APPLICATION NOTE

ICP - Mass Spectrometry

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Analysis of Nanoparticles in Biological Tissues using SP-ICP-MS

Introduction

The use of engineered nanoparticles (ENPs) in consumer products is well documented and has raised concern of the eventual fate and potential toxicity of these materials at the end of their consumer-product life[1,2]. It is likely that these materials will eventually find their way into environmental systems through food packaging and manufacturing, food products or waste disposal[3]. The analysis of ENPs is focused on three metrics: particle size, particle number, and particle mass distributions. Each of these metrics is very important for assessing environmental effects, and ultimately the risk associated with the use of these materials in consumer products. While established methods exist for the determination of mass distributions of metals in tissue samples, few robust methods have been developed to detect and characterize nanomaterials, especially particle number and size distributions[4].

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The recent development of the analytical technique single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) has provided an analytical means to directly quantify particle size, mass and number distributions at environmentally relevant concentrations (ng/L). This is the first analytical technique capable of determining all three nano-specific metrics from a single sample. The utility of this technique has been proven in numerous sample matrices including wastewater, EPA moderately hard water and tissue samples, with the aid of tissue digestion[5–8].

The use of SP-ICP-MS to detect ENPs in aqueous samples is straightforward, with sample preparation only consisting of dilution prior to analysis. However, the analysis of ENPs in tissues is more difficult and requires a digestion step prior to analysis. Traditional digestion procedures focus on using strong acids to liberate the desired elements from tissues. However, this type of digestion is incompatible with ENP analysis, as ENPs present would likely dissolve.

Instead, a different approach is to use strong bases or enzymes to digest tissues, ideally liberating ENPs without altering them. The medical community initially developed these non-traditional extractions for analysis of artificial joint wear particles[9,10]. These extractions have been applied in an attempt to extract and analyze ENPs. One approach used for ENP extraction is chemical digestion using the strong base tetramethylammonium hydroxide (TMAH)[5–8]. TMAH extraction has proven to yield high recoveries of both particle number and total mass, as compared to tissue digestion using sonication and water, and is a promising technique for analyzing ENPs in biological samples[5]. This work will describe the process of tissue extraction coupled with SP-ICP-MS analysis using the PerkinElmer NexION® 350Q ICP-MS.

Experimental

Materials

Citrate-coated Ag ENPs (60 and 70 nm) used in this work were purchased from NanoComposix (San Diego, CA). Ground beef was purchased from a local supermarket (Golden, CO) and used as a model mammalian tissue. *Lumbriculus variegatus (L. variegatus, a* species of aquatic worm) were purchased from aquatic foods (Fresno, CA) and were used as an environmentally and toxicologically relevant tissue.

All extracts containing ENPs were diluted in NanoPure™ (NP) water immediately prior to SP-ICP-MS analysis (water or digested tissues).

Instrumentation

Analysis using SP-ICP-MS was conducted following the method described by Pace et al. 2011[11] using the size based efficiency approach. All samples were analyzed using a NexION 350Q ICP-MS (PerkinElmer, Shelton, CT). ICP-MS operating conditions are shown in Table 1. Silver was measured at AMU 107. All data was measured in triplicate, though raw data shown is for one run, while the size distribution is a composite of all three runs.

Sample Preparation

Tissue digestion was conducted using the method described by Gray et al. 2013[5]. Briefly, the organic base (TMAH) was used to digest tissues and liberate ENPs from tissues at a solvent to tissue ratio of 20:1. The TMAH digestion solution was 20 %TMAH w/w. This concentration was selected based on spike recovery optimization tests using model mammalian tissue. Sample digestions were performed over 24 hours at room temperature, the first hour of which all samples were sonicated. Digested tissues were diluted to 1 %TMAH prior to analysis. This TMAH concentration was maintained if further dilution was required.

Table 1. Operating conditions for SP-ICP-MS analysis.

Parameter	Values
SP-ICP-MS Instrument	PerkinElmer NexION 350Q ICP-MS
Plasma Power	1600 W
Nebulizer, Spray Chamber, and Flow	Meinhard, Cyclonic, 1 mL/min
Efficiency Calibration	Particle Size Method
Masses Monitored	^{107}Ag, ^{197}Au
Dwell Time per AMU	50 µs
Readings per Sample	60 sec

Bioaccumulation experiments (70 nm Ag ENPs) were conducted using *L. variegatus.* The exposure period was 24 hours in EPA moderately hard water at a concentration of 5 mg/L. Silica sand was used in each beaker to provide adequate substrate for the worms to live in. The test was conducted in an incubator which was maintained at 20 °C with a 16:8 light/dark photoperiod. At the conclusion of the exposure, all worms were depurated for 24 hours prior to analysis to allow gut-associated ENPs to be cleared.

Results and Discussion

Tissue Spike Recovery

Spike digestion experiments showed that TMAH succesfully decomposed all of the model mamalian tissue prior to analysis. Figure 1 shows the resulting raw data for an Ag ENP standard run in water (A) compared to the same Ag particle spiked and extracted from tissue (B). These two figures clearly show similarites between the number of particles observed, their average intensity, and the background count mean (representing dissolved Ag). The similarity observed between detected pulses is as expected, as each sample was spiked with an identical mass concentration (and particle number concentration) of the same ENP. Further, TMAH digestion does not adversely affect particle stability through aggregation and particle settling (loss of pulses). Finally, the silver background signal did not increase between the water-based standard and the extracted tissues. This indicates that the extraction procedure did not cause any ENP dissolution as compared to the standard in water. It is possible that similar dissolution could have occurred in both the standard and TMAH digestion. However, this has not been observed for particle standards in water[12].

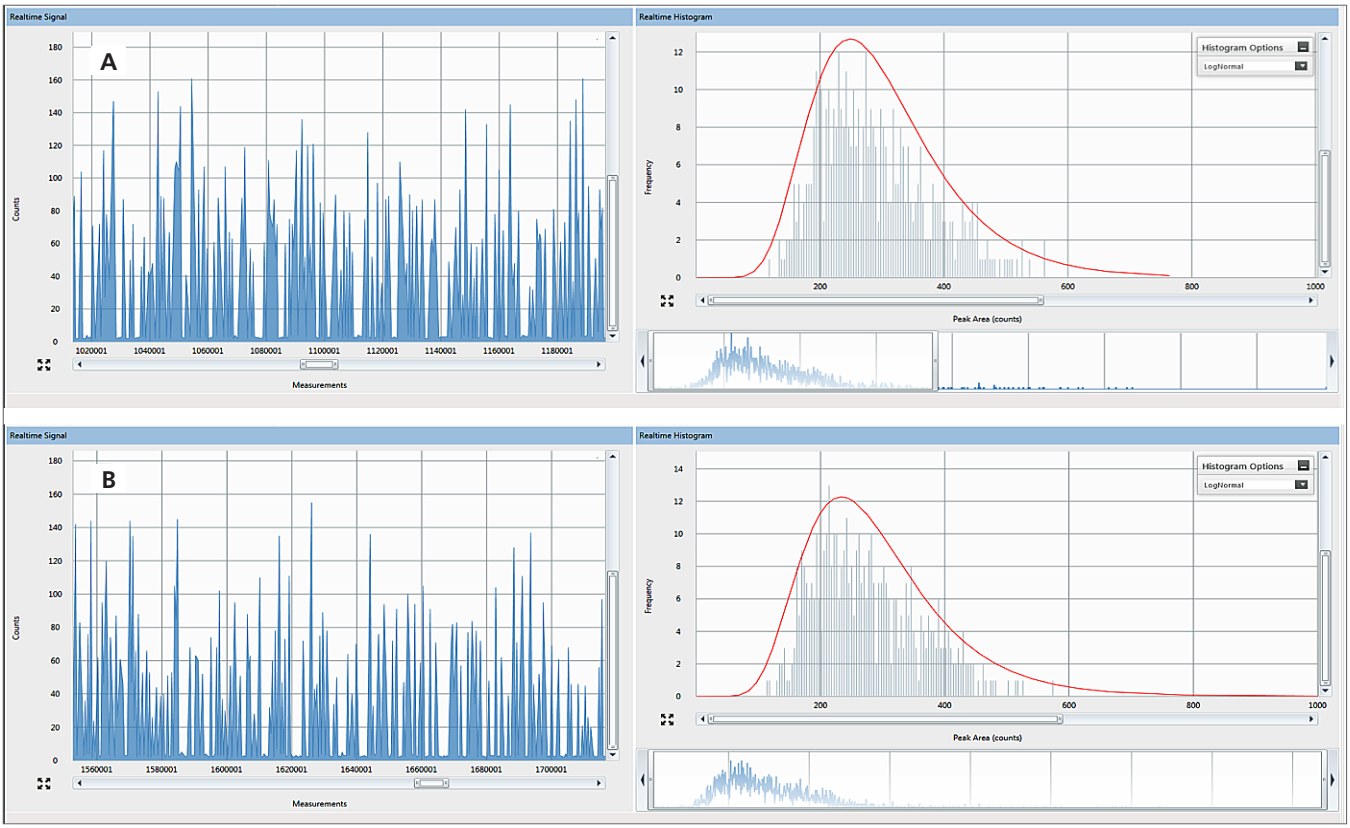

Figure 1. Raw counts for 60 nm Ag ENPs spiked into (A) nanopure water and (B) ground beef at an aqueous concentration of 19 µg/L and a tissue concentration of 19 µg/kg w/w tissue.

Biological Uptake

L. variegatus was readily digested using the TMAH procedure, allowing for SP-ICP-MS analysis of tissues. Ag ENPs were detected in *L. variegatus* after 24 hours of depuration, proving that this technique can be applied to liberate and analyze bioaccumulated ENPs. The pulses observed in Figure 2A, can clearly be observed above a very low Ag^+ background. The observed pulses in Figure 2A were converted to a size distribution (Figure 2B), showing a peak mode of 55 nm. The observed size distribution was slightly smaller than the manufacturer-reported diameter. However, this size distribution is closer to what was observed using TEM analysis (data not shown). The observed tissue concentration was 7.1 µg/kg with essentially all of the Ag present as ENPs.

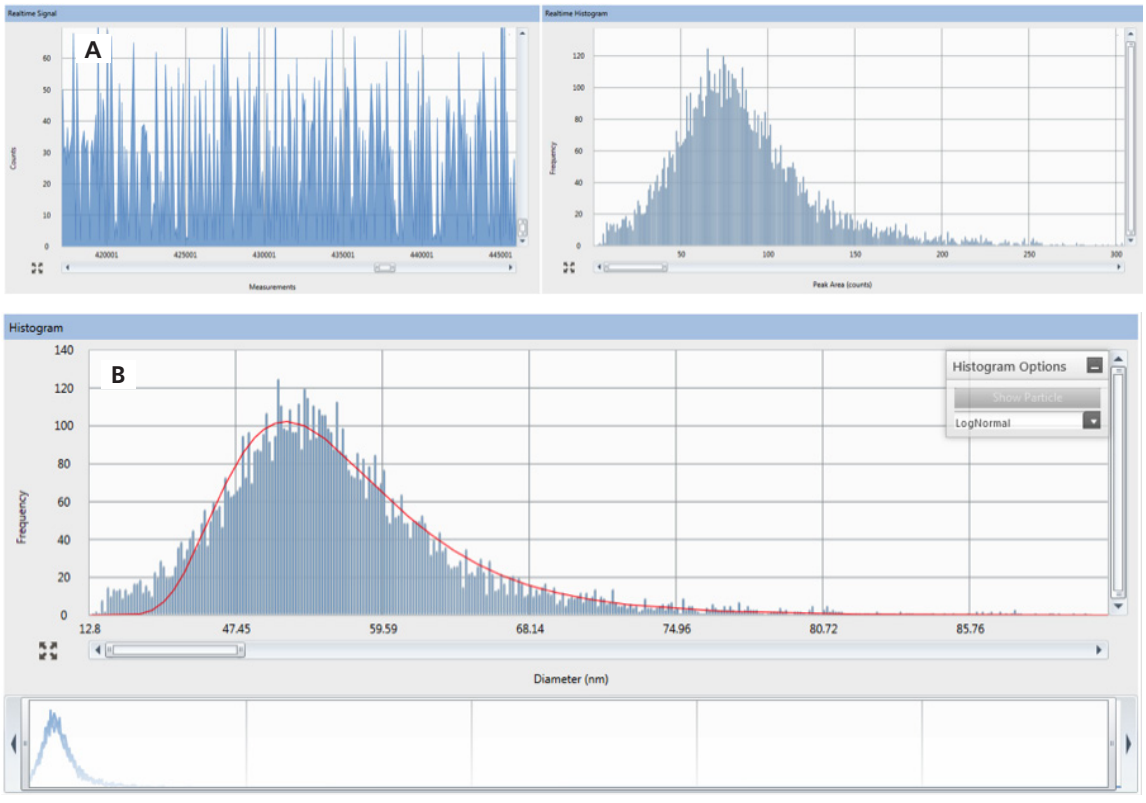

Figure 2. Raw counts (A) and size distribution (B) for ENPs that were acummulated by the aquatic worm, *L. variegatus*. Organism exposure was conducted at 5 µg/L as ENP of 70 nm Ag.

Conclusion

This work has shown that ENPs can be extracted from biological tissues and analyzed using SP-ICP-MS. Further, this approach is valid for ENPs bioaccumulated in tissues at low concentrations due to the senstivity of ICP-MS. The extraction procedure did not visibly change the background for a 60 nm Ag ENP extracted from water compared to ENPs extracted with TMAH from model mamalian tissue. ENPs were also sucessfully extracted from *L. variegatus* exposed to 5 µg/L Ag ENPs in water. These experiments prove that TMAH extraction coupled to SP-ICP-MS can be used to successfully liberate ENPs from tissues. ENP count distributions can ultimately be converted to size distributions, allowing for size, number, and mass distributions to be determined using this analysis technique. The applicability of this digestion technique beyond these tissue matrices is unknown and should be investigated for any new tissue sample.

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