APPLICATION NOTE

ICP - Mass Spectrometry

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Determination of Gold and Silver Nanoparticles in Blood Using Single Particle ICP-MS

Introduction

Rapid development of nanotechnologies and their potential applications in clinical research have raised concerns about the adverse effects of nanoparticles (NPs) on human health. The small size of nanoparticles implies enhanced reactivity due to their larger surface area per volume. While these properties may enhance the desired effects, they may also introduce new, unwanted toxic effects[1]. Two metal NPs, gold (Au) and silver (Ag), have been intensively studied – Au NPs, due to their desired intrinsic properties such as high chemical stability, well-controlled size and surface functionalization; while Ag NPs, due to their antibacterial effect, are often applied in wound disinfection, coatings of medical devices and prosthesis, and commercially in textiles, cosmetics and household goods[2]. As a result, concerns have been raised about the migration of Ag NPs from things like bandages or medical devices into open wounds, and thus the blood stream. These concerns emerge from recent publications showing that NPs can directly be taken up by the exposed organs and are able to translocate using the blood stream to secondary organs, such as the central nervous system, potentially affecting the growth characteristics of embryonic neural precursor cells[3].

Therefore, the need exists for researchers to detect and measure NPs in blood. This work explores the ability of single particle ICP-MS (SP-ICP-MS) to detect and measure gold and silver nanoparticles in blood.

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Experimental

Samples and Sample Preparation

A blood Standard Reference Material (Seronorm™ Trace Elements in Whole Blood, Level I) was diluted 20 times with tetramethylammonium hydroxide (TMAH) + 0.1% Triton-X. Gold and silver nanoparticles (gold - 30 and/or 60 nm, NIST® 8012, 8013; silver - 40 and/or 60 nm, Ted Pella™ Inc.) were added to each blood sample at various concentrations. To break up any agglomerated particles, the stock solutions were sonicated for five minutes prior to spiking in the blood. The blood samples were manually shaken prior to analysis.

Instrumentation

All samples were run on a PerkinElmer NexION® 350D ICP-MS using the Nano Application Module in Syngisitx™ software. Instrumental conditions are shown in Table 1. Calibrations were carried out with both dissolved and particulate gold or silver. Table 2 shows the calibration standards used for each element.

Two rinses were used between samples: 1% HCl + 0.1% Triton-X was aspirated to dissolve/remove any residual gold particles, followed by deionized water to remove traces of the hydrochloric acid. A 1% HNO_3 + 0.1% Triton-X solution was used as a rinse solution for silver particles. This two-solution rinse approach was found essential as residual acid could dissolve particles in the sample. Each rinse solution was aspirated for one minute.

Table 1. NexION 350 ICP-MS Parameters.

Parameter	Value
Nebulizer	Glass concentric
Spray Chamber	Glass cyclonic
RF Power	1600 W
Nebulizer Gas Flow	Optimized for maximum Au signal
Dwell Time	100 µs
Quadrupole Settling Time	0 µs
Data Acquisition Rate	10,000 points/sec
Analysis Time	60 sec

Table 2. Calibration Standards for Au and Ag Nanoparticle Analysis.

Gold				
Particle Standard	Particle Size (nm)	Approx. Particle Concentration (Particles/mL)	Dissolved Standard	Concentration (µg/L)
1	10	100,000	1	1
2	30	100,000	2	1.5
3	60	100,000	3	5
Silver				
Particle Standard	Particle Size (nm)	Approx. Particle Concentration (Particles/mL)	Dissolved Standard	Concentration (µg/L)
1	40	100,000	1	1
2	60	100,000	2	5

Results

Initial tests were performed with gold nanoparticles. Figure 1 shows a blood sample spiked with a mixture of 30 and 60 nm Au NPs (approximately 100,000 particles/mL each). There are clearly two size distributions, indicating that both particle sizes are seen. This sample was analyzed three times consecutively, with the measured particle sizes shown in Table 3. These results demonstrate both accuracy and repeatability.

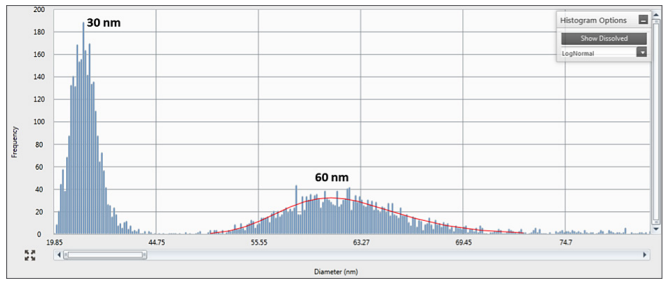

Figure 1. Size distribution of 30 and 60 nm Au nanoparticles (100,000 particles/mL each) in blood (20x dilution).

Table 3. Analysis of 30 and 60 nm Au Nanoparticle Mixture in Blood.

Replicate	Nominal Size (nm)	Most Frequent Size (nm)	Mean Size (nm)	Particle Concentration (Particles/mL)
1	30	30	31	108,710
	60	61	62	107,490
2	30	31	31	102,878
	60	61	62	101,294
3	30	31	32	102,017
	60	61	62	103,467

Next, 40 and 60 nm Ag NPs were added to blood sample so that the final, total particle concentration was about 200,000 particles per milliliter. Figure 2 shows the detected particle distribution, and Table 4 shows the results from three consecutive analyses. From Figure 2, it is evident that there are more 40 nm particles than 60 nm. Despite this difference, the measured size is accurate and reproducible for both size particles.

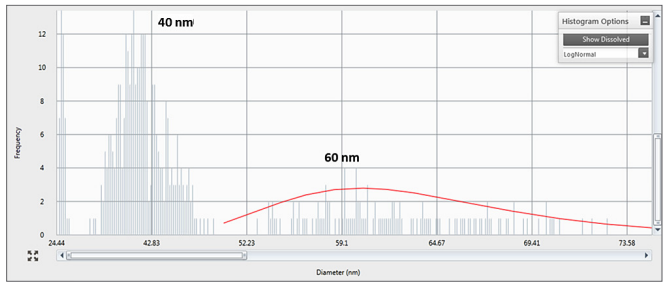

Figure 2. Size distribution of 40 and 60 nm Ag nanoparticles (100,000 particles/mL each) in blood (20x dilution).

Table 4. Analysis of 40 and 60 nm Ag Nanoparticle Mixture in Blood.

Replicate	Nominal Size (nm)	Most Frequent Size (nm)	Mean Size (nm)	Particle Concentration (Particles/mL)
1	40	41	42	100,024
	60	60	63	97,483
2	40	41	42	101,967
	60	61	63	98,957
3	40	41	42	102,263
	60	60	63	99,069

To see if lower particle concentrations could be detected in blood, only 40 nm Ag particles were spiked into the blood samples at a nominal concentration of 50,000 particles per milliliter, half the concentration of the previous analysis. Figure 3 shows that the particles are detected, while the data in Table 5 demonstrate that even at low concentrations, Ag NPs can be accurately and reproducibly measured in blood.

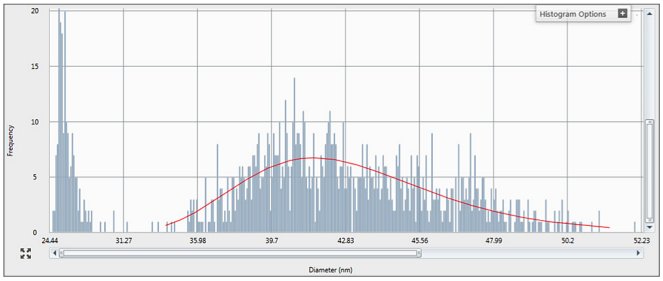

Figure 3. Size distribution of 40 nm Ag nanoparticles in blood, at a concentration of 50,000 particles/mL.

Table 5. Analysis of 40 nm Ag Nanoparticles in Blood at 50,0000 Particles/mL.

Replicate	Nominal Size (nm)	Most Frequent Size (nm)	Mean Size (nm)	Particle Concentration (Particles/mL)
1	40	42	43	50,242
2	40	42	43	50,775
3	40	42	43	50,486

Conclusion

This work demonstrates the ability of SP-ICP-MS to rapidly and accurately detect and measure gold and silver nanoparticles in whole blood, both at low concentrations and in mixtures. These measurements were accomplished with simple sample preparation (requiring only dilution) using PerkinElmer's NexION 350 ICP-MS and Syngistix Nano Application Module, offering continuous data acquisition and instant particle counting and sizing for research applications.

Reference

1. Chen X, Schluesener HJ (2008) Nanosilver: a nanoproduct in medical application. Toxicol Lett 176: 1–12.
2. Sintubin L, Verstraete W, Boon N (2012) Biologically produced nanosilver: Current state and future perspectives. Biotechnol Bioeng 109: 24222–22436.
3. Soderstjerna E, Johansson F, Klefbohm B, Johansson UE (2013) Gold- and Silver Nanoparticles Affect the Growth Characteristics of Human Embryonic Neural Precursor Cells. Plosone 8-3:58211.

Consumables Used

Component	PerkinElmer Part #
Green/orange peristaltic pump tubing	N0777042
Meinhard™ Type C0.5 glass nebulizer	N8145012
Baffled glass cyclonic spray chamber	N8145014
Quartz ball joint injector, 2.0 mm	WE023948
Quartz torch	N8122006
Nickel sampler cone	W1033612
Nickel skimmer cone	W1026356

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