

# Rapid size characterization of silver nanoparticles by single particle ICP-MS and isotope dilution

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The increasing application of silver nanoparticles (AgNP) in consumer products and their potential release into the environment call for intensive investigation of their toxicity, stability, and fate. Analytical methods that are able to detect and characterize AgNPs at low concentrations and in complex matrices are needed. Single particle inductively coupled plasma mass spectrometry (spICP-MS) has in recent years emerged to a reliable technique suitable to quantify and size nanoparticles at low concentrations. However, the size determination by means of spICP-MS depends on external calibration with nanoparticle or element standard solutions. Here, a new approach is introduced using internal calibration with isotope dilution to determine the size of different AgNPs. External calibration becomes unnecessary, leading to a more rapid nanoparticle characterization and more robustness towards matrix effects. The power of the method is shown by determining the size of 50 nm citrate capped and 80 nm PVP capped AgNPs spiked with <sup>109</sup>Ag enriched silver standard. The method is highly reproducible and shows good agreement with results obtained by established methods. The successful size determination of AgNPs in wastewater and river water using this method demonstrates its practicability even in samples with high matrix loads. Sample preparation only requires the addition of an isotope enriched standard, which makes the method interesting for long-term studies when AgNPs have to be characterized on many different days. As an example, the size alteration of AgNPs under different conditions was monitored over a period of four days, employing the developed method of internal calibration with isotope dilution.

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## Introduction

The use of nanomaterials in consumer products has increased tremendously in the past years. Silver nanoparticles (AgNP) are integrated into a wide range of products for antibacterial purposes, including socks, bandages, food containers, washing machines, deodorants, and refrigerators. Typically, AgNPs have a structure with a silver Ag(0) core of varying size and shape, and an organic coating with varying molecular weight and functional groups. These surface capping agents, such as polyvinylpyrrolidone (PVP) or citrate, stabilize the nanoparticles and inhibit aggregation.<sup>1</sup>

AgNPs released from domestic and industrial sources will for the most part enter sewer systems, where they will be carried to wastewater treatment plants.<sup>2</sup> There is evidence that a high proportion of silver is removed during wastewater treatment.<sup>3</sup> Due to the high affinity of silver for sulphur, the AgNPs may be transformed into silver sulphide (Ag<sub>2</sub>S) and subsequently incorporated into sewage sludge.<sup>4–6</sup> However, there have been few published data to show whether all AgNPs are removed

during sewage treatment and how much silver is released into the aquatic environment.<sup>5</sup> The release of AgNPs and/or their transformation products into the environment could have impacts on aquatic organisms and human health. It is imperative to monitor the fate of these nanomaterials in wastewater and in the aquatic environment.

The analysis of AgNPs and other nanoparticles has in the past been carried out employing a variety of instruments and methods, including transmission electron microscopy (TEM),<sup>7,8</sup> energy dispersive X-ray spectroscopy (EDX),<sup>9,10</sup> ultrafiltration,<sup>11</sup> asymmetric flow field-flow fractionation (AF4)<sup>12–14</sup> and reverse phase liquid chromatography (RPLC)<sup>15</sup> with subsequent quantification by inductively coupled plasma mass spectrometry (ICP-MS), to name a few. However, poor detection limits of these methods, laborious sample preparation or susceptibility towards complex matrices such as wastewater prohibited the analysis of samples with low AgNP concentrations in typical environmental samples.

Single particle (sp) ICP-MS is an emerging analytical technique that allows not only quantification and sizing of nanoparticles, but also the simultaneous quantification of the respective dissolved metal. In sp mode, nanoparticles are introduced into the ICP-MS individually and are detected as one time-resolved peak for each particle. The intensity of the respective peaks gives information about the particle size, the

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number of peaks gives information about the particle concentration. At the same time, dissolved silver is detected as a constant signal and can therefore be distinguished from the particle signal.

The feasibility of spICP-MS for the analysis of colloids, including titanium and aluminum suspensions, was first proposed by Degueldre *et al.* in 2003.<sup>16</sup> Subsequent studies by these researchers described the concept in more experimental detail and developed particle quantification and sizing techniques.<sup>16–19</sup> Engineered nanoparticles, including silver,<sup>11,20–31</sup> gold,<sup>20,24,29,30</sup> titanium oxide,<sup>27</sup> cerium oxide,<sup>27</sup> and zinc oxide<sup>27</sup> have since been characterized successfully using this analytical approach.

The size determination of the respective nanoparticles can be carried out using aqueous standards of nanoparticles with well-defined diameters. However, not all nanoparticles are available in the necessary range of sizes or as mono-dispersed material. Pace *et al.* introduced a sizing method using ordinary element standards of dissolved metals to create an external calibration curve.<sup>23</sup> By including parameters such as nanoparticle transport efficiency, dwell time, and sample uptake rate into the calculations, the nanoparticle mass and size can be determined by relating the signal intensity of the nanoparticle peaks to the external calibration curve.<sup>23</sup>

To the best of our knowledge, all strategies described in the literature to determine the size of nanoparticles in aqueous solutions are based on external calibrations using standards prepared from either nanoparticles or dissolved metals. This can be problematic when measuring samples in complex matrices like wastewater, as the analyte response may depend on the matrix resulting in inaccurately calculated concentrations. When determining concentrations of dissolved analytes, this problem can be avoided by several quantification strategies. One common approach to compensate for matrix effects is by isotope dilution analysis (IDA).<sup>32</sup>

The present study describes the application of the IDA approach using <sup>109</sup>Ag for determining the size of AgNPs in aqueous matrices by means of spICP-MS. As proof of principle, the mass and diameter of AgNPs were determined with conventional external calibration and compared to results obtained with internal calibration after spiking with <sup>109</sup>Ag. This new method was applied to characterize AgNPs spiked into the influent and effluent of a wastewater treatment plant and in river water. In addition, the IDA approach was used to monitor changes in the size and concentration of AgNP aged in water over 4 days.

## Experimental

### Chemicals and consumables

Suspensions of AgNPs with spherical shape and diameters of 50 nm and 80 nm, respectively, were purchased from nanoComposix (San Diego, CA, USA). The particles were coated with citrate (50 nm) or PVP (80 nm) capping agent. The suspensions were stabilized with 378 mg L<sup>-1</sup> citrate and 1–3 µg L<sup>-1</sup> PVP, respectively. Aqueous Ag(I) standard for ICP-MS (1000 mg L<sup>-1</sup>) was obtained from SPC Science (Baie D'Urfé, QC, Canada). <sup>109</sup>Ag (99.4%) enriched silver was purchased in metallic

form from Isotex (San Francisco, CA, USA). Nitric acid (65%) was purchased from BDH Chemicals through VWR International (Radnor, PA, USA). Nylon membrane syringe filters (45 µm) were also obtained from VWR International. All chemicals were used in the highest quality available. Water was purified with a Milli-Q Element system (Millipore, Billerica, MA, USA).

### Stock and standard solutions

A 1000 mg L<sup>-1</sup> stock solution of <sup>109</sup>Ag was prepared by dissolution of 50 mg of the isotope enriched metal in 50 mL of 4% nitric acid. The stock solution was subsequently diluted to a 100 µg L<sup>-1</sup> working solution. A working suspension of AgNPs with a concentration (*c*<sub>Ag</sub>) of 20 µg L<sup>-1</sup> was freshly made every day by dilution of the nanoComposix stock suspension with water. The Ag ICP-MS standard solution was diluted to a 100 µg L<sup>-1</sup> working solution.

### Sampling

Grab samples of wastewater (influent and effluent) were collected in July 2013 during dry weather at the wastewater treatment plant for the city of Peterborough, ON, Canada. The plant serves a population of approximately 75 000 people and uses secondary treatment with activated sludge. A grab sample of Otonabee River water was taken from the river bank in July 2013 during dry weather. All samples were collected in polyethylene (PE) bottles and were stored in darkness at 4 °C.

### Instrumentation and set-up

**Transmission electron microscope (TEM).** Measurements were carried out with a Philips CM200 TEM. This unit is equipped with a LaB<sub>6</sub>-cathode thermionic gun. Operating voltage was set to 200 kV, the point-to-point resolution was 0.24 nm and the line resolution 0.17 nm. Data were evaluated optically/manually from the photomicrographs obtained from the Gatan CCD camera (2k × 2k).

**Inductively coupled plasma mass spectrometry (ICP-MS).** A ThermoFisher (Bremen, Germany) XSeries 2 ICP-MS was used for spICP-MS analysis. The samples were introduced into the plasma with a borosilicate glass conical nebulizer (1 mL min<sup>-1</sup>, AHF, Tübingen, Germany) *via* a conical spray chamber with impact bead (AHF). Prior to measurements, the operating conditions were optimized with Ag(I) ICP-MS standard (5 µg L<sup>-1</sup>) to maximize the signal intensity. Standard measurements were carried out using the self-aspiration mode of the nebulizer. The nebulizer gas flow was set to 0.85 L min<sup>-1</sup>. Environmental samples are transported with a peristaltic pump and mixed in-line with <sup>109</sup>Ag spike solution *via* a T-piece prior to introduction into the plasma. In this case the nebulizer gas flow was set to 0.93 L min<sup>-1</sup>. The plasma was run at a RF power of 1450 W. Cool gas was operated with a flow of 15 L min<sup>-1</sup>. The spray chamber was cooled externally to 4 °C. The run time was 300 s for AgNP suspensions and dissolved Ag(I) solutions.

### Sample preparation and experiments

**TEM.** TEM analysis was carried out with undiluted nanoComposix stock suspensions (50 nm, 80 nm). Prior to measurement, copper TEM grids were soaked in the suspensions and then dried.

**spICP-MS general.** The size determination with external calibration as well as with isotope dilution requires the determination of the particle transport efficiency. A suspension of 80 nm PVP capped AgNPs with a defined concentration (*i.e.* 200 ng L<sup>-1</sup>) was prepared in water. The suspension was analyzed every three to four hours in sp mode. This run was also used to determine the isotopic abundances and ratios of <sup>107</sup>Ag and <sup>109</sup>Ag in the nanoparticles. The isotope abundance of Ag in the spike solution was also determined daily. A 5 µg L<sup>-1</sup> solution of the <sup>109</sup>Ag enriched spike was prepared in water. Three replicates of the solutions were recorded in sp mode. The exact flow rate of the nebulizer was also determined daily, both for uptake with self-aspiration and uptake *via* the T-piece and peristaltic pump. During analyses of all samples and calibration solutions and suspensions, the intensities of <sup>107</sup>Ag and <sup>109</sup>Ag were recorded in the same run. The respective quadrupole settling time is 1.9 ms, leading to an actual runtime of about 109 s per isotope per run.

**Dwell time optimization.** A range of dwell times from 1–100 ms have been reported in the literature for spICP-MS measurements. Three dwell times of 1 ms, 5 ms, and 10 ms were tested for best performance. A suspension of 200 ng L<sup>-1</sup> AgNPs (80 nm, PVP coated) was analyzed three times at each dwell time, respectively.

**Isotope spiking of AgNPs in water.** To investigate the feasibility of isotope spiking, suspensions were prepared in water of 100 ng L<sup>-1</sup> 50 nm citrate capped AgNPs and 200 ng L<sup>-1</sup> 80 nm PVP capped AgNPs. The 50 nm suspension was spiked with 100 ng L<sup>-1</sup> of <sup>109</sup>Ag enriched silver and the 80 nm suspension was spiked with 250 ng L<sup>-1</sup> of the isotope enriched Ag. These concentrations ensure a <sup>109</sup>Ag intensity that is high enough to obtain a measurable difference in intensity between the two isotopes needed for IDA, and at the same time allowing definite peak identification in spite of increased signal noise. The spiked suspensions were analyzed ten times in sp mode consecutively. The procedure was repeated on a different day with freshly prepared suspensions to obtain two independent sets of analyses. For the conventional sizing approach using external calibration, Ag(I) standard solutions were made by diluting the working solution to  $c_{\text{Ag}} = 0, 0.10, 0.25, 0.50, 0.75, 1.00$  and  $2.00 \mu\text{g L}^{-1}$ .

**Wastewater and river water.** Samples of wastewater and river water were filtered through 0.45 µm nylon syringe filters. Then, aliquots of the 80 nm PVP capped AgNP working suspension were added to the samples to reach a final nominal concentration of approximately 3 mg L<sup>-1</sup>. Separately, a 250 ng L<sup>-1</sup> <sup>109</sup>Ag enriched solution (*i.e.* spike solution) was prepared in water. A T-piece was employed to allow mixing of the sample and spike solution directly before introduction into the ICP-MS. The sample was pumped through tubing with a diameter of 0.38 mm and the spike solution was pumped through tubing with a diameter of 1.85 mm. The peristaltic pump was set on 5 rpm, resulting in a rate of uptake of the sample of  $q = 0.21 \text{ mL min}^{-1}$  and a rate of uptake of the spike solution of  $q = 3.29 \text{ mL min}^{-1}$ . In addition to spiking the sample with isotope enriched standards, this set-up allows dilution of the sample without laborious sample preparation and without altering the sample prior to analysis.

**Stability monitoring.** The stability of PVP coated AgNPs (100 ng L<sup>-1</sup>, 80 nm) was monitored over four days under different conditions. Sample 1 and sample 2 were prepared in purified water and stored at room temperature and at 4 °C, respectively. Sample 3 was prepared in purified water with a pH of 4.1, adjusted with nitric acid, and was also stored at room temperature. Samples were run frequently over 4 days in sp mode using the previously described conditions. Directly before measurement, a 5 mL aliquot of the samples was mixed with 5 mL of a 500 ng L<sup>-1</sup> <sup>109</sup>Ag standard solution.

**ICP-MS data acquisition and processing.** Data from the ICP-MS were processed using PlasmaLab software, version 2.5.9.300 (ThermoFisher). Raw data were exported as Thermo Electron Glitter Format V1.1. The numerical results were subsequently imported into the software OriginLab8 (OriginLab Corp., Northampton, MA, USA) for further processing.

For the determination of mean peak intensities of the AgNPs, <sup>107</sup>Ag and <sup>109</sup>Ag intensities were plotted as histograms with increments of 1000 cps for measurement with a dwell time of 5 ms (500 cps increment for 10 ms and 2000 cps increment for 1 ms dwell time). The histograms were then in the respective intensity range fitted to a Gaussian function to determine the mean intensity of the nanoparticles in the samples.

The mean background intensity was determined by plotting <sup>107</sup>Ag and <sup>109</sup>Ag intensities with an increment of 200 cps, and proceeded as described above. The increment was chosen to be lower, because the intensity of the <sup>107</sup>Ag background usually ranges between 0 and 3000 cps, depending on the age of the sample.

The mean intensities of the Ag(I) solutions used for external calibration, isotope ratio determination with Ag ICP-MS standard and <sup>109</sup>Ag spike were determined by averaging the single readings from each analysis.

## Results and discussion

### TEM

TEM measurements yielded an average diameter of 50.2 nm for the 50 nm nanoparticles and 80.65 nm for the 80 nm nanoparticles. The photomicrographs shown in Fig. 1 clearly illustrate that the size distribution is very high, which is consistent with the manufacturer's information. Also, the AgNPs are not

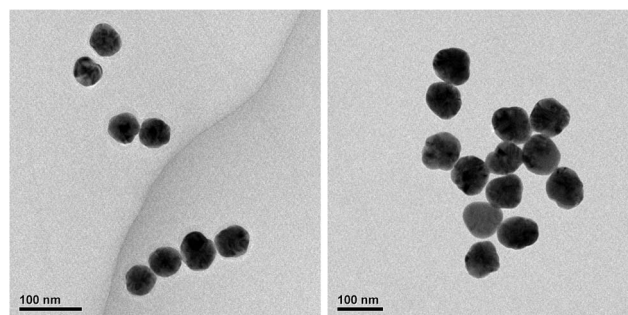


Fig. 1 TEM photomicrographs of AgNPs with nominal diameters of 50 nm (left) and 80 nm (right).

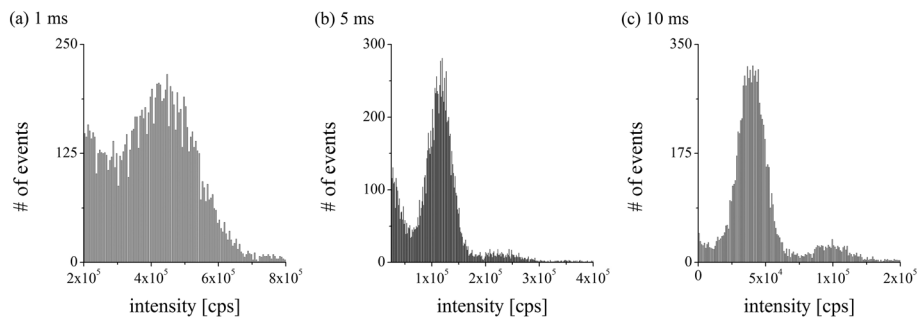


Fig. 2 Histograms of the peak intensity of 80 nm PVP capped AgNPs run with dwell times of (a) 1 ms, (b) 5 ms, and (c) 10 ms.

perfectly spherical. This is important since spICP-MS might work with any NP shape, but the mathematical processing mainly refers to spherical NPs.

### Dwell time optimization

Fig. 2 shows the histograms of the peak intensities of the 80 nm PVP capped AgNPs analyzed with dwell times of 1 ms, 5 ms, and 10 ms, respectively. The mean intensities of the particles are highest for measurements carried out with a dwell time of 1 ms. However, the histograms show that the lower minimum of the intensity distribution is not clearly defined, and the distribution could not be fitted to a Gaussian function. Most likely, the low dwell time prohibits the ICP-MS from detecting the entire ion cloud for each single particle in individual data acquisition. The histogram of the measurement carried out with a dwell time of 10 ms shows a low mean intensity, but there is a clear minima for the intensity distribution (Fig. 2). A second smaller peak at approximately twice the intensity of the main peak, suggests frequent particle coincidence. This could be avoided by diluting suspensions to lower particle concentrations, which would mean higher run times to achieve statistically evaluable intensity distributions.

Measurement with a dwell time of 5 ms led to a high mean intensity of the particles (Fig. 2). The histogram indicates infrequent coincidences and a distribution that can easily be fitted to a Gaussian function. Therefore, for all subsequent measurements, a dwell time of 5 ms was used. The limit of detection regarding particle size under these conditions is 40 nm. This is adequate for the conducted size determination of 50 nm and 80 nm AgNPs, as well as the stability monitoring of 80 nm particles over a couple of days.

### Size determination of AgNPs in purified water

The transient signal intensity for the 200 ng L<sup>-1</sup> 80 nm AgNPs suspension in water is displayed in Fig. 3. Histograms of binned intensities of <sup>107</sup>Ag and <sup>109</sup>Ag are also shown. Fitting the histograms to a Gaussian function generated mean intensities for both peaks.

The background intensity (dissolved silver concentrations) has to be subtracted to avoid inaccurate calculations. For <sup>107</sup>Ag measurements, the mean background is easily determined by fitting a Gaussian curve to the background intensity distribution, as described in the Experimental section (data processing). However, determining the background of the <sup>109</sup>Ag signal is not straightforward, because the constant silver signal is mainly derived from the dissolved <sup>109</sup>Ag spike. Therefore, the <sup>109</sup>Ag background was calculated by multiplying the value of the <sup>107</sup>Ag background with the ratio of silver natural isotopic abundances (<sup>109</sup>Ag/<sup>107</sup>Ag), determined daily. This procedure is based on the assumption that the background silver contamination is of natural isotope abundance (only possible if the abundance of the spiked enriched isotope is close to 100%), leading to a <sup>107</sup>Ag background signal that is slightly higher than the <sup>109</sup>Ag background.

The following IDA calculations are based on eqn (1). The derivation of this equation (symbols are described below) is described in detail by Rodríguez-González *et al.*<sup>32</sup>

$$c_s = c_{sp} \frac{m_{sp}}{m_s} \frac{M_s}{M_{sp}} \frac{A_{sp}^b}{A_s^a} \left( \frac{R_m - R_{sp}}{1 - R_m R_s} \right) \quad (1)$$

Eqn (1) can be used to determine the unknown concentration of a dissolved analyte ( $c_s$ ) based on the concentration of a

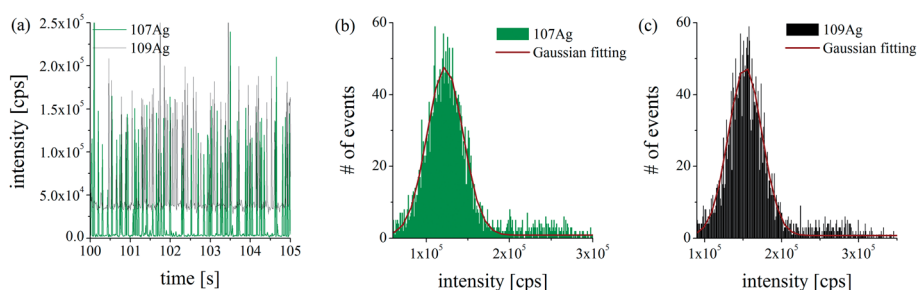


Fig. 3 Transient signal and histograms of the peak intensity of both silver isotopes of 80 nm PVP capped AgNPs run in sp mode.



dissolved standard of an enriched isotope of the respective analyte ( $c_{sp}$ ). In our experiments, we used a dissolved standard of  $^{109}\text{Ag}$ . However, the aim is to determine the mass of the AgNPs. Therefore, a calculation has to be integrated that correlates the concentration of the dissolved analyte to a total analyte mass per reading.

Pace *et al.* developed a protocol for determining the particle mass by using external calibration with dissolved standards.<sup>23</sup> Eqn (2) shows how the mass per particle ( $m_p$ ) can be calculated from the respective analyte concentration introducing transport efficiency  $\eta$ , dwell time  $d_t$ , and sample uptake rate  $q$  as additional parameters.

$$m_p = \eta q d_t c_s \quad (2)$$

An increase of the transport efficiency generally leads to a higher signal of the dissolved analyte, whereas the nanoparticle signal does not change when detected individually (however, increased transport efficiency would increase the number of measured particles). Pace *et al.* describe in detail, how eqn (2) accounts for the differences in mass delivery of dissolved analyte and nanoparticles.<sup>23</sup>

Eqn (1) and (2) can now be combined to determine the mass per particle  $m_p$  based on IDA.

$$m_p = \eta q d_t c_{sp} \frac{m_{sp}}{m_s} \frac{M_s}{M_{sp}} \frac{A_{sp}^b}{A_s^a} \left( \frac{R_m - R_{sp}}{1 - R_m R_s} \right) \quad (3)$$

The concentration of the enriched isotope spike is  $c_{sp}$ . The values  $m_{sp}$  and  $m_s$  are the mass of the spike and the sample respectively.  $A_{sp}^b$  represents the isotopic abundance of  $^{109}\text{Ag}$  in the spike (*i.e.* 0.994) and  $A_s^a$  represents the isotopic abundance of  $^{107}\text{Ag}$  in the original sample.  $A_{sp}^b$  and  $A_s^a$  are calculated with inputs of the isotope ratio, determined by analyzing daily a fresh AgNP suspension and the  $5 \mu\text{g L}^{-1}$  spike solution, as described in the Experimental section. This procedure circumvents mass discrimination effects which otherwise could cause major errors in the calculation.  $M_s$  is the atomic weight of Ag in the sample and  $M_{sp}$  is the atomic weight of Ag in the spike. The isotope ratio in the mixture of sample and spike is  $R_m$ , which is the ratio of the mean  $^{107}\text{Ag}$  and  $^{109}\text{Ag}$  nanoparticle peak intensities (background corrected).  $R_{sp}$  is the isotope ratio  $^{107}\text{Ag}/^{109}\text{Ag}$  in the spike and  $R_s$  is the isotope ratio for  $^{109}\text{Ag}/^{107}\text{Ag}$  in the unspiked sample. The ratios are determined daily by measuring the intensities of both silver isotopes in a fresh AgNP suspension and a spike solution as described above.

The transport efficiency  $\eta$  can be calculated with eqn (4).<sup>23</sup> For the present study,  $\eta$  was determined daily with the frequency  $f$  of AgNP peaks in this run, the sample uptake rate  $q$ , and the known particle number concentration  $N_p$  in the AgNP suspension.

$$\eta = \frac{f}{q N_p} \quad (4)$$

Assuming a spherical geometry, eqn (5) can be used to calculate the particle diameter from the determined

particle mass, using the known density of silver,  $\rho = 1.049 \times 10^{-14} \mu\text{g nm}^{-3}$ .

$$d = \sqrt[3]{\left[ \frac{6m}{\pi\rho} \right]} \quad (5)$$

The following example shows the calculations in four steps used for one of the replicate analyses of the 80 nm PVP AgNP suspension:

Step 1:

- Determine mean  $^{107}\text{Ag}$  and  $^{109}\text{Ag}$  intensities of nanoparticle peak by Gaussian fitting

int.  $^{107}\text{Ag}$  = 117 800 cps

int.  $^{109}\text{Ag}$  = 147 500 cps

- Determine mean background intensity of  $^{107}\text{Ag}$  by Gaussian fitting

bckg.  $^{107}\text{Ag}$  = 1500 cps

- Background correction

int.  $^{107}\text{Ag}$  = 117 800 cps – 1500 cps = 116 300 cps

int.  $^{109}\text{Ag}$  = 147 500 cps – 1500 cps  $\times$  0.968 = 146 050 cps

Step 2:

- Calculate  $\eta$  by employing eqn (4)

$f$  = 23.367 particles per s

$q$  = 0.0215 mL s<sup>-1</sup>

$N_p$  = 71 051 particles per mL

$\eta$  = 0.015

- Determine  $R_m$

$R_m$  = 116 300 cps/146 050 cps = 0.796

- Determine  $R_{sp}$  and  $R_s$

$R_{sp}$  = 0.006

$R_s$  = 0.968

Step 3:

- Calculate the particle mass by employing eqn (3)

$m_p$  =  $2.811 \times 10^{-9} \mu\text{g}$

Step 4:

- Calculate the particle diameter by employing eqn (5)

$m$  = 79.98 nm

The results of the measurements of the 80 nm PVP-capped and the 50 nm citrate-capped AgNPs in water are summarized in Table 1. Results for the silver mass and the diameter of the AgNPs are shown for analyses using both our new method using an enriched isotope spike as an internal standard and the

**Table 1** Mean diameters and relative standard deviations (RSD) of AgNPs suspended in water, determined with internal calibration with a  $^{109}\text{Ag}$  enriched isotope and with conventional external calibration

	50 nm, citrate capped		80 nm, PVP capped	
<b>Internal calibration</b>				
Mean diameter	50.16 nm	51.07 nm	80.20 nm	79.85 nm
RSD	1.51%	1.31%	1.28%	1.45%
<b>External calibration</b>				
Mean diameter	50.40 nm	50.89 nm	79.70 nm	79.06 nm
RSD	1.14%	1.14%	0.23%	1.28%

conventional method using external calibration as described by Pace *et al.*<sup>23</sup>

Relative standard deviations (RSD) between replicate measurements were low (<1.6%) for the measurements with internal calibration. Therefore, the new quantitation procedure was highly reproducible for calculating the size of AgNPs. More importantly, there was little discrepancy between the nanoparticle sizes determined by the new method and by the established external calibration method. We assume that the little remaining deviation is mostly attributed to small variations in transport efficiency or small inaccuracies in the Gaussian fitting of the intensity histograms. Since the RSDs of the results of the new method and the method based on external calibration are similar, we propose that the employment of the enriched isotope spike does not lead to additional uncertainty in the size determination.

The excellent correlation between both methods suggests that spiking with <sup>109</sup>Ag enriched silver is well suited for AgNP sizing. Also, these measurements were consistent with the mean diameters determined by TEM.

One difference between conventional isotope dilution analysis of dissolved elements and that of nanoparticles is homogeneity. When detecting metal ions in solution, one can easily determine the ratio of two isotopes although they are (usually) determined consecutively with a short settling time in between individual isotope mass measurements. In sp mode, however, each data reading represents the intensity of one particular NP. Since the NPs vary in mass, consecutively measured <sup>107</sup>Ag and <sup>109</sup>Ag signals represent two different particles, which are not necessarily of similar size. It is therefore very important to collect sufficient data of multiple NPs to allow adequate averaging of peak intensities. Only then a correct determination of the mean isotope ratio of AgNPs can be ensured.

The fact that consecutively measured <sup>107</sup>Ag and <sup>109</sup>Ag signals represent two different particles also prohibits the determination of the isotope ratio of individual NPs. It is therefore difficult to determine the size distribution in a solution of nanoparticles with the presented new approach. In a suspension of NPs with different sizes, the various mean diameters can only be determined if the maxima in the intensity distribution can be fitted with distinct Gaussian curves.

### Wastewater and river water

Samples in aqueous matrices should be altered as little as possible to avoid potential transformation of the analyte. In the case of the AgNPs on hand, dilution should be avoided to prevent dissolution of the particles. For this reason, wastewater and river water samples for this experiment were only filtered and spiked with AgNPs. As described in the Experimental section, a T-piece was employed to mix the sample flow with the <sup>109</sup>Ag spike solution directly before entering the ICP-MS. The respective uptake rates of sample and spike led to a dilution factor of ~20.

Particle mass, particle volume and diameter of AgNPs were calculated for spiked (80 nm PVP capped) water and wastewater samples as described above. The mean diameters of AgNPs determined from 10 replicate measurements of spiked

**Table 2** Mean diameters and relative standard deviations (RSD) of AgNPs in spiked samples of wastewater influent and effluent, and river water, determined by internal calibration with a <sup>109</sup>Ag enriched isotope

	Influent	Effluent	River water
Mean diameter	79.96 nm	80.22 nm	78.79 nm
RSD	1.90%	1.17%	0.74%

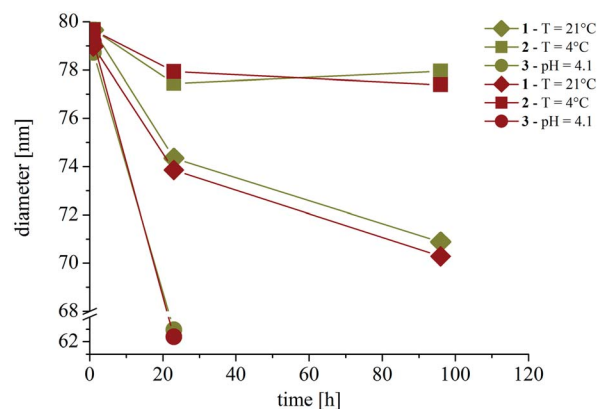
wastewater influent, effluent, and river water sample are presented in Table 2.

The relative standard deviations between replicate measurements were <1.9%. The new procedure for sizing nanoparticles by spiking with isotope enriched silver was highly reproducible, even in complex wastewater matrices. The mean calculated sizes showed only a slight deviation from the expected value of 80 nm. This approach offers a powerful alternative to characterize AgNPs rapidly, without additional calibration solutions and without matrix interferences.

AgNP concentrations in wastewater and river water were determined by counting NP peaks in each run. With the knowledge of transport efficiency  $\eta$ , sample uptake rate  $q$ , and dilution factor, the particle and corresponding Ag concentration, was calculated. In the influent, an average concentration of 3.00 mg L<sup>-1</sup> was determined, in the effluent 2.75 mg L<sup>-1</sup>, and in river water 3.16 mg L<sup>-1</sup>. These concentrations correlate to the expected values. Please be aware that all water samples were spiked with AgNPs in order to demonstrate the feasibility of the method.

### Stability monitoring

The impacts of engineered nanoparticles on the aquatic environment are dependent on their long-term stability. Several previous studies have shown that the stability of AgNPs in suspension is highly dependent on the ambient conditions, including the pH, as well as the concentration of chloride and dissolved organic matter.<sup>33–36</sup>



**Fig. 4** Changes in the mean diameter of AgNPs in suspension over time under different storage conditions. Results of sizing with internal isotope dilution are shown in green, and the sizing using external calibration in brown.

With previous long-term studies of the fate of nanoparticles in suspension conducted using spICP-MS, laborious preparation of external standards was necessary. Here, the rapid monitoring of AgNP diameters by means of spICP-MS with isotope dilution is presented.

Fig. 4 shows the diameters of the AgNPs stored under different conditions and changes over time. For comparison, the nanoparticle size was determined by external calibration and with internal calibration based on isotope spiking, as described above.

In all samples, a decrease of diameter was observed over time (Fig. 4). This is most likely due to dissolution. An increase in the constant signal resulting from dissolved silver supports this assumption.

As can be clearly seen from sample 2, storage at colder temperatures significantly improves the stability (Fig. 4). The lowering of pH by the addition of acid, as in sample 3, leads to a more rapid decrease in size compared to unaltered purified water (sample 1). These observations are in accordance with previous observations.<sup>33,35</sup>

Similar diameters were calculated for the AgNPs in the different samples using either daily external calibration or internal isotope dilution. These results suggest that internal calibration with isotope enriched silver is an excellent strategy to monitor the dissolution of AgNPs in aqueous media avoiding laborious and time consuming sample preparation. Only the addition of a known amount of isotope enriched silver is necessary. This can be of major importance when similar studies are carried out over a period of many days.

## Conclusions

This new approach for calculating the diameter of nanoparticles is based on isotope dilution analysis with addition of isotope enriched material. Using this analytical method, we were able to accurately estimate the mean particle diameter of AgNP suspensions prepared in purified water, river water and wastewater, with RSDs of replicate measurements below 1.9%. There was excellent agreement between the analytical results using this new calibration method and the conventional procedure. This method has the advantage of avoiding time-consuming preparation of samples and measurement of external standard solutions. In addition, matrix effects are overcome when using internal calibration with isotope dilution. This is especially crucial when characterizing nanoparticles in complex environmental samples. The sample preparation is easy and only needs the addition of the isotope enriched standard. This new characterization procedure is ideal for long-term fate studies that require the analysis of samples on many different days. Using this method, we were able to demonstrate reductions in the size of AgNPs in aqueous suspensions over time as a result of dissolution of the core material.

The presented method allows rapid size determination of suspensions of NPs with a distinct size. However, it is difficult to investigate complex size distributions. Although this is a disadvantage compared to established methods, the new presented method will be of great benefit for the analysis of matrix loaded environmental samples and stability experiments that focus on NPs with definite diameters.

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