	60 s
Mass monitored (MS/MS mode)	^{107}Ag or ^{197}Au

Data analysis

In this work, the newly developed Single Nanoparticle Application Module of the ICP-MS MassHunter software (G5714A) was used for data analysis.

Standard and Sample preparation

The gold NP standard (60 nm, RM 8013) was diluted to a concentration of 50 ng/g for the evaluation of the nebulization efficiency. This parameter has to be determined in order to calculate the number concentration and the size of the nanoparticles. Prior to their analysis, the gold nanoparticles were placed in an ultrasonic bath for 10 min.

NIST AgNP RMs were diluted 2×10^{10} times (required for sp ICP-MS analysis) and silver was added to the Ag NP in suspension in a concentration of 5 ng/g to generate a solution containing both ionic silver and AgNPs. Prior to their analysis, the silver nanoparticles were manually shaken.

A calibration curve for ionic silver in water was prepared daily water by gravimetric dilution of SRM 3151 *Silver (Ag) Standard Solution* at the following concentrations: 1, 2, 5, and 10 ng/g.

A standard addition calibration curve was prepared daily by spiking different concentrations of ionic silver in aqueous solution (1, 2, 5, and 10 ng/g) with 0.06 ng/g of silver nanoparticles.

Results and discussion

To investigate whether spICP-MS is feasible to able to distinguish between dissolved or ionic silver and nanosilver (AgNP), the NIST reference material (RM 8017) was used as a model sample as it can be spiked with ionic silver; no significant amount of dissolved (ionic) silver was found to be present in a suspension of this material.

By knowing the instrument response factor (using the slope of the calibration curve with ionic silver), the nebulization efficiency and the geometry of the particles, it is possible to determine the AgNP diameter (equation 1 and 2) by spICP-MS[7].

$$m_p = \frac{I_p\, t_d}{RF_{ion}} \times \frac{V\,\eta_n}{60} \times \frac{M_p}{M_a}$$

equation 1

$$d_p = \sqrt[3]{\frac{6\,m_p}{\pi\,\rho_p}} \times 10^4$$

equation 2

Where m_p= particle mass (ng); I_p = particle signal intensity in the sample (cps); RF_{ion} = ICP-MS response for ion standard (cps/ppb); t_d = dwell time (s); V = sample flow (mL min^{-1}); η_n = nebulization efficiency; M_p = molar mass nanoparticle material; Ma =molar mass analyte measured; d_p= particle diameter in the sample; ρ_p = particle density (g mL^{-1})

Figure 1 shows the size distribution of the investigated AgNP (spiked with ionic Ag) suggesting a nominal diameter of 71 ± 2 nm (precision as SD, n=6) , as obtained with spICPMS; which is in agreement with the reference values obtained by microscopic techniques (74.6 ± 3.8 nm by Transmission Electron Microscopy and 70.1 ± 6.0 nm by Atomic Force Microscopy)[8].
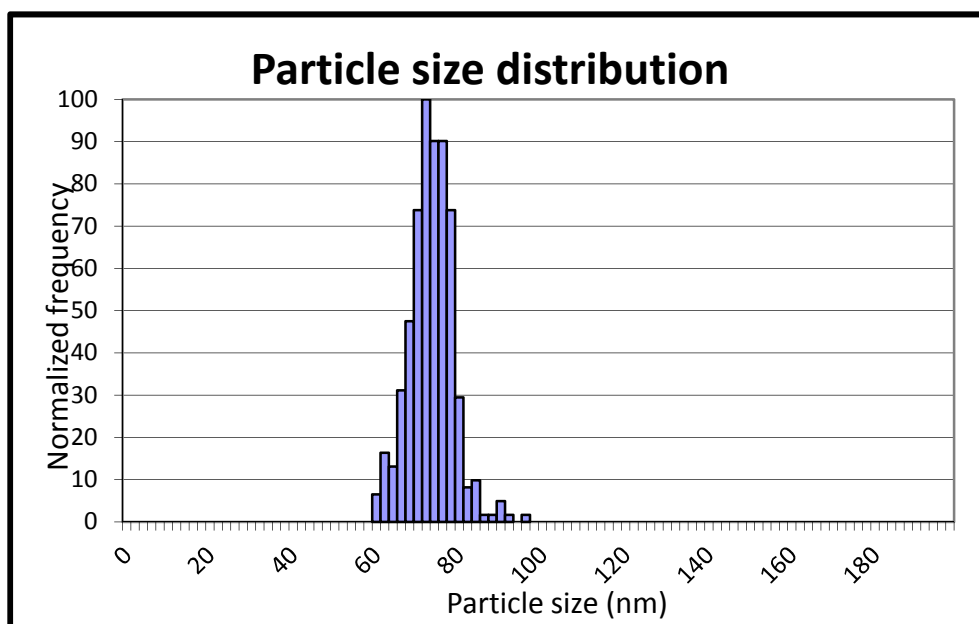
Figure 1: Particle size distribution of NIST AgNPs (RM 8017) with 75 nm nominal size

Additionally, as the sample is highly diluted (2×10^{10} times) the number of particles is sufficiently low to correlate each data with a unique particle, which can provide information about the number-based concentration. This sample was found to have a concentration 5×10^{17} particles/L, whereas the theoretical value is 5.5×10^{17} particles/L, indicating that the recoveries are quantitative: 95 ± 8% (precision as SD, n=6) .

One of the attractive characteristics of the spICPMS is that the single particle events for AgNPs can easily be distinguished from the signal produced by ionic silver, enabling quantification of both forms in a single run (Figure 2). For this, NIST AgNPs were spiked with 5 ng/g of ionic silver. Two calibration approaches were used in order to quantify the amount of ionic silver in the samples (Table 2):

a) External calibration with ionic silver standards

b) Standard addition calibration where a suspension containing 0.06 ng/g Ag NPs was spiked with increasing concentrations of ionic silver

Table 2. Results of the quantification of ionic silver in a solution containing NIST AgNPs

Calibration strategy	Ionic Ag added (ng g^{-1})	Ionic Ag measured (ng g^{-1})	Recoveries (%)
External Calibration	5.09	4.9 ± 0.2	96 ± 4
Standard addition	5.09	0.7 ± 0.1	14 ± 1

The results in the table 2 show that quantitative recoveries of the ionic silver can be achieved (96 ± 4 %) when standard addition calibration approach is applied. This is due to the presence of matrix effects. This is shown in the Figure 3 where the slope of the standard addition is 9 times smaller than the slope of the external calibration. The maximum level of ionic silver that can be separated from AgNPs, without an additional fractionation/separation step, was 10 ng/g using the proposed approach.
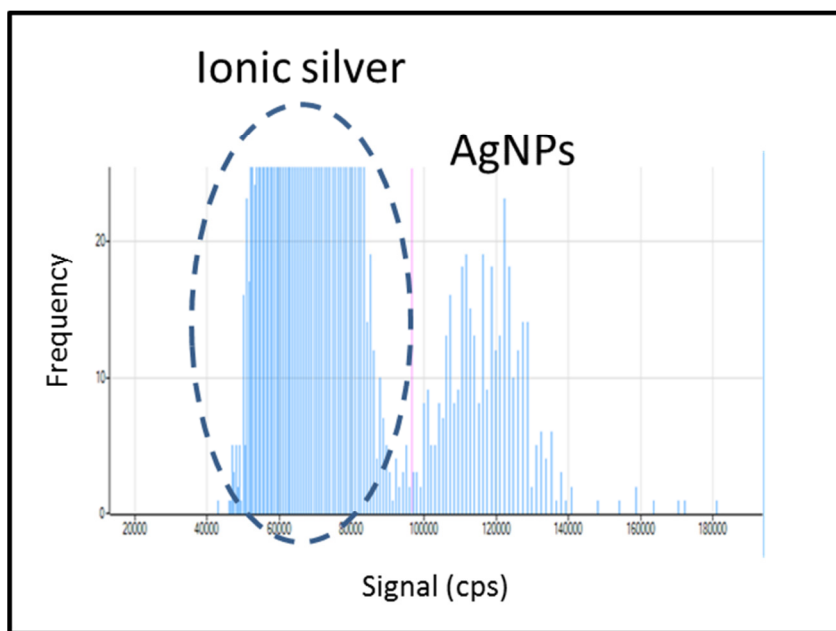
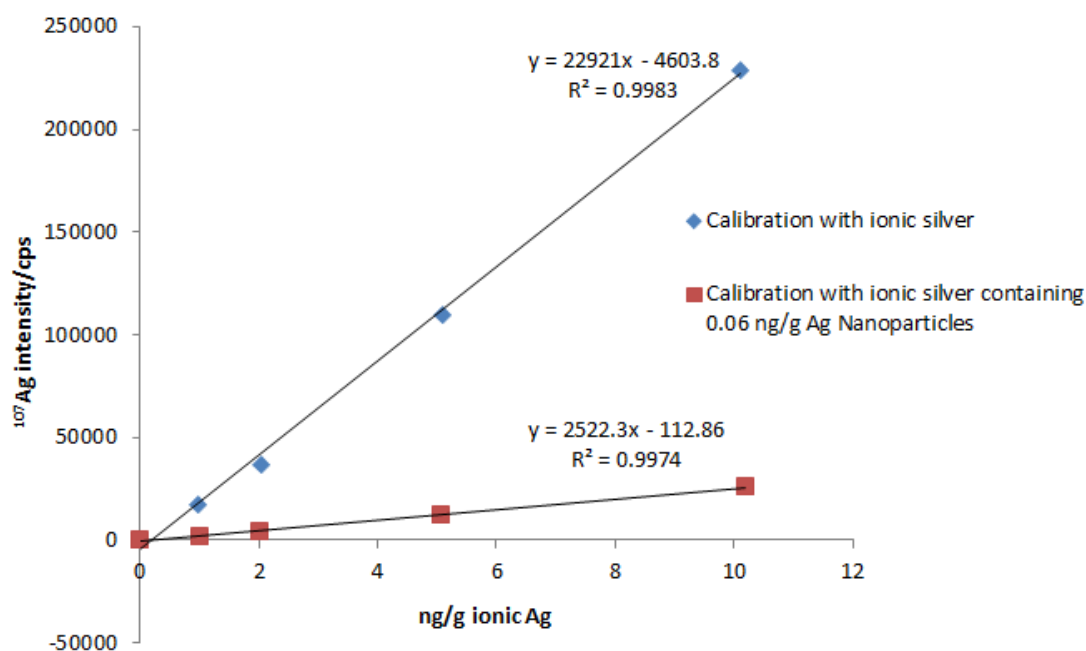**Figure 2:** Signal distribution of NIST AgNPs spiked with 5 ng/g ionic silver

Figure 3: Calibration strategies to quantify ionic silver

Conclusions and future work

The work described above is only a preliminary investigation, but demonstrates the feasibility of spICP-MS for the characterisation, in terms of both size and number-based particle concentration, of AgNPs in samples that contain low levels of ionic silver (< 10 ng/g), whilst simultaneously allowing the quantification and differentiation of the ionic silver from

AgNPs to be performed in a run of just one minute using this technique. In those samples where the levels of ionic silver is much higher in comparison with that of the nanoparticles, additional separation techniques, prior to spICP-MS measurements, such as Field Flow Fractionation (FFF), will need to be investigated since they could offer an attractive approach to achieve particle selectivity for the determination of number-based concentration using spICP-MS. Future work will involve further validation and characterisation of the developed method (e.g. for measurement uncertainty).

[1] EC Commission Recommendation, 2011/696/EU; European Union: Luxembourg, 2011

[2] http://www.nanotechproject.org/, PEN, The project on emerging nanotechnologies.

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