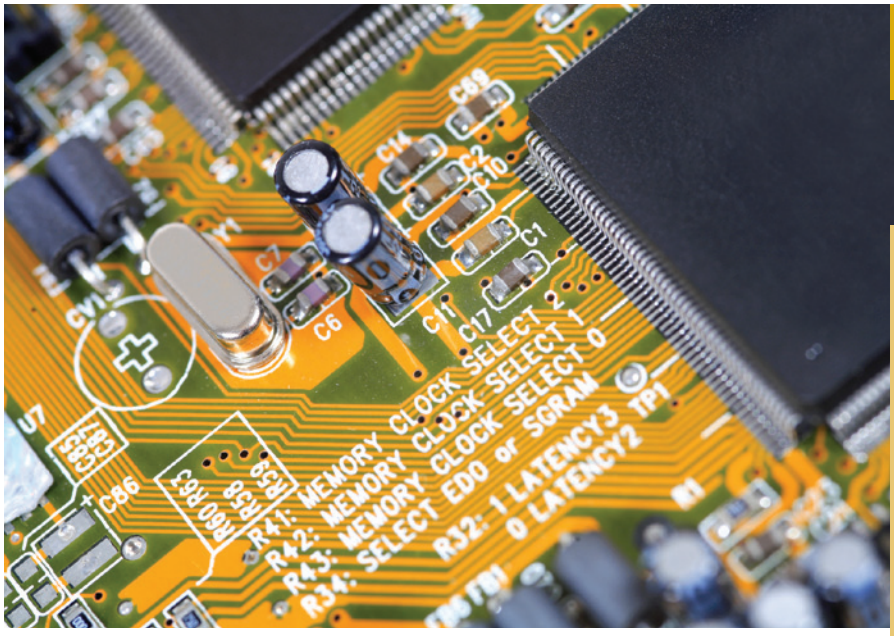

APPLICATION NOTE

ICP-Mass Spectrometry

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Coupling Flow Field Flow Fractionation to ICP-MS for the Detection and Characterization of Silver Nanoparticles

Introduction

Analysis of nanomaterials should include characterization of composition as well as size. Many techniques are capable of sizing nano-size particles, such as dynamic light scattering (DLS), UV/Vis spectrophotometry, and transmission electron microscopy (TEM), yet provide no information on the composition of the particle and/or are time intensive and costly. Inductively coupled plasma-mass spectrometry (ICP-MS), however, is a standard instrument in many analytical laboratories and is the method of choice for analysis of most elements across the periodic chart. The multi-element capability of the ICP-MS, low detection limit (ppt), and wide dynamic range (10^9 orders of magnitude) also make it ideal for application to the measurement of inorganic engineered nanoparticles (ENPs). While ICP-MS can be used directly to obtain concentrations of nanoparticulate-associated elements, more information on characteristics of ENPs can be obtained by coupling a size-separation step prior to ICP-MS analysis. The most versatile size-separation technique for this application is field flow fractionation (FFF). Although FFF is a powerful nanoparticle sizing technique, many common detectors used in conjunction with FFF do not provide the needed compositional information of the particles. Therefore, the resultant hyphenated technique of FFF-ICP-MS provides nanoparticle sizing, detection, and composition analysis capabilities at the parts per billion (ppb) level, which is critical to environmental investigations of nanomaterials. Furthermore, the similar flow conditions required by both ICP-MS and FFF make interfacing relatively simple.

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Nanometrology

Nanotechnology has great potential in both industrial and commercial sectors, producing useful products for society either when used alone or when integrated with other material into products (e.g. consumer goods, foods, pesticides, pharmaceuticals, and personal care products, among others). Nanotechnology, defined as the control of matter between 1 and 100 nm where unique phenomena occur because of their small size, has seen great innovation and study in recent years.

Several classes of ENPs contain metals that make them particularly suitable for characterization by ICP-MS methods. For example, quantum dots (QDs) often contain cadmium (Cd), selenium (Se), tellurium (Te) and zinc (Zn), among others. QDs are the smallest ENPs having semiconductor properties and are investigated for their use in transistors, solar cells, and LEDs.

Despite rapid development, early public acceptance, and acknowledgement of probable release of nanoproducts to the environment, the potential for adverse environmental effects has not yet been established. In the case of QDs, most of the constituent elements can be toxic to organisms.

This knowledge gap exists, in part, because of the innate difficulties of detection, characterization, and quantification of ENPs, particularly in environmental and biological samples. There are universal calls for improvements in nanometrology. Many techniques are capable of sizing nano-sized particles (nanoparticles and quantum dots) in simple laboratory systems, including dynamic light scattering (DLS), transmission electron microscopy (TEM), and disc centrifugation (DCS), among others. Yet these methods provide little or no information on the composition of the particle. These techniques are also often not sensitive enough to work at environmentally or biologically relevant concentrations (sub-µg/L). Finally, these techniques lack specificity, which means they are not able to distinguish ENPs from other matrix constituents, such as natural particles, humic substances, and cellular debris. Coupling FFF with ICP-MS (or ICP-OES/AES), however, garners element-specific information at trace concentration levels[1] when studying metal-containing NPs.[2] Furthermore, the capability of multi-metal analysis is an added benefit when coupling FFF with mass spectrometry.

Experimental

Materials

Silver nanoparticles of 20 and 40 nm (Nanocomposix, San Diego, CA, USA) were acquired in stock suspensions at a nominal concentration of 20 mg Ag/L and were stabilized in aqueous 2 mM citrate, per the manufacturer. Nano-Ag suspensions were made by diluting the stock solutions with 18.2 M-ohm Nanopure water to final concentrations, ranging from 10 to 500 µg/L. Aqueous Ag standards (High-Purity Standards, Charleston, SC, USA), used for calibration, were diluted in 1% nitric acid (Optima grade) to concentrations ranging from 1 to 100 µg/L.

Red mercaptoundecanoic acid (MUA)-coated CdSe/ZnS quantum dots (NN-Labs, Fayettville, AR, USA) were investigated in the second study where the hydrodynamic diameter was 25 nm, with a metal core stated as 5 nm. Stock solutions were diluted approximately 1000-fold, using deionized water to concentrations ranging from 4.6×10^{13} to 1.8×10^{16} particles/L.

Instrumentation

An ELAN® 6100 ICP-MS (PerkinElmer, Shelton, CT, USA) was used for all analyses. Standard operating and tuning procedures were used. Only one silver isotope was monitored (^{107}Ag) with a dwell time of 2000 ms, alternating with a Bi internal standard with a dwell time of 1000 ms, resulting in a data point being collected at a rate of approximately one every three seconds. The total number of readings per sample was chosen such that the data were collected for the entire length of the fractogram, which, depending on experimental conditions, ranged from 40 to 60 minutes.

An AF2000 asymmetrical FFF instrument (Postnova Analytics, Salt Lake City, UT, USA) was used for the silver experiments. A 10 kDa regenerated cellulose membrane was used and was replaced approximately every 25 runs. The carrier fluid consisted of 0.01% FL-70 surfactant and 0.025% sodium azide (an antibacterial agent). The FFF instrument was directly plumbed into the ICP-MS. The channel flow conditions allowed direct connection of the FFF effluent to the ICP-MS nebulizer without a flow splitter. Asymmetrical flow field flow fractionation (AF4) runs were programmed to start with a 10 min relaxation period (focusing step), followed by 40 min elution (0.7 mL/min cross flow and 1.0 mL/min detector flow) with 10 min flush (field-off) between each experimental run. The detector flow can be diverted to a number of instruments, such as ICP-MS, for characterization after AF4 separation. The carrier fluid used to flush the channel after analysis is typically also analyzed in order to determine the unfractionated portion of analyte. Details of AF4 and ICP-MS run conditions are given in Tables 1 and 2 (Page 3).

Size characterization of quantum dot samples was accomplished by an F1000 symmetrical FFF instrument (Postnova Analytics) equipped with 1 kDa regenerated cellulose membrane. The ICP-MS (ELAN 6100, PerkinElmer) was used to measure the concentrations of ^{64}Zn, ^{114}Cd, and ^{82}Se, with ^{209}Bi as the internal standard. Carrier fluid also consisted of 0.01% FL-70 and 0.1 mM sodium azide. Pumps delivered the carrier fluid at a channel flow rate of 1.0 mL/min and recirculated the cross flow at a rate of 0.9 mL/min. A 20 µL injection loop was used for sample injection.

The outlet flow from the FFF passed through a fluorescence detector and then to the ICP-MS. Details of FFF and ICP-MS run conditions are shown in Tables 1 and 2. An example of the online addition of fluorescence and ICP-MS detectors is given in Figure 1. More details on FFF theory, instrumental setup, and coupling to various detectors can be found in PerkinElmer white paper "An Introduction to Flow Field Flow Fractionation and Coupling to ICP-MS".

Table 1. **FFF Parameters.**

Parameter	Ag Nanoparticles	CdSe / Zn Quantum Dots
Instrument	Postnova AF2000 (asymmetrical)	Postnova F1000 (symmetrical)
Channel Size	355 x 60 x 40 mm	20 x 270 mm
Membrane Type	Regenerated Cellulose	
Membrane Porosity	10 kDalton	1 kDalton
Spacer Width	500 µm	254 µm
Sample Injection Volume	100 µL	20 µL
Detector Flow	1 mL/min	
Cross Flow	0.7 mL/min	0.9 mL/min
Injection Delay	1 min	15 sec
Equilibration Time	10 min	2 min
Flush Time	10 min	N/A
Carrier Fluid	0.1% FL-70, 0.025% NaN_3	0.01% FL-70, 1 mM NaN_3

Table 2. **ICP-MS Parameters.**

Parameter	Ag Nanoparticles	CdSe / Zn Quantum Dots
Instrument	PerkinElmer ELAN 6100 ICP-MS	
Nebulizer	Cross Flow	
Spray Chamber	Scott Double Pass	
Neb Gas Flow	Optimized for <3% Oxides	
Sample Flow	1 mL/min	
RF Power	1000-1300W	
Dwell Time	3000 ms	4000 ms
Analytes	Ag107; Bi109	Zn66; Cd111; Se82; Bi209
Total Analysis Time	60 min	30 min

Daily Standards

For the silver nanoparticle study, to ensure the reproducibility of results from day to day, a daily standard was prepared for AF4-ICP-MS analysis that consisted of a mixture of 20 and 40 nm Ag nanoparticles at 100 µg/L each. This sample was run at the beginning and end of each day, determining if there was a shift in retention time of the particles or change in ICP-MS response (percent recovery). If the retention times did not drift over the course of the day for the standard mixture, we presumed that sample runs were not affected by matrix/membrane or particle/membrane interactions throughout the day.

Although size can be directly computed from retention time using FFF theory, for this study we made a linear plot of particle size versus retention time. The linear equation from this plot could then be applied to sample runs to convert elution time to particle diameter.

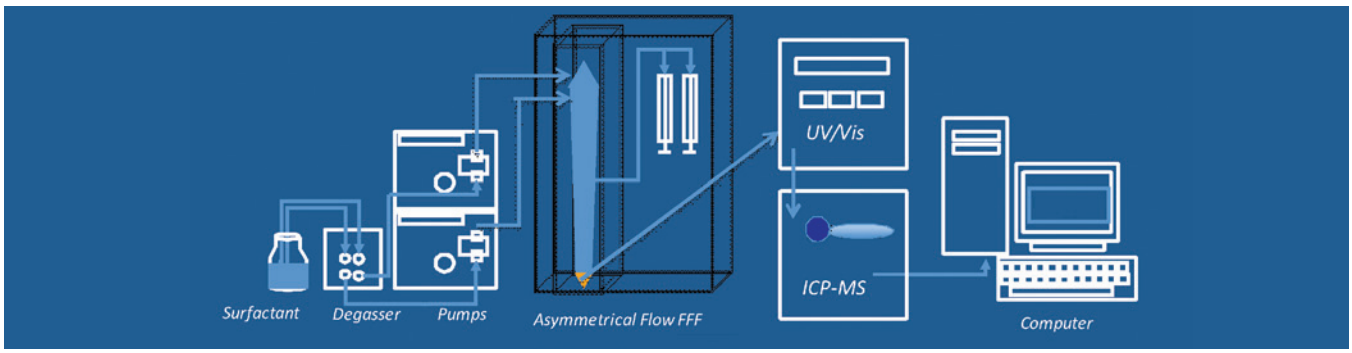

Figure 1. Schematic of AF4-ICP-MS analysis with online addition of UV/Vis analysis.

Analytical Results

Resolution and detection limit

There are a number of parameters in the AF4 method that contribute to both detection limit and resolution, the most important of which is the cross flow parameter of the FFF. Under the flow conditions used, we see nearly baseline separation between the 20 nm and 40 nm Ag nanoparticles (Figure 2), with the void peak (unresolvable material) present on the far left of the fractogram. An increase in cross flow would allow better separation for smaller particles, down to as small as 3-5 nm. However, with increasing cross flow, the analysis time increases and there is a higher chance of particle/membrane interaction, which leads to a decrease in recovery. Therefore, a balance needs to be struck to achieve the best results when considering these multiple factors. Based on the data for 25 and 100 ppb Ag, the detection limit under the current run conditions is estimated to be approximately 5 ppb. The concentration detection limit was determined by running serial dilutions through the FFF-ICP-MS until no discernable analyte peaks were detected above the background.

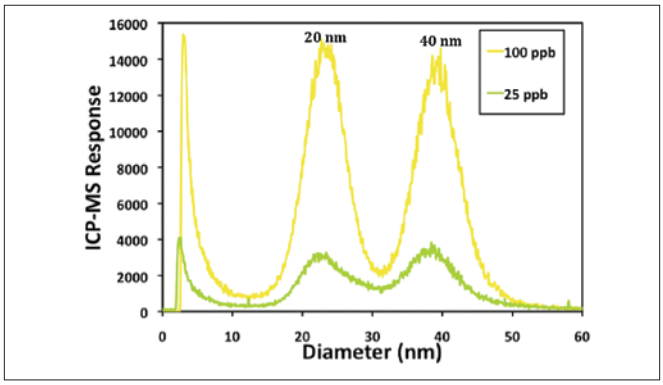

Figure 2. Analysis of 25 and 100 ppb nano-Ag mixture.[3]

Mixed Metal Analysis with Flow FFF-ICP-MS

The results for the symmetrical flow FFF-ICP-MS characterization of a commercial CdSe/ZnS/MUA quantum dot is shown in Figure 3. Here, it is demonstrated that the manufacturer incorrectly described some quantum dot characteristics, specifically the metal content in the dots.[4] It was found that the MUA coated quantum dots had a significantly higher (9:1) Cd:Se ratio than the expected nearly 1:1 molar ratio.

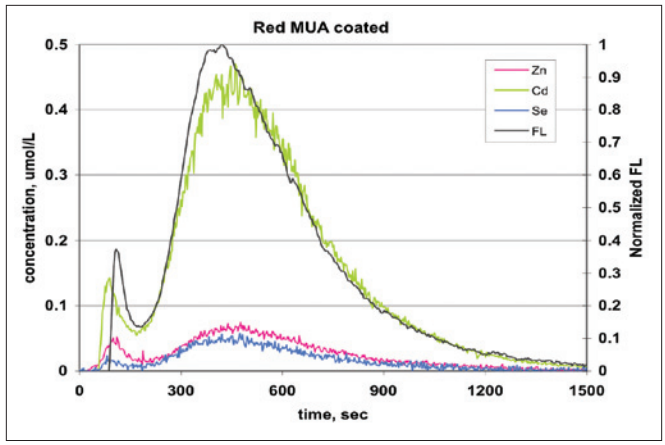

Figure 3. Symmetrical FFF-ICP-MS fractogram of red-emitting MUA coated CdSe/ZnS quantum dots (QDs). The first small peak is the void peak, which contains unfractionated materials. The larger analyte peak shows that all three metal signals are associated with the fluorescent signal (FL) from the QD. The particle size at the peak maximum was calculated to be 23 nm.[4]

It was proposed, but not confirmed, that the excess Cd was associated with the MUA coating as part of an incomplete washing process during manufacturing. This additional Cd demonstrated much higher than expected toxicity. The symmetrical flow FFF-ICP-MS characterization proved integral in demonstrating that Cd was, at least initially, integral to the quantum dot and not simply excess Cd in solution (Figure 3). This element-specific information would be difficult, if not impossible, to acquire using chemical analysis approaches.

Conclusions

Concerning engineered nanoparticles, nanometrology is a growing field that is certain to benefit from on-line flow FFF-ICP-MS analysis. The combination of continuous fractionation using FFF, with the sensitive, multi-elemental capability of ICP-MS, will provide increased knowledge about size-dependent variations in composition and trace element interactions at environmentally and biologically relevant concentrations. Although method development can at times be a lengthy process, the multitude of run conditions, such as flow rates, carrier fluid composition, as well as membrane type and porosity, lends itself to the flexibility needed to fractionate a variety of particles under a number of conditions. The universality of this will undoubtedly soon make flow FFF-ICP-MS an integral part of the standard methods with which to study nanoproducts in this fast growing field.

Acknowledgements

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