

Electronic Supplementary Information

Critical considerations for the determination of nanoparticle number concentrations, size and number size distributions by single particle ICP-MS

Francisco Laborda,* Javier Jiménez-Lamana, Eduardo Bolea and Juan R. Castillo

Group of Analytical Spectroscopy and Sensors (GEAS)
Institute of Environmental Sciences (IUCA)
University of Zaragoza
Pedro Cerbuna 12, 50009 Zaragoza, Spain.
E-mail: flaborda@unizar.es; Fax:+34 976761292; Tel: +34 976762252

S.1. Analytical signals in SP-ICPMS

SP-ICPMS signals are usually recorded as a function of time during several seconds or minutes. Figure S1.a shows a typical time scan, consisting of a number of spikes above a steady baseline. Whereas each spike is due to the pack of ions from a nanoparticle, the baseline is due to the background or the presence of dissolved forms of the element measured. Raw time scans can be processed by plotting the number of pulses versus each pulse intensity, obtaining histograms as shown in figure S1.b. The main advantage of working with frequency histograms is that these two components can be easily identified: at low intensities, the continuous contribution due to the background or the dissolved element, and at higher intensities, the contribution of the nanoparticles. Moreover, the first contribution is produced by a conventional counting process, which follows Poisson statistics and hence the signal distribution is Poissonian, whereas the signal distribution for the nanoparticles reflects their original distribution, which tends to be log normal [1].

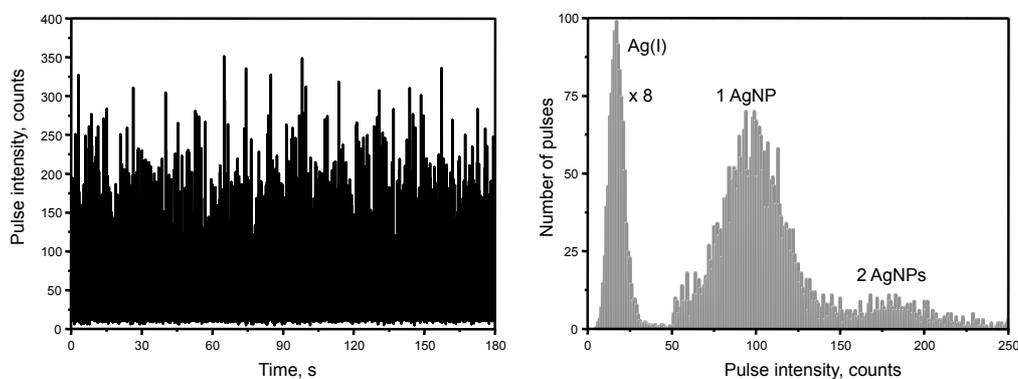


Fig. S1 (a) Time scan of a silver nanoparticle suspension containing silver (I). (b) Pulse intensity frequency histogram of data from (a).

S.2. Threshold for detecting nanoparticle events: Critical value (S_C)

The threshold for detecting nanoparticle events has been calculated as a critical value (S_C) with respect to the signals distribution corresponding to the background/dissolved analyte. This critical value has been defined as the signal intensity at which the fraction of the distribution excluded is equal or below to the confidence level α , which represents the proportion of false positives, and it is given by equation 9:

$$S_C = \mu_B + z_{1-\alpha} \sqrt{\mu_B + 1} \quad (\text{S-1})$$

where μ_B is the mean signal intensity (counts) of the background signal and $z_{1-\alpha}$ the $(1-\alpha)$ quantile of the standard normal distribution.

Equation S-1 was applied at three different background levels (1, 10 and 100 counts) which followed Poisson distributions. Different $z_{1-\alpha}$ coefficients were selected and S_C , α and the percentage of false positives were calculated for each situation. False positives were calculated for a typical situation in SP-ICPMS (10 000 readings of background and 1000 readings of nanoparticles), assuming that signal intensity frequencies below 1 involve no events detected (shaded areas in figure S2). Figures S-2.d, e and f show that by applying threshold coefficients higher than 4, no events are detected at signal intensities higher than S_C for the three cases shown. Quantitative simulated data are summarized in table S1.

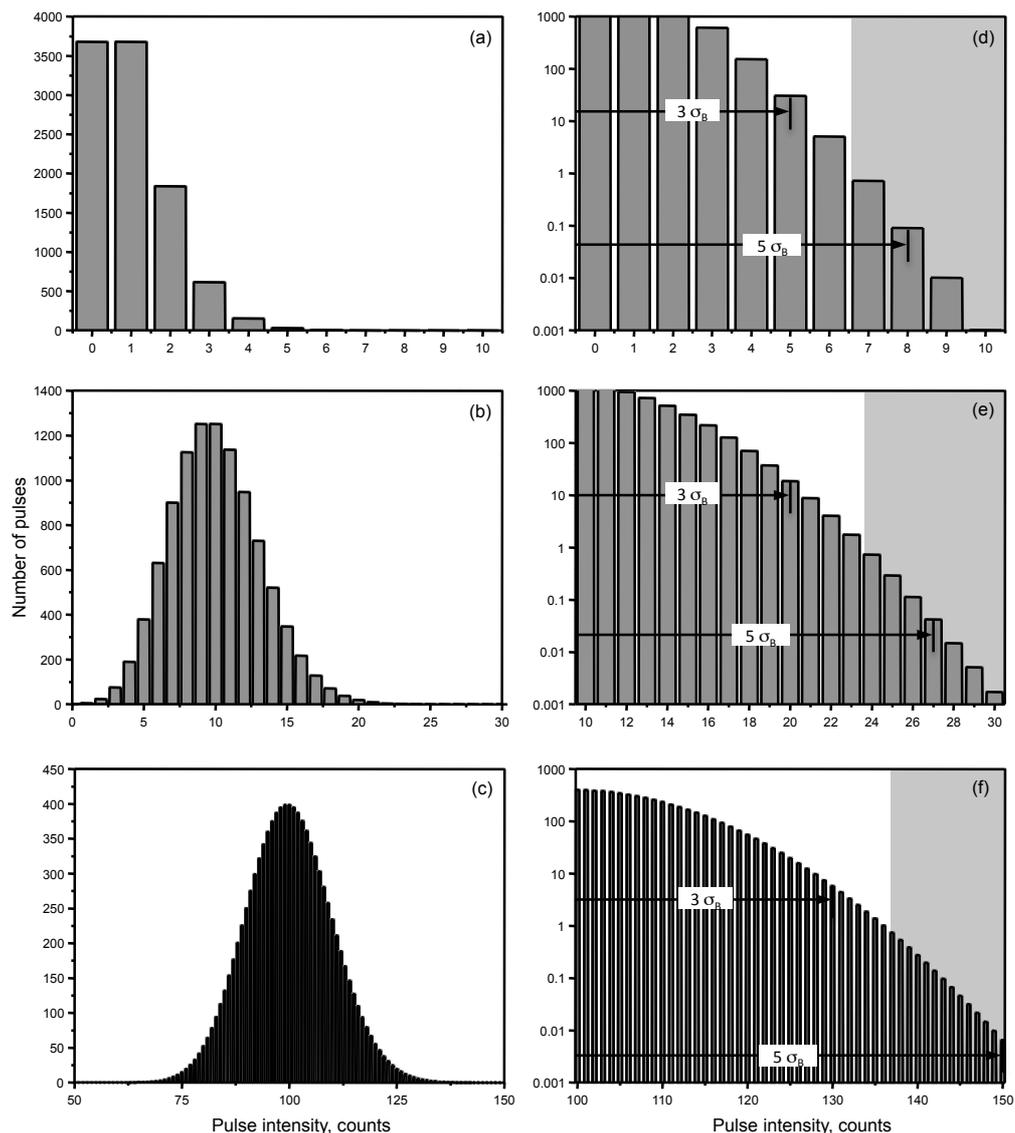


Figure S2. Poisson distribution for simulated background signal of 1 (a), 10 (b) and 100 (c) counts and 10 000 readings. Occurrence of false positives for simulated background signal of 1 (d), 10 (e) and 100 (f) counts. The shaded areas include signal intensities corresponding to less than one event.

Table S1. Critical values (S_c), fraction of the Poisson distribution excluded (α) and percentage of false positives for different threshold criteria and Poissonian background signals (μ_B). Percentage of false positives calculated for a ratio of background:nanoparticle readings 10:1.

Threshold		simulated data			experimental data	
		μ_B (counts)	10	100	1	15
	σ_B (counts)	1.41	3.32	10.05	1.41	4.00
$\mu_B+3\sigma_B$	S_c (counts)	5	20	130	5	27
	α (%)	0.06	0.16	0.17	0.03	0.26
	false positives (%)	0.5	1.6	1.7	0.3	2.6
$\mu_B+4\sigma_B$	S_c (counts)	7	23	140	7	31
	α (%)	0.001	0.01	0.006	0.0	0.01
	false positives (%)	0.0	0.0	0.0	0.0	0.1
$\mu_B+5\sigma_B$	S_c (counts)	8	27	150	8	35
	α (%)	0.0001	0.0002	0.0001	0.0	0.0
	false positives (%)	0.0	0.0	0.0	0.0	0.0

S.3. Uncertainty associated to the counting of nanoparticles

The uncertainty associated to the counting of nanoparticles has two main components: (i) a random error, due to the counting process and governed by Poisson statistics, and (ii) a systematic error, due to the occurrence of 2-nanoparticle events, which are counted as one nanoparticle. The random error ($\sigma_N=\sqrt{N}$) can be expressed as a relative standard deviation (rsd_N):

$$rsd_N = \frac{\sigma_N}{N} = \frac{\sqrt{N}}{N} = \frac{1}{\sqrt{Q_{NP}t_i}} = \frac{1}{\sqrt{t_i}} Q_{NP}^{-\frac{1}{2}} \quad (S-3)$$

where N is the total number of nanoparticles ($=Q_{NP}t_i$), Q_{NP} the flux of nanoparticles into the plasma and t_i the total acquisition time.

Poisson statistics can be used to estimate the probability of one or more than one nanoparticle arriving to the plasma during a measurement period (dwell time), being detected as a single event. The cumulative probability to detect nanoparticle events with n nanoparticles ($n \geq 1$) is given by:

$$\sum_{n=1}^{\infty} \frac{(Q_{NP}t_{dwell})^n}{n!} e^{-Q_{NP}t_{dwell}} \quad (S-4)$$

Assuming that only 1 and 2-nanoparticle events are significant:

$$Q_{NP}t_{dwell}e^{-Q_{NP}t_{dwell}} + \frac{(Q_{NP}t_{dwell})^2}{2}e^{-Q_{NP}t_{dwell}} \quad (S-5)$$

Then, if the total number of reading is given by t_i/t_{dwell} , the nanoparticles counted assuming that 2-nanoparticle events are counted as one nanoparticle (N_{count}) is given by:

$$N_{count} = \frac{t_i}{t_{dwell}} \left(Q_{NP}t_{dwell}e^{-Q_{NP}t_{dwell}} + \frac{(Q_{NP}t_{dwell})^2}{2}e^{-Q_{NP}t_{dwell}} \right) \quad (S-6)$$

and the absolute bias due to the counting process:

$$bias = \left| \frac{N_{count} - N}{N} \right| \quad (S-7)$$

Considering that $e^{-Q_{NP}t_{dwell}} \approx 1$, the bias can be approximated to:

$$\text{bias} = e^{-Q_{\text{NP}} t_{\text{dwell}}} \left(1 + \frac{t_{\text{dwell}}}{2} Q_{\text{NP}} \right) - 1 \approx \frac{t_{\text{dwell}}}{2} Q_{\text{NP}} \quad (\text{S-8})$$

S.4. Uncertainty associated to the determination of nanoparticle number concentration

If the relationship between the total number of events (N) and the nanoparticle number concentration (N_{NP}) is linear, equation 5 can be expressed as $y = b_N x$, where b_N is the slope of the number concentration calibration, y represents the signal measured (number of events) and x the number concentration.

The sources of the uncertainty associated to the determination of nanoparticle number concentration are: (i) the counting of the nanoparticle events in the sample, $u(\text{sample})_N$, and (ii) the number concentration calibration, $u(\text{cal})_N$. Two calibration strategies have been considered: a multiple-point calibration, based on the bracketing calibration with three standards, and a single-point calibration.

The uncertainty associated to the counting of nanoparticle events is given by the random error, which is governed by Poisson statistics:

$$u(\text{sample})_N = \frac{\sigma_N}{b_N} = \frac{\sqrt{N}}{b_N} \quad (\text{S-9})$$

For a multiple point calibration, the uncertainty associated to the calibration, $u(\text{cal})_N^{\text{mp}}$, is given by [2]:

$$u(\text{cal})_N^{\text{mp}} = \frac{s_{\text{res}}}{b_N^{\text{mp}}} \sqrt{\frac{1}{n} + \frac{(y_{\text{sample}} - \bar{y})^2}{b_N^{\text{mp}2} \sum (x_i - \bar{x})^2}} \quad (\text{S-10})$$

where s_{res} is the residual standard deviation of the regression, b_N^{mp} the slope of the multiple-point regression, n the number of calibration standards, y_{sample} the number of events for a specific sample and \bar{y} and \bar{x} the centroids of the regression. A detailed description of the parameters involved can be found in [2].

The total uncertainty for the predicted number concentration obtained by multiple point calibration is:

$$u(\text{total})_N^{\text{mp}} = \sqrt{u(\text{sample})_N^2 + u(\text{cal})_N^{\text{mp}2}} \quad (\text{S-11})$$

A single-point calibration is performed by measuring one standard (x_{std}). Its signal (y_{std}), allows to obtain the response factor between signal and concentration (b_N^{1-p}). The uncertainty of this single-point calibration ($u(\text{cal})_N^{1-p}$) depends on the uncertainty of the signal from the standard, $u(y_{\text{std}})$, and the uncertainty of the concentration of the standard, $u(x_{\text{std}})$, it is given by [2]:

$$u(\text{cal})_N^{1-p} = \sqrt{\frac{x_{\text{sample}}^2}{x_{\text{std}}^2} + \frac{u(y_{\text{std}})^2}{b_N^{1-p2}} + u(x_{\text{std}})^2} \quad (\text{S-12})$$

The total uncertainty for the predicted number concentration obtained by single point calibration is:

$$u(\text{total})_N^{1-p} = \sqrt{u(\text{sample})_N^2 + u(\text{cal})_N^{1-p2}} \quad (\text{S-13})$$

Therefore, the difference between the two methods comes from the way the calibration is performed, as it can be seen in the table S2. Relative uncertainties have been calculated at the critical number concentration calculated in paragraph 4.1.3, corresponding to $2.8 \times 10^7 \text{ L}^{-1}$ and 800 nanoparticle events.

Table S2. Relative standard uncertainties ($100 \times u_N/N$) in the determination of nanoparticle number concentration for the two calibrations used.

Source	Relative uncertainty (%)	
	Calibration	
	3-points	1-point
sample	3.5	3.5
calibration	2.8	3.7
total	4.5	5.1

S.5. Nanoparticle size determination by using dissolved standards

When size standards are not available, the application of equation (6) to determine nanoparticle diameters would imply to know all the parameters involved. Whereas the parameters related to the element and the nanoparticle are known, K_{ICPMS} must be estimated. Pace et al. [3] developed a procedure based on the use of dissolved standards of the analyte to determine the nanoparticle diameter, in fact, the mass of analyte per nanoparticle. The procedure assumes that once in the plasma, atoms from a dissolved standard solution and from a nanoparticle behave comparably for the same element.

The relationship between the mass concentration and the mass measured per event for a dissolved analyte (m_{dis}) is given by:

$$m_{dis} = \eta_{neb} Q_{sam} t_{dwell} C^M \quad (S-14)$$

If equation (2) is expressed as counts:

$$r_{dis} = \eta_{neb} Q_{sam} t_{dwell} K_{ICPMS} K_M C^M \quad (S-15)$$

Substituting equation (S-14) in (S-15) gives:

$$r_{dis} = K_{ICPMS} K_M m_{dis} \quad (S-16)$$

which is analogous to equation (7) and can be used for calibration purposes. Thus a calibration performed with dissolved standard (equation S-15) can be transformed into equation (S-16) if η_{neb} , Q_{sam} and t_{dwell} are known. Once estimated $K_{ICPMS} K_M$, equations 6 and 7 can be used for calculation of the diameter of the analyte mass per nanoparticle, respectively.

S.6. Uncertainty associated to the determination of median nanoparticle diameters

The relationship between the pulse intensity (r_{NP}) and the nanoparticle diameter (d) is given by equation 6. This equation can be expressed as $y = b_{size} x^3$, where b_{size} is the slope of the size calibration, y represents the signal measured (counts) and x the diameter.

When nanoparticle diameters are determined by calibration with one size standard, the contributions to the total uncertainty involve: (i) the uncertainties in the measurement of the median pulse intensities of the sample and the standard, and (ii) the uncertainty associated to the size of the standard used. The total relative uncertainty associated can be calculated by applying the rules of propagation of the uncertainty to the expression $x_{sample} = x_{std} (y_{sample}/y_{std})^{1/3}$:

$$\frac{u(x_{sample})}{x_{sample}} = \sqrt{\frac{1}{9} \left(\frac{u(y_{sample})^2}{y_{sample}^2} + \frac{u(y_{std})^2}{y_{std}^2} \right) + \frac{u(x_{std})^2}{x_{std}^2}} \quad (S-17)$$

The uncertainty associated to the measurement of the pulse intensity of both the sample and the standard is considered to be due to counting statistics, other sources related to the introduction and ionization of nanoparticles in a plasma have not been included [4]. This relative uncertainty is given by:

$$\frac{u(r_{NP})}{r_{NP}} = \frac{\sqrt{r_{NP}}}{r_{NP}} = \frac{1}{\sqrt{r_{NP}}} \quad (S-18)$$

Equation S-17 can be expressed in terms of the pulse intensities of the size standard ($r_{NP, std}$) and the sample ($r_{NP, sample}$), and the size of the standard (d_{std}) as:

$$\frac{u(\text{total})_{size}^{SS}}{d} = \sqrt{\frac{1}{9} \left(\frac{1}{r_{NP, sample}} + \frac{1}{r_{NP, std}} \right)^2} + \frac{u(d_{std})^2}{d_{std}^2} \quad (\text{S-19})$$

Relative standard uncertainties for samples of different sizes are shown in table S3. Calibration was performed with a 60 nm silver nanoparticle suspension (uncertainty provided by the manufacturer: ± 4 nm).

Table S3. Relative standard uncertainties associated to the determination of median nanoparticle diameters by calibration with one size standard

Diameter (nm)	Relative uncertainty (%)
40	13.2
60	10.2
80	9.0

When nanoparticle diameters are determined by using dissolved standards, following the method developed by Pace et al. [3], there are two contributions to the total uncertainty: (i) the measurement of the median pulse intensity of the sample, and (ii) the determination of K_{ICPMS} .

K_{ICPMS} is calculated from equation S-15 as:

$$K_{ICPMS} = \frac{r_{dis}}{\eta_{neb} t_{dwell} Q_{sam} C_M K_M} \quad (\text{S-20})$$

by using a standard solution of the analyte and knowing the nebulization efficiency and the sample flow rate. Thus, the sources of uncertainty associated to the determination of K_{ICPMS} , $u(K_{ICPMS})$, involve the measurement of a dissolved standard, $u(r_{dis})$, the determination of the nebulization efficiency, $u(\eta_{neb})$, the determination of the sample flow rate, $u(Q_{sam})$, and the uncertainty associated to the concentration of the standard, $u(C_M)$. The relative uncertainty is given by:

$$\frac{u(K_{ICPMS})}{K_{ICPMS}} = \sqrt{\frac{u(r_{dis})^2}{r_{dis}^2} + \frac{u(\eta_{neb})^2}{\eta_{neb}^2} + \frac{u(Q_{sam})^2}{Q_{sam}^2} + \frac{u(C_M)^2}{C_M^2}} \quad (\text{S-21})$$

The different contributions related to the determination of K_{ICPMS} are listed in table S-4.

Table S4. Relative standard uncertainty associated to the determination of K_{ICPMS} .

Source	Relative uncertainty (%)
r_{dis} ^a	1.0
η_{neb} ^{b,c}	3.9
Q_{sam} ^c	2.2
C_M ^d	0.21
K_{ICPMS}	4.6

^a counting statistics ($=1/(r_{dis})^{1/2}$)

^b calculation procedure based on [3]

^c experimental determination ($n=10$)

^d calculation procedure based on [2]

The total relative uncertainty can be calculated by applying the rules of propagation of the uncertainty to the expression $x_{sample} = (y_{sample}/b_{size})^{1/3}$:

$$\frac{u(x_{sample})}{x_{sample}} = \sqrt{\frac{1}{9} \frac{u(y_{sample})^2}{y_{sample}^2} + \frac{1}{9} \frac{u(b_{size})^2}{b_{size}^2}} \quad (\text{S-22})$$

Considering that the uncertainty associated to the measurement of the pulse intensity of the sample is due to counting statistics (equation S-18) and the uncertainty of the slope corresponds to the uncertainty of K_{ICPMS} (equation S-21), equation S-22 can be expressed as:

$$\frac{u(\text{total})_{\text{size}}^{\text{ds}}}{d} = \sqrt{\frac{1}{9} \frac{1}{r_{\text{NP, sample}}} + \frac{1}{9} \frac{u(K_{\text{ICPMS}})^2}{K_{\text{ICPMS}}^2}} \quad (\text{S-23})$$

The relative standard uncertainties associated to the determination of different nanoparticle diameters are summarized in table S-5.

Table S5. Relative standard uncertainties associated to the determination of nanoparticle diameters by using dissolved standards and the method developed by Pace et al. [3].

Source	Relative uncertainty (%)		
	40 nm	60 nm	80 nm
sample	10.0	5.5	2.5
K_{ICPMS}	1.5	1.5	1.5
total_{ds}	10.2	5.7	3.0

References

- [1] F. Laborda, J. Jiménez-Lamana, E. Bolea and J. R. Castillo, *Journal of Analytical Atomic Spectrometry*, 2011, **26**, 1362–1371.
- [2] F. Laborda, J. Medrano and J. R. Castillo, *Journal of Analytical Atomic Spectrometry*, 2004, **19**, 1434–1441.
- [3] H. E. Pace, N. J. Rogers, C. Jarolimek, V. A. Coleman, C. P. Higgins, and J. F. Ranville, *Analytical Chemistry*, 2011, **83**, 9361–9369.
- [4] J. W. Olesik and P. J. Gray, *Journal of Analytical Atomic Spectrometry*, 2012, **27**, 1143–1155.