

# Optimizing Instrument Operation and Data Processing for Single Particle Inductively Coupled Plasma Mass Spectrometry Measurement of Gold Nanoparticles

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## ABSTRACT

Inductively coupled plasma mass spectrometry (ICP-MS) operated in single particle mode is an attractive analytical tool capable of both sizing and counting metal containing nanoparticles in aqueous suspensions. In this study, we have evaluated the performance of single particle-ICP-MS (spICP-MS) for gold nanoparticle (AuNPs) characterization. Instrument operating parameters, test solution concentration and data processing were optimized for accurate size and particle number concentration measurement. We demonstrate that short measurement dwell time induces severe particle splitting between adjacent dwells, and the optimal dwell time in current study, limiting both splitting and coincident particle events, is 10 ms. A pre-established protocol for particle size calculation using the measured aerosol transport efficiency and soluble standard calibration was examined over a range of AuNPs diameters from 10 nm to 200 nm. Finally, size measurements were performed on heterogeneous Au solutions containing both soluble Au and AuNPs.

**Keywords:** Single particle-ICP-MS, nanomaterial characterization, gold nanoparticles

## 1 INTRODUCTION

The increasing application of engineered nanomaterials (ENMs) in commercial products raises concern about the emission of nanoparticles into the environment, where the fate, transport, stability, and potential risks of ENMs remain largely unknown [1]. Full-scale risk assessment of ENMs in natural environments needs reliable techniques for detecting, quantifying and characterizing nanoparticles under relevant exposure conditions. The “*in situ*” measurement is challenging for most routine nanoscale characterization tools due to the extremely low environmental concentrations of ENMs (predicted to be on the order of ng/L [2]), the compromised sensitivity in complex environmental matrix, and the interference from natural colloids [3]. There is a need in the nanotechnology community for improved nanomaterial characterization metrology.

In recent years, inductively coupled plasma mass spectrometry (ICP-MS) operated in time-resolved analysis (TRA) mode has emerged as a promising analytical tool

capable of both sizing and counting metal containing nanoparticles in aqueous suspensions [4]. The theory of single particle ICP-MS (spICP-MS) for characterizing colloids in aqueous solutions was firstly proposed by Degueldre *et al.* [4-7]. Briefly, introduction of a dilute suspension of nanoparticles into the ICP-MS will produce a signal intensity spike each time an individual particle enters the plasma, is ionized, and detected as a cluster of ions. Using a sufficiently low particle number concentration and short data detection window (dwell time), each intensity spike represents a single particle event. The frequency and intensity of the pulses can then be used to derive the particle number concentration and particle mass (size). The major advantages of spICP-MS over other material characterization techniques are its superior sensitivity and element specificity that may overcome the challenges of extremely low concentration and complex matrix effects in environmental sample measurements [8]. Single particle-ICP-MS also offers simultaneous detection of both soluble and particulate species, as well as size distribution analysis for polydisperse nanoparticles.

In this study, we systematically examined the use of spICP-MS for detecting and quantifying nanoparticles. We used AuNPs as the model material, and investigated the effects of instrument operating parameters, test solution concentration and data processing on the performance and outcome of spICP-MS. A pre-established protocol for particle size calculation was evaluated over a wide range of diameters from 10 nm to 200 nm. Finally, the feasibility of spICP-MS in characterizing heterogeneous samples was tested with Au mixture solutions containing both soluble and nanoparticulate Au.

## 2 MATERIALS AND METHODS

### 2.1 Materials<sup>1</sup>

Monodisperse citrate stabilized spherical AuNPs were used in this spICP-MS study. Gold nanoparticle reference materials RM8011, RM8012, and RM8013 (nominal diameters of 10 nm, 30 nm and 60 nm respectively, NIST, MD), and AuNP suspensions (nominal diameters of 20 nm,

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<sup>1</sup> The identification of any commercial product or trade name does not imply endorsement or recommendation by the National Institute of Standards and Technology.

80 nm, 100 nm, and 200 nm, Ted Pella, CA) were used. The NIST 10 nm, 30 nm and 60 nm reference materials have certified Au mass fractions of  $(51.56 \pm 0.23) \mu\text{g/g}$ ,  $(48.17 \pm 0.33) \mu\text{g/g}$  and  $(51.86 \pm 0.64) \mu\text{g/g}$  respectively, where the expanded uncertainty (95% confidence interval) is calculated according to the ISO Guide [9]. The Ted Pella gold nanoparticles were supplied at  $57 \mu\text{g/g}$ , as reported by the vendor. NIST SRM 3121 Gold (Au) Standard Solution (Lot No. 991806) was used to prepare soluble gold calibration solutions. This SRM lot has a certified Au mass fraction of  $(9.89 \pm 0.02) \text{mg/g}$ , where the uncertainty is expanded at a confidence level of approximately 95%. The matrix of the SRM is approximately 10% (volume fraction) HCl.

## 2.2 Single Particle ICP-MS

A quadrupole ICP-MS (XSeries 2, ThermoFisher Scientific, Waltham, MA, USA) with a PFA-ST nebulizer (Elemental Scientific, Omaha, NE, USA) and an impact bead spray chamber was used for single particle analysis. The instrument was tuned daily for maximum  $^{115}\text{In}$  sensitivity and minimum  $^{156}\text{CeO}/^{140}\text{Ce}$  oxide level. The sample uptake rate was measured every day in triplicate by weighing a vial containing DI water before and after 5 min of aspiration, and was relatively constant at (0.18 to 0.19) mL/min. AuNP suspensions were prepared by diluting the stock suspensions with de-ionized (DI) water to particle concentrations in the range of  $2.4 \times 10^4 \text{mL}^{-1}$  to  $1.2 \times 10^5 \text{mL}^{-1}$ . Soluble gold calibration standards containing (0 to 28) ng/g Au were prepared in thiourea solution (2.4 % (volume fraction) HCl, 0.04 % (volume fraction)  $\text{HNO}_3$  and 0.5 % (mass fraction) thiourea). Additionally, mixtures of soluble Au (0.041 ng/g) and 60 nm AuNPs ( $2.4 \times 10^4 \text{mL}^{-1}$ ), and 30 nm AuNPs and 60 nm AuNPs (both at  $2.4 \times 10^4 \text{mL}^{-1}$ ) were prepared in DI water for examining the effectiveness of spICP-MS in characterizing complex samples.

## 2.3 Data Acquisition and Evaluation

For single particle measurement,  $^{197}\text{Au}$  was recorded in transient time resolved analysis (TRA) mode, and the duration of each run was 60 s with quadrupole settling time of 10 ms and dwell time of (1 to 20) ms. Time resolved intensities, in units of counts per second (cps), were exported to Excel (Microsoft, Bellevue, WA, USA) and converted to counts per event by multiplying by the dwell time. Typical intensity-time profiles for DI water blank, soluble Au solution and 60 nm AuNPs suspension are shown in Figure 1. Differentiation of particle pulses from the background was accomplished using a five times standard deviation threshold [10] in the following manner. The mean particle count ( $\bar{I}$ ) and standard deviation ( $\sigma$ ) are calculated for the data set, and all data  $5 \times \sigma$  above the mean are collected as nanoparticle events and removed from the data set. This selection process is repeated by

calculating a new  $\bar{I} + 5 \times \sigma$  of the remaining data. Nanoparticle pulses are collected in this way until no more data points can be identified as particle signal. The remaining data represent the instrument background and dissolved Au fraction.

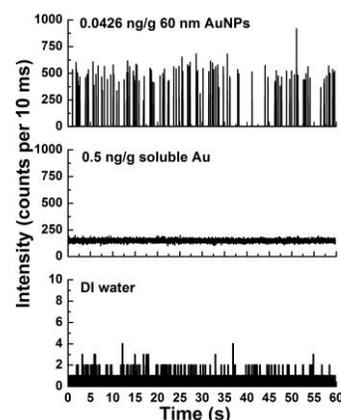
At optimized sample measurement conditions, each intensity spike is ideally produced by a single particle. Therefore the frequency ( $f_p$ , number of pulses/min) and intensity ( $I_p$ , counts/event) of those spikes can be used for quantifying number concentration ( $N$ , number of particles/mL) and AuNP mass ( $m_p$ ) and/or diameter ( $d_p$ ), respectively. An established protocol [8] was used to correlate particle size with experimental pulse intensity *via* the soluble calibration curves. Intensity-mass calibration curves were established using soluble Au standards (0 ng/g to 28 ng/g) and AuNPs (30 nm and 60 nm), respectively. Assuming nanoparticulate Au behaves the same as dissolved Au in the plasma, the ratio of the slope of the soluble calibration curve ( $S_{sol}$ ) over the slope of the particle calibration curve ( $S_p$ ) will give the effective transport efficiency ( $\eta_{tran}$ ). The number concentration and particle mass/size can then be determined by the following equations:

$$N = \frac{f_p}{v_{liq} \times \eta_{tran}} \quad (1)$$

$$m_p = \frac{(I_p - I_{bgd})}{S_{sol}} \times \eta_{tran} \quad (2)$$

$$d_p = \sqrt[3]{(6m_p / \pi\rho)} \quad (3)$$

where  $v_{liq}$  (mL/min) is the sample uptake rate,  $I_{bgd}$  (counts/event) is the average background intensity, and  $\rho$  is Au density. The sizes calculated from each particle pulse were binned to create a size distribution histogram.



**Figure 1.** Single particle-ICP-MS measurements ( $^{197}\text{Au}$ ) of DI water, 0.5 ng/g soluble Au in thiourea solution, and 0.0426 ng/g ( $2.4 \times 10^4 \text{mL}^{-1}$ ) 60 nm AuNPs in water. Dwell time: 10 ms.

**Table 1.** Effect of dwell time on spICP-MS measurements of 60 nm AuNPs\*.

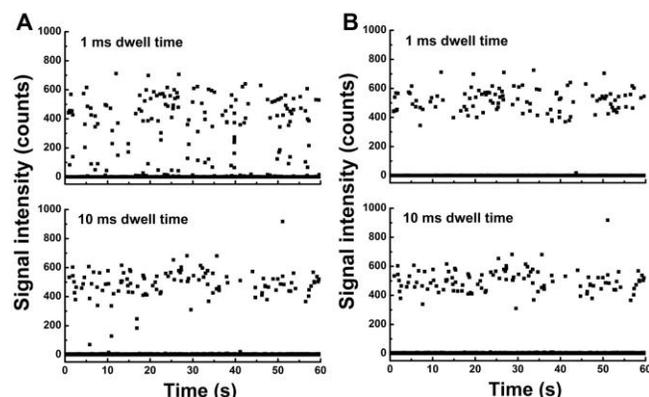
Dwell time (ms)	Observed particle events (number/60 s)	Observed average particle intensity (counts/event)	Percentage of incomplete pulses (%)	Corrected average particle intensity (counts/event)
1	198±8	281.04±5.08	30.4±1.3	508.42±13.26
5	138±16	485.82±15.72	7.7±1.4	526.40±8.89
10	133±12	474.19±8.65	3.9±1.3	493.48±6.04
15	141±10	509.70±14.83	3.2±0.3	526.48±16.83
20	97±8	515.55±17.9	2.3±1.1	527.99±13.77

\*The uncertainty (68.3% confidence interval) is calculated from 2-4 replicates.

### 3 RESULTS AND DISCUSSION

#### 3.1 Optimization of Dwell Time

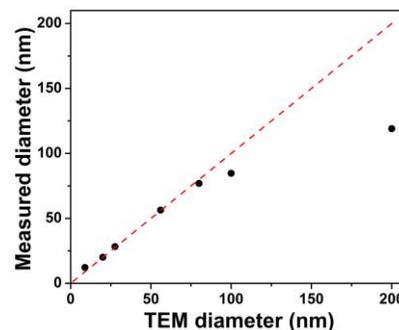
The effect of dwell time on spICP-MS data quality was first evaluated using 60 nm AuNPs, as summarized in Table 1. The average particle intensity is significantly lower at 1 ms dwell compared with measurements using longer dwell times. However, spICP-MS theory suggests the intensity of a single particle event is independent of dwell time for a given particle size if dwell time is sufficiently greater than the duration of a particle event (typically 0.1 ms to 0.5 ms [10]). After careful examination of the 1 ms data set, we identified 60 false particle events out of 198 observed events due to signal splitting between adjacent dwells. To our knowledge, this is the first quantitative demonstration of partial ion counting in spICP-MS measurement. The average particle intensity after correction for those incomplete data points (Figure 2) is comparable to intensities measured using 5 ms to 20 ms dwell intervals. The incidence of signal splitting greatly declines at longer dwell times (Table 1), but we start to observe increased particle coincidence with dwell times of 15 ms or longer. A dwell time of 10 ms is a good compromise to minimize both particle splitting and coincidence, and is used in the following discussions.



**Figure 2.** Time-resolved intensity for 60 nm AuNPs before (A) and after (B) correction of split pulses. AuNPs were used at  $2.4 \times 10^4$  mL<sup>-1</sup>.

#### 3.2 Single Particle-ICP-MS for Particle Sizes and Number Concentration

Using the calculated transport efficiency and soluble calibration curve, the size distribution and particle number concentration of suspended nanoparticles can be determined by spICP-MS [8]. This protocol uses an assumption that the nanoparticles behave the same as their dissolved analog once they are introduced into the plasma. The feasibility of such protocol in sizing and quantifying AuNPs was tested over a wide range of particle sizes at Au mass concentration as low as 1 pg/g. The observed number concentrations are comparable with the actual input particle number for (20 to 100) nm AuNPs (data not shown).



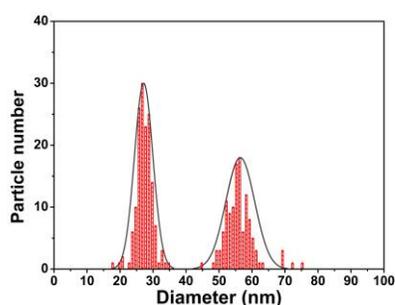
**Figure 3.** Average particle size of AuNPs measured by spICP-MS. The diagonal dotted red line represents ideal 1:1 correlation between vendor reported TEM and spICP-MS experimental particle diameters. 10 nm AuNPs were used at  $1.2 \times 10^5$  mL<sup>-1</sup> and the other AuNPs were used at  $2.4 \times 10^4$  mL<sup>-1</sup>. Dwell time: 10 ms.

Figure 3 gives the mean particle diameters obtained for (10 to 200) nm AuNPs by spICP-MS. The experimental particle sizes show good agreement with vendor reported diameters up to 80 nm, but deviates from the reported values at higher sizes. We characterized 100 nm and 200 nm AuNPs by scanning electron microscopy (SEM) and found particle sizes in the reported range. We considered the possibility that the efficiency of either transporting or ionizing nanoparticles in ICP-MS may decrease when

particle size exceeds certain limit; here our experiment suggests 100 nm, which may indicate a limitation of spICP-MS for sizing large particles.

### 3.3 Characterization of Complex Samples

The ability of spICP-MS to characterize heterogeneous samples was further investigated with solutions containing both soluble and nanoparticle Au, or AuNPs of different sizes. Separation of 60 AuNPs signals from soluble Au background was successfully achieved using the  $5 \times \sigma$  particle selection procedure as described above, allowing precise quantification of both soluble Au and AuNPs (data not shown). Differentiation of a mixture of 30 nm and 60 nm AuNPs by spICP-MS is shown in Figure 4. Complete resolution of the two sizes is achieved, suggesting the potential application of spICP-MS for detecting and characterizing complex samples.



**Figure 4.** Size distribution for a mixture suspension containing 30 nm and 60 nm AuNPs. Gaussian fitting for size distribution is shown in black line. Dwell time: 10 ms, both AuNPs were used at  $2.4 \times 10^4$  mL<sup>-1</sup>, bin size: 1 nm. The TEM measured diameter of 30 nm and 60 nm AuNPs are  $(27.6 \pm 2.1)$  nm and  $(56.0 \pm 0.5)$  nm, respectively, where the uncertainties are expanded to approximately 95% confidence.

## 4 CONCLUSIONS

In this study, precise counting and sizing of AuNPs were achieved by spICP-MS at pg/g levels. Dwell time was found to contribute significantly to the quality of data, and 10 ms was optima. spICP-MS signals deriving from AuNPs can be resolved from a soluble Au background signals when the size exceeds 20 nm. We demonstrate that soluble and nanoparticulate forms of Au can be simultaneously detected and accurately quantified in a mixture, indicating spICP-MS can be a potential tool for studying some of the important environmental transformations, such as particle dissolution. Our study also shows measurement artifacts may arise unless instrument operating conditions and data processing protocol are carefully selected. Single particle-ICP-MS measurement of large particles (> 100 nm) with mass calibration using soluble Au solutions may not be reliable for nanoparticle aggregates, which is a common

form of ENMs in the environment. The performance of spICP-MS at such a size range warrants further investigation. Overall, spICP-MS offers a significant improvement in studying the fate and transformation of ENM in complex environmental and biological systems, but care needs to be paid to experimental design and data interpretation.

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