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. Silver and gold are among the most used nanomaterials in consumer products and biomedical applications. Since humans can be exposed through various scenarios (consumer products, environment, workplace) and routes (respiratory, gastrointestinal tract, skin), there is an urgent need for analytical methods that can 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 as the ionic concentration (µg/mL), the particles' concentration (part/mL), size and size distribution in the same sample analysis. In addition, SP-ICP-MS can detect low 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 researchers studying the fate/transformation of nanoparticles in various sample matrices.4, 5

The present work explores the ability of SP-ICP-MS to assess the fate of nanoparticles (NPs) in biological fluids, building on the initial work which demonstrated the ability of SP-ICP-MS to detect gold and silver nanoparticles in blood.6 The transformation (fate) of silver and gold nanoparticles in blood has a light-protecting role in preventing dissolution of Ag to the dilution solution and not to the matrix (blood). It seems that the decrease in size of the primary distribution is due mainly to the biological matrix (urine or blood).

**Results and Discussion**

The fate of Ag and Au NPs was studied in blood and urine by following the change in size of the NPs, as well as the concentration of the ionic species which could be generated by partial or complete dissolution of NPs. 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 NPs is caused by the biological matrix (urine or blood) or by the dilution solution, blank samples were prepared for 40 nm Ag NPs (40 nm Ag blank) and 30 nm Au NPs (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 NPs 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 NPs. A very slight increase of the ionic Ag concentration (Ag⁺) was found for the blank. From these observations and considering the complex medium in which the NPs are being diluted (blood), it could be hypothesized that smaller-size NPs can undergo, to some extent, dissolution and that ions thus generated can reform smaller-size NPs. Other phenomena can occur, like sedimentation and deposition of NPs 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.

**Experimental**

**Instrumentation**

All samples were analyzed with a PerkinElmer NexION™ 300S/350S ICP-MS. The Nano Application Module from Synergistix™ 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 wash and remove any residual nanoparticles. This rinse solution was followed by deionized water to remove the acid.

**Table 1. NexION 300S/350S ICP-MS Parameters.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample uptake rate</td>
<td>0.44 mL/min</td>
</tr>
<tr>
<td>Nebulizer</td>
<td>Quartz concentric</td>
</tr>
<tr>
<td>Spray chamber</td>
<td>Quartz cyclonic</td>
</tr>
<tr>
<td>RF power</td>
<td>1600 W</td>
</tr>
<tr>
<td>Nebulizer gas flow</td>
<td>Optimized for maximum Ag and Au signal</td>
</tr>
<tr>
<td>Dwell time</td>
<td>50 µs</td>
</tr>
<tr>
<td>Analysis time</td>
<td>60 sec</td>
</tr>
</tbody>
</table>

**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 NPs (Ag - 40 and 80 nm from Ted Pella Inc., Redding, California, USA; Au - 30 and 60 nm, NIST™ 8012, 8013 from National Institute for Standards and Technology, Gaithersburg, Maryland, USA) to reach a concentration of 250,000 particles/mL. A control sample (blank) was prepared with 40 nm Ag and 30 nm Au NPs 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 research 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 Au standard solution (VHG Labs, Manchester, New Hampshire, USA) and a 10,000 ppm Ag standard solution (SCP Science, Baie D’Urfé, Quebec, Canada), respectively. Calibration curves for particles were built with 40 and 60 nm Ag NPs and 30 and 60 nm Au NPs respectively, at 250,000 particles/mL.
A more pronounced decrease in size was observed for Ag NPs in urine (Figure 2), especially for 80 nm Ag NPs. 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 NPs is negligible.

A linear fit for the curves that show the decrease in NP size over time (Figures 1 and 2) allows us to estimate the dissolution rate of the NPs in a certain matrix. When comparing the slopes of the curves for the same NP, e.g. 80 nm Ag NPs 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.

Similar trends were found for Au NPs 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 NPs is negligible.

Again, when comparing the slopes of the curves for the same NP, e.g. 60 nm Au NPs 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 NPs is 1.5 times faster in blood than in urine.

**Conclusion**

The fate/transition of nanoparticles in biological fluids is of crucial importance to help researchers understand their behavior in the body. The present study demonstrates the capability of SP-ICP-MS to assess the fate of Ag and Au NPs 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 NPs 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. The analysis of blood or urine samples should be done right after dilution to avoid any transformation of NPs.
References


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