

Tracking Nanoparticles in Biological Fluids using Single Particle ICP-MS

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ABSTRACT

Single Particle-ICP-MS is a new operating mode in ICP-MS that is dedicated to the analysis of engineered nanoparticles (ENPs) commonly used in Nanomedicine and drug delivery systems. Single Particle (SP-ICP-MS) allows the differentiation between ionic and particulate signals, quantitation of both ionic and particulate fractions, measurement of particle composition, concentration (part/mL), particle size, and size distribution, and assists in monitoring agglomeration.

The present work explores the use of SP-ICP-MS as a metrology tool for the analysis of engineered nanoparticles in biological matrices (blood, urine). Preliminary results show that Au ENPs (30 and 60 nm) are stable in diluted blood and urine. No change in the size of Au ENPs and/or concentration was noticed during the 24h study period.

SP-ICP-MS is rapidly becoming a key analytical instrument in assessing the fate, behavior and distribution of (ENPs) in several types of matrices [1,2] including biological fluids such blood and urine.

Keywords: Single Particle ICP-MS, Nanoparticles, Biological Matrices, Fate on Nanoparticles.

1 INTRODUCTION

Nanotechnology is growing fast, with currently more than 1600 nanomaterials-based consumer products available on the market, as indicated by Nanotechnology Consumer Products Inventory [3]. Silver and gold are among the most used nanomaterials in consumer products and biomedical applications [4-5]. Since humans can be exposed through various scenarios (consumer products, environment, workplace) and routes (respiratory, gastrointestinal tract, skin), there is an urgent need to detect and quantify nanoparticles in biological fluids.

Single particle ICP-MS (SP-ICP-MS) is capable of quantitatively differentiating between ionic and particulate fractions providing the user with several important measurements such the ionic concentration ($\mu\text{g/mL}$), the particles concentration (part/mL), size and size distribution in the same sample analysis. In addition, SP-ICP-MS can detect small concentrations of particles (as might be expected in biological fluids), and can perform rapid analyses (typically 1-2 minutes). With such capabilities

SP-ICP-MS is becoming “The” technique of choice for studying the fate/transformation of nanoparticles in various sample matrices [6-7].

The present work explores the ability of single particle ICP-MS (SP-ICP-MS) to assess the fate of nanoparticles (NP) in biological fluids, building on the initial work which demonstrated the ability of SP-ICP-MS to detect gold and silver nanoparticles in blood [8]. The transformation (fate) of silver and gold nanoparticles in blood and urine was tracked over 76 hours by following the change in size, the ionic concentration as well as the aggregation/agglomeration of the nanoparticles.

2 EXPERIMENTAL

2.1 Experimental

All samples were analyzed with a PerkinElmer NexION 300S ICP-MS. The Nano Application Module from Syngisix software was used for data acquisition and automated data treatment. Instrumental conditions used for all measurements are shown in Table 1. Operating conditions were optimized in order to get the maximum Ag⁺ and Au⁺ intensities. A rinse solution (1% HNO₃ + 1% HCl + 0.1% Triton-X) was aspirated for 1 minute between each sample in order to dissolve and remove any residual nanoparticles. This rinse solution was followed by deionized water to remove the acid.

Table 1. NexION 300S ICP-MS Parameters.

Parameter	Value
Sample Uptake Rate	0.44 mL/min
Nebulizer	Quartz concentric
Spray Chamber	Quartz cyclonic
RF Power	1600 W
Nebulizer Gas Flow	Optimized for maximum Ag and Au signal
Dwell Time	50 μ s
Analysis Time	60 sec
Parameter	Value

2.2 Materials and Sample Preparation

Blood samples were prepared by diluting 20 times a certain volume of human blood from a non-exposed person with an aqueous solution of 0.5% ammonium hydroxide + 0.1% octylphenol ethoxylate (Triton-X). Urine samples were prepared by diluting 20 times a certain volume of human urine from a non-exposed person with an aqueous solution of 0.5% HNO₃. Diluted blood and urine were then spiked with a 5 minute sonicated stock solution of Ag or Au NP (Ag - 40 and 80 nm from Ted Pella Inc., Redding, CA; Au - 30 and 60 nm, NIST 8012, 8013 from National Institute for Standards and Technology, Gaithersburg, MD) to reach a concentration of 250 000 particles/mL. A control sample (blank) was prepared with 40 nm Ag and 30 nm Au NP following the same procedure, but no matrix (urine or blood) was added. The dilution solutions (ammonium hydroxide for blood and nitric acid for urine) were chosen based on their regular use in the analysis of metals and metalloids in blood and urine respectively. The samples were manually shaken prior to analysis.

Calibration curves for dissolved species (from 0 to 5 ppb) were prepared using a 1000 ppm VHGAu standard solution and a 10000 ppm SCP Science Ag standard solution, respectively. Calibration curves for particles were built with 40 and 60 nm Ag NP and 30 and 60 nm Au NP respectively, at 250 000 particles/mL.

2.3 Results and discussion

The fate of Ag and Au NP was studied in blood and urine by following the change in size of the NP, as well as the concentration of the ionic species which could be generated by partial or complete dissolution of NP. The NP concentration in each sample was followed over time in order to attempt a correlation with the change in size and increase in concentration of the ionic species. In order to check if a change in size and concentration of NP is caused

by the biological matrix (urine or blood) or by the dilution solution, blank samples were prepared for 40 nm Ag NP (40 nm Ag blank) and 30 nm Au NP (30 nm Au blank) following the same procedure as for the other samples but without adding the biological matrix (urine or blood).

As shown in Figure 1, a decrease in NP size is noticed for both 40 and 80 nm Ag NP in diluted blood after 76 hours. A similar trend is observed for the blank sample, with a slightly more pronounced decrease when comparing with samples. This suggests that the decrease in size of the primary distribution is due mainly to the dilution solution and not to the matrix (blood). It seems that blood has a light-protecting role in preventing dissolution of Ag NP. A very slight increase of the ionic Ag concentration (Ag⁺) was found for the blank. A secondary size distribution is also observed, which might suggest a slight agglomeration/aggregation of NP.

From these observations and considering the complex medium in which the NP are being (diluted blood), it could be hypothesized that NP can undergo to some extent dissolution and that ions thus generated can reform NP. Other phenomena can occur, like sedimentation and deposition of NP in the tubes, giving rise to a non-homogeneous solution, especially in the blank sample where the lack of blood leads to a less stable solution. In such a case, the NP will not be detected

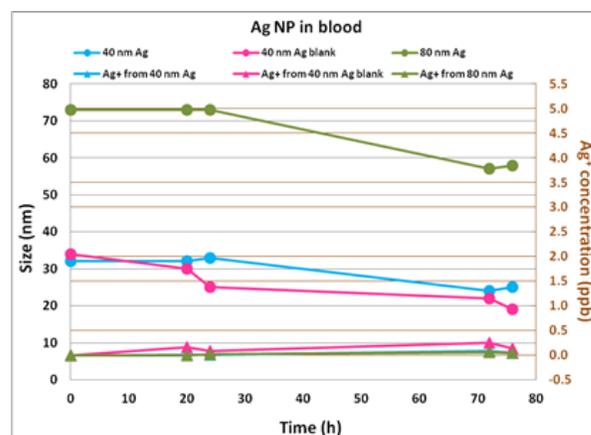


Figure 1. Size change for Ag NP (40 and 80 nm) and Ag⁺ concentration over time in blood

A more pronounced decrease in size was observed for Ag NP in urine (Figure 2), especially for 80 nm Ag NP. The blank sample seems to be slightly more affected than the other samples. This can be expected as the acidic solution used to dilute urine (diluted nitric acid) has the potential to dissolve metals. However, the increase in Ag⁺ due to dissolution of NP is negligible.

A linear fit for the curves that show the decrease in NP size over time (Figures 1 and 2) allows estimation the

dissolution rate of the NP in a certain matrix. When comparing the slopes of the curves for the same NP, e.g. 80 nm Ag NP in blood (Figure 1, slope: -0.2414, R2 = 0.92) and urine (Figure 2, slope: -0.6743, R2 = 0.96), it is clear that the dissolution rate is different in both matrices. A three times higher dissolution rate is obtained in urine than in blood.

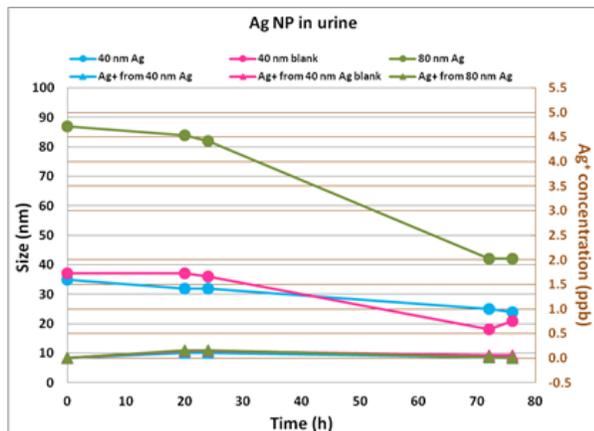


Figure 2. Size change for Ag NP (40 and 80 nm) and Ag⁺ concentration over time in urine.

Similar trends were found for Au NP in blood and urine (Figures 3 and 4). The decrease in NP size is due mainly to the dilution solution, as confirmed by the blank sample in both cases. The increase in Au⁺ due to dissolution of NP is negligible.

Again, when comparing the slopes of the curves for the same NP, e.g. 60 nm Au NP in blood (Figure 3, slope: -0.182, R2 = 0.86) and urine (Figure 4, slope: -0.118, R2 = 0.97), it can be seen that the dissolution rate is different in both matrices. Actually, the dissolution rate for 60 nm Au NP is 1.5 times faster in blood than in urine.

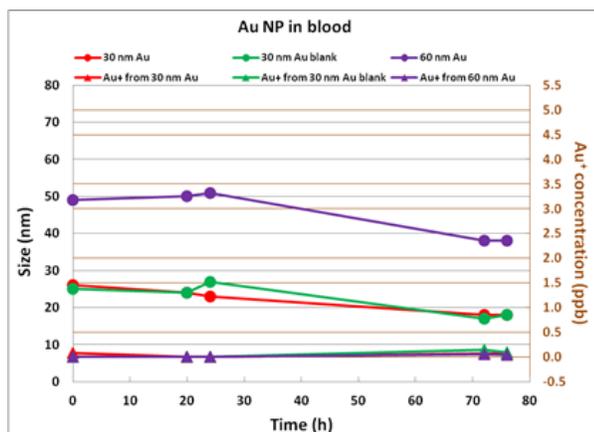


Figure 3. Size change for Au NP (30 and 60 nm) and Au⁺ concentration over time in blood

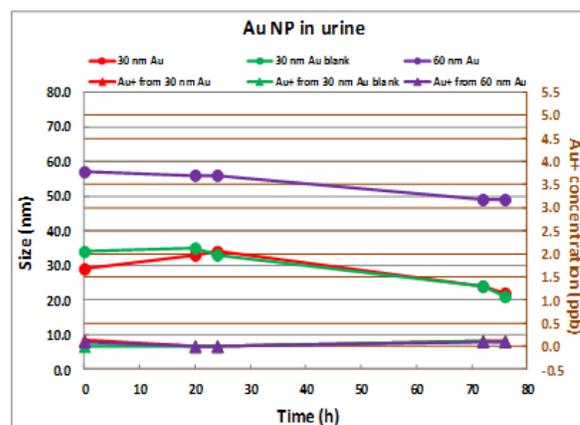


Figure 4. Size change for Au NP (30 and 60 nm) and Au⁺ concentration over time in urine.

3 CONCLUSION

The fate/transformation of nanoparticles in biological fluids is of crucial importance for i) understanding their behavior in the body and ii) assessing their potential health effects. The present study demonstrates the capability of SP-ICP-MS to assess the fate of Ag and Au NP in blood and urine, two important biological fluids from a toxicological point of view. The change in size and the ionic concentration were tracked over 76 hours. A certain decrease in size was noticed for all NP in blood and urine with a more or less significant increase in the ionic concentration. No significant aggregation/agglomeration was observed. From the results obtained with the blank samples it is clear that decrease in size occurs because of the dilution solution employed to dilute blood and urine, probably by dissolution. This indicates that the long-time stability of those NP could be compromised. Obviously, the analysis of diluted blood or urine samples should be executed right after diluting the sample to avoid transformation of NP.

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