Supplementary information:

Performance of single-cell ICP-MS for quantitative biodistribution studies of silver interactions with bacteria

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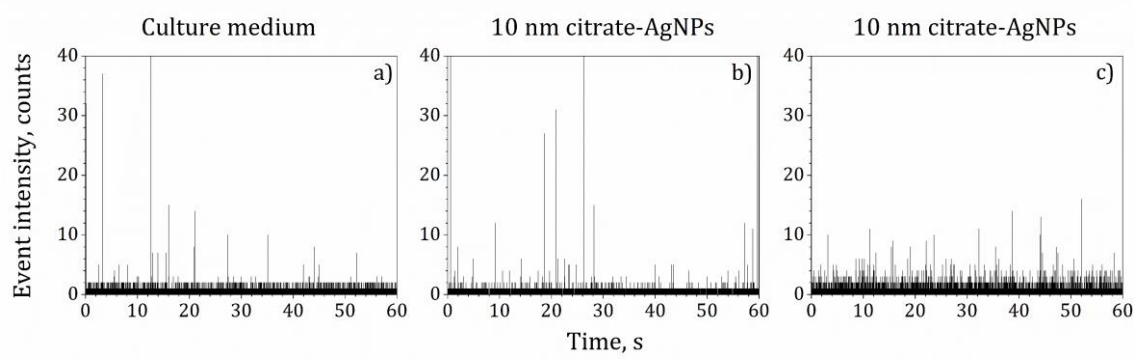
1. Effectiveness of washing processes

After optimisation of SC-ICP-MS method, samples of bacteria exposed to silver were analysed by this technique. Furthermore, in order to test the effectiveness of PBS washing process during sample preparation to remove remaining silver that had not interacted with bacteria, a control of culture medium exposed to silver nanoparticles was analysed. MH + 2% Tween 80 medium was exposed to 10 nm silver nanoparticles as described in the experimental section and the samples were subjected to three washing steps with PBS. Figure S1.a and b show the time scans obtained by SC-ICP-MS for a control culture medium and the medium exposed to silver nanoparticles, respectively. Taking the control culture medium as reference, a similar number of events were obtained in the culture medium exposed to silver nanoparticles, confirming the absence of particles in the sample. Under the measurement conditions, the size critical value was 14.3 nm, indicating that a part of the 10 nm silver nanoparticle distribution would not be detected even if nanoparticles would not have been removed after washing. Size critical value was calculated from the expression proposed by Laborda *et al*.[1] In the case of a sample of 10 nm silver nanoparticles suspended in the culture medium (without incubation process) (Figure S1.c), a higher number of events was observed, corresponding to nanoparticles bigger than 14.3 nm. This difference between time scans confirmed that three washing steps allow to remove remaining silver nanoparticles.

Figure S1. Time scans of SC-ICP-MS for samples in MH + 2% Tween 80 medium: a) culture medium control, b) culture medium exposed to 10 nm AgNPs (three washing steps) and c) 10 nm AgNPs in culture medium (no incubation).



2. Bacteria FESEM images

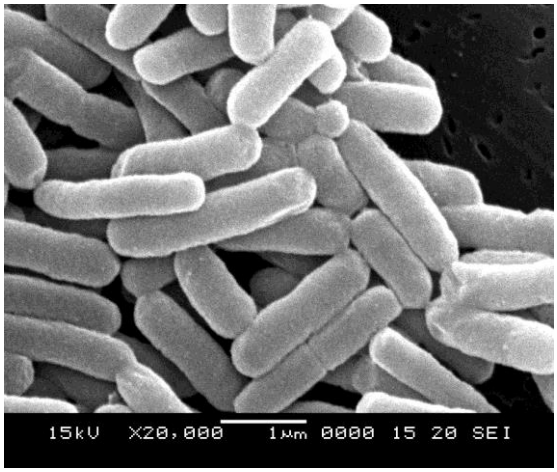


Figure S2. FESEM images from *E. coli* bacteria (x20000).

3. Detection of bacteria exposed to silver by SC-ICP-MS

Figure S3. Time scans of SC-ICP-MS for *E. coli* bacteria: a) bacterial control, b) bacteria exposed to Ag(I) (Y_c = 2 counts; $Y_{C,N}$ = 28 events) and c) bacteria exposed to 10 nm AgNPs (Y_c = 1 counts; $Y_{C,N}$ = 28 events). Instrument: NexION 2000 B Perkin Elmer ICP-MS.

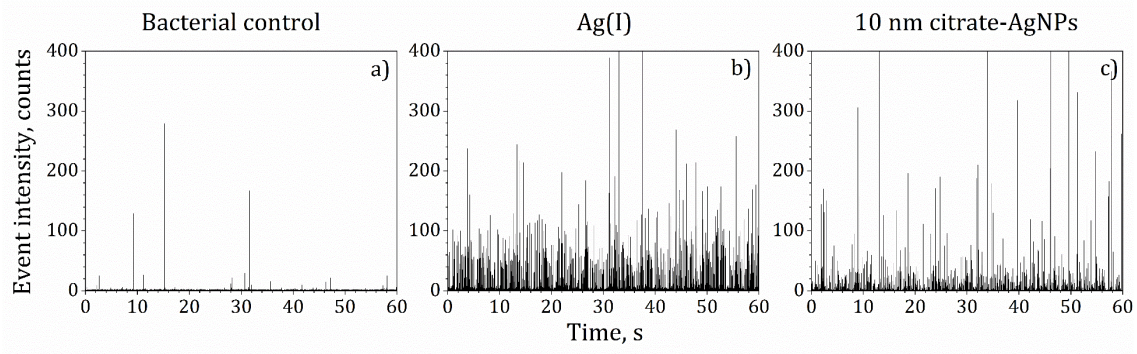
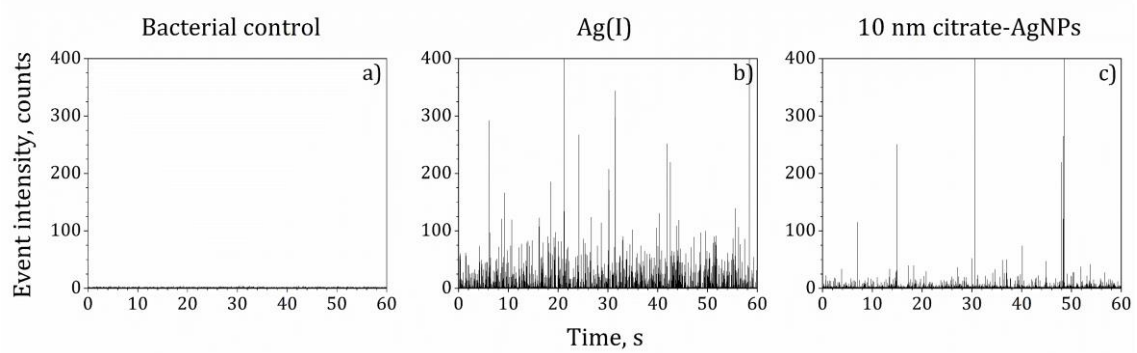
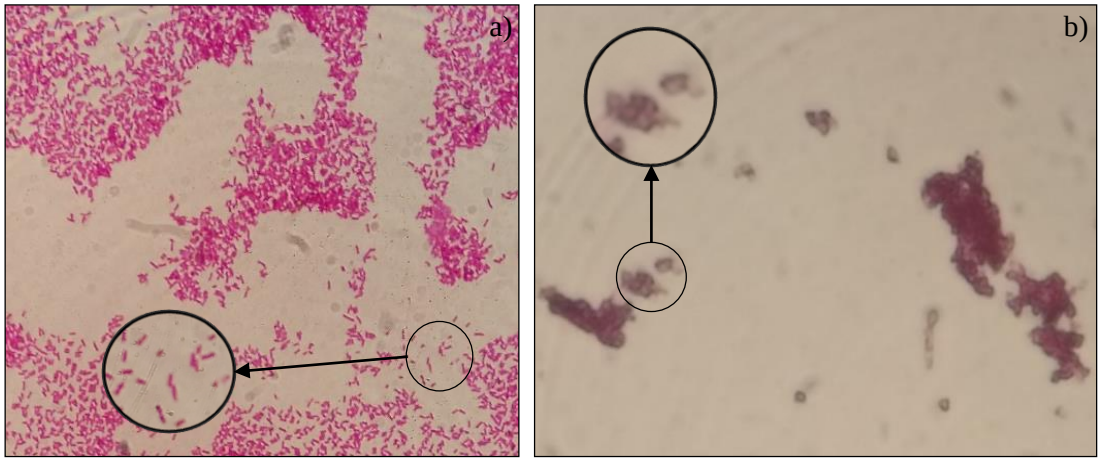


Figure S4. Time scans of SC-ICP-MS for *E. coli* bacteria: a) bacterial control, b) bacteria exposed to Ag(I) (Y_c = 1 count; $Y_{C,N}$ = 12 events) and c) bacteria exposed to 10 nm AgNPs (Y_c = 1 count; $Y_{C,N}$ = 12 events). Instrument: Agilent 8900 ICP-MS.



4. Determination of intracellular silver in bacteria by SC-ICP-MS

Figure S5. a) *E. coli* bacteria with cell wall and b) spheroplasts after removal of the outer membrane after enzymatic digestion with lysozyme.



5. Method validation

SC-ICP-MS methods were validated through their specific performance parameters, which were adapted to the peculiarities of the analysis of bioparticles due to the lack of certified reference materials for bioparticles.[2] Thus, properly characterised commercial nanoparticles were used (60 nm silver nanoparticles). These nanoparticles were chosen as they do not pose problems with their discrimination from the baseline signals. Since nanoparticles and Eu-based microspheres behave similarly in the system, as demonstrated in this work and others[3], and their size and density of the latter are similar to those of bacteria, the performance parameters estimated from the nanoparticles can be transferred to the analysis of bacteria. Performance characteristics of the SC-ICP-MS methods developed in this work are summarised in Table S1.

The mass per particle limit of detection (LOD_{mass}) was considered equal to the critical value, as proposed in Laborda *et al*[1], being calculated from 5 times the standard deviation of the baseline by using the following expression:

$$LOD_{mass} = X_C^{mass} = \frac{5\sigma_B}{\frac{2}{w}K_{ICPMS}K_M t_{dwell}} \quad (1)$$

where w is the duration of a bioparticle event, K_{ICPMS} is the detection efficiency, which represents the ratio of the number of ions detected versus the number of analyte atoms of the measured isotope introduced into the ICP; and K_M $(= AN_{Av}/M_M)$ is a factor related to the element measured, where A is the atomic abundance of the isotope considered, N_{Av} the Avogadro number,

and M_M the atomic mass of the element. K_{ICPMS} was estimated from the slope of a mass concentration calibration with dissolved silver once the transport efficiency was known.

Best number concentration limits of detection (LOD_{number}) were calculated for ideal blanks where no particles would be detected. Under such conditions the LOD_{number} involves the detection of 3 bioparticles and the figure of merit is calculated as:

$$LOD_{number} = X_D^{number} = \frac{3}{\eta_{neb} Q_{sam} t_i} \quad (2)$$

where η_{neb} is the analyte transport efficiency, Q_{sam} the sample introduction flow rate and t_i the total acquisition time. In any case, number concentration LODs can be enhanced by using longer acquisition times. A spreadsheet is available [4] for calculation of the detection limits from equations (1) and (2).

Under the conditions of this study, mass per particle LOD obtained was 7 ag, corresponding to an equivalent spherical metallic silver nanoparticle of ca. 11 nm, whereas number concentration LOD was $5x10^5$ L^{-1}.

Total mass concentrations determined by SC-ICP-MS involve the sum of the masses of analyte measured in all the bioparticles detected. The corresponding LOD and LOQ were calculated by multiplying the standard deviation of the blank signals processed like the samples by 3 or 10, respectively, and considering the final dilution applied. The standard deviation was calculated by measuring five replicates of ultrapure water blanks. LOD and LOQ values were 38 and 128 ng L^{-1}, respectively.

Precision was estimated as repeatability and expressed as relative standard deviation. For this purpose, ten replicates of 60 nm AgNP were analysed and mass per particle, total mass and number concentrations calculated. Then, relative standard deviation of the ten replicates was calculated obtaining repeatability values of 1.1%, 2.0% and 3.5% for mass per particle, number concentration and total mass concentration, respectively. Trueness was estimated by the performance of recovery tests. For this purpose, and due to the lack of reference material for bioparticles, commercially available 60 nm AgNP of known concentration were used. Recovery values were obtained by comparing the theoretical and the obtained ones. In this sense, recovery values of 93 ± 1, 110 ± 3 and 96 ± 3 % were obtained for mass per particle, number concentration and total mass concentration, respectively.

Validation of ICP-MS in standard mode was also performed. Total mass concentration LOD and LOQ were calculated in the similar way than in SC-ICP-MS mode. The standard deviation was calculated by measuring five replicates of method blanks. LOD and LOQ values were 150 and 510 ng L^{-1}, respectively. Total mass concentration repeatability was calculated by analysing five replicates of 60 nm silver nanoparticles. A repeatability value around 3.6% was obtained. Finally, the recovery of the total mass concentration, which was determined by analysing 60 nm silver nanoparticles, was 97 ± 3%.

When compared to the standard mode, apart from providing individual bioparticle information, SC-ICP-MS, allows to obtain lower LOD and LOQ for total mass concentrations because of the inherent higher detection capability in single particle mode. The repeatability was similar operating in both modes and the recovery was within the accepted range at the concentration levels applied.

Table S1 Validation parameters for SC-ICP-MS based methods. Isotope monitored: ^{107}Ag. Instrument: Perkin Elmer NexION 2000 B. Nebulisation system: high efficiency glass concentric micronebuliser and Asperon^{TM} spray chamber

Parameter	Single cell mode	Standard mode
Single particle information		
Mass per particle LOD	7 ag	-
Number concentration LOD	$5x10^5$ L^{-1}	-
Mass per particle repeatability	1.1%	-
Number concentration repeatability	2.0%	-
Mass per particle trueness	93 ± 1%	-
Number concentration trueness	96 ± 3%	-
Total mass concentration		
LOD	38 ng L^{-1}	150 ng L^{-1}
LOQ	128 ng L^{-1}	510 ng L^{-1}
Repeatability	3.5%	3.6%
Trueness	110 ± 3%	97 ± 3%

References

1 F. Laborda, A. C. Gimenez-Ingalaturre, E. Bolea and J. R. Castillo, *Spectrochim Acta Part B At Spectrosc*, 2020, **169**, 105883.

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3 F. Laborda, C. Trujillo and R. Lobinski, *Talanta*, 2021, **221**, 121486.

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