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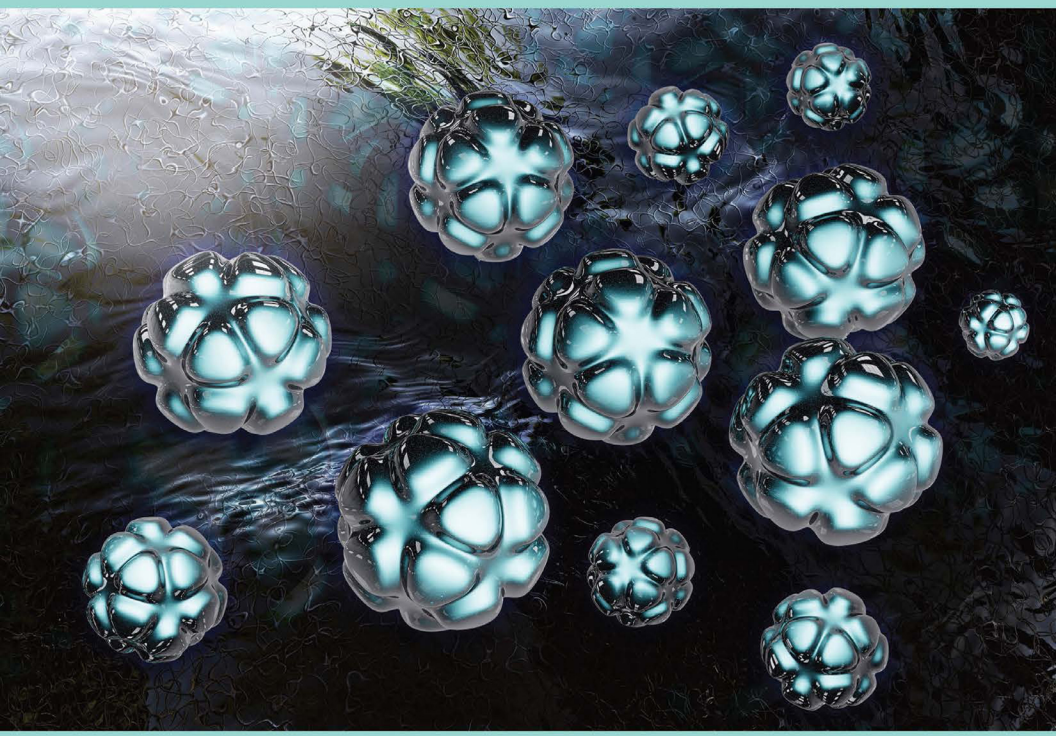

Nanoparticle Analysis using the Sensitivity of ICP-MS

Essential Knowledge Briefings

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INTRODUCTION

The term nanoparticle refers to any particle with size ranging from 1 to 100 nm. Nanoparticles are increasingly being used in diverse products such as food additives, pharmaceuticals and drug delivery systems, cosmetics, and consumer goods and in materials such as optical and electronic devices. For example, nanoscale particles of titanium dioxide or zinc oxide are replacing the larger particles that have been used in sunscreens for decades. The larger particles are effective at scattering or absorbing UV radiation from sunlight, but they also reflect visible light, making them appear white. Nanoparticles not only retain the UV absorbency but also absorb or scatter visible wavelengths, so they appear transparent. Nanoparticles are also used in healthcare, where silver nanoparticles are added to sterile dressings and hand sanitizers for their antibacterial properties (Figure 1).

In addition to deliberately manufactured or engineered nanoparticles (ENPs), man-made nanoparticles can also be generated by the normal functioning of certain mechanical systems, such as friction surfaces, electric motors, and vehicle exhaust catalytic converters.

Due to their tiny size – in the range between simple molecules (such as glucose) and single-celled organisms (such as viruses) – nanoparticles have physical and chemical properties that can be very different from the bulk material. These differences are due to the very high surface area to mass ratio of nanoparticles; since the surface area of a sphere increases by the square of the diameter, 1 g of 100 nm particles has a total surface area 100 000 000 times greater than 1 g of 1 mm particles. Furthermore, the properties of nanoparticles are highly dependent on particle size, so measuring mean particle size and particle size distribution is critical to understanding the potential uses and effects of nanoparticles. However, much remains unknown about their toxicological properties and fate in the environment, leading to growing concerns about their potential impact on health and wildlife. In view of these possible risks, it is vital that scientists and regulators understand the characteristics of the nanoparticles within a commercial product or an environmental, biological, or food sample. This requires sensitive and selective analytical tools that can analyze nanoparticles in both their original manufactured state and in complex natural samples.

(a)

(b)

(c)

(d)

Source: Image Courtesy of 5Gyres.org

(e)

(f)

(g)

Figure 1. Nanoparticles, produced unintentionally or engineered, may be present in these products.

Inductively coupled plasma mass spectrometry (ICP-MS) offers many advantages as an analytical tool for nanoparticle characterization. ICP-MS instruments consist of a high-temperature plasma-based ion source (the ICP) coupled to a mass spectrometer (the MS). The ICP source decomposes, vaporizes, atomizes, and ionizes the atoms of the elements contained in a sample. The ions are then transferred into a high vacuum region where they are separated and detected by the mass spectrometer, providing information on the elemental composition of the sample.

ICP-MS can measure nanoparticles directly in a sample solution, using single particle inductively coupled plasma mass spectrometry (spICP-MS). The attraction of spICP-MS is that, in addition to detecting nanoparticles based on their elemental composition, the ICP-MS measurement also provides information on the size, size distribution, and number of nanoparticles in the sample, plus the concentration of the element dissolved in the solution. All these information are obtained from a single, rapid analysis, which explains why ICP-MS is proving to be a key tool for characterizing nanomaterials.

Alternatively, the ICP-MS instrument can be connected to a separation technique such as field-flow fractionation (FFF) or capillary electrophoresis (CE) to characterize the nanoparticle content of a bulk sample. The two approaches both have benefits and limitations, but they provide different information and can therefore be complementary.

This Essential Knowledge Briefing provides an introduction to the use of ICP-MS for nanoparticle analysis and explains how the technique works in single particle mode and when coupled to a separation technique. The specific advantages of ICP-MS for detecting and characterizing nanoparticles are discussed, and some potential applications are highlighted.

The briefing goes on to outline the key parameters that affect performance and how to optimize the system to obtain the best results. It also identifies and addresses the challenges of nanoparticle analysis using ICP-MS, while two case studies illustrate how ICP-MS is being used by scientists in their research. Finally, the briefing reveals how the technique is poised to develop and advance in terms of both technology and applications over the next few years.

HISTORY AND BACKGROUND

The driving force behind the development of ICP-MS was the need for a technique that could perform rapid, trace-level elemental analysis, with simpler spectra and fewer interferences than existing optical techniques. Following the initial development of plasma-source mass spectrometry in the 1970s, the first paper reporting the use of an ICP as the ion source was published in 1980. The first commercial ICP-MS instruments appeared just three years later, and the first computer-controlled, benchtop ICP-MS instrument – the HP 4500 – followed in 1994.

The potential of the new technique was quickly apparent, and ICP-MS was rapidly adopted as a highly useful analytical technique for a wide variety of research and industrial applications. Early applications included analyzing metal contaminants in semiconductors; monitoring toxic trace metals in water, soil, and food; measuring rare earth elements in geological samples; and identifying the isotopes produced from nuclear reactions. Nowadays, in addition to characterizing nanoparticles, ICP-MS is used in applications such as routine environmental monitoring, protein quantification in life science, and pharmaceutical production control, and as a sensitive, selective detector for chromatography.

The ICP is a high-temperature plasma formed by ionizing a stream of argon gas in a quartz tube called a torch. The plasma is created by a strong magnetic field produced by a radio frequency (RF) current applied to an induction coil that encircles the open end of the torch. Because the plasma is formed at atmospheric pressure, the gas density is relatively high so the plasma achieves a very high temperature (6000–10 000 K).

Samples for ICP-MS analysis are typically in liquid form, although solids and gases can be analyzed with suitable sample introduction systems. Liquid samples are first converted into a fine aerosol mist by a stream of argon gas passing through a nebulizer. An enclosed spray chamber then removes the larger droplets, allowing the finer aerosol droplets to be carried to the plasma torch.

The ICP torch typically consists of three concentric tubes, each carrying an argon gas stream. The outer tube carries the argon flow that supports

the plasma, while the middle tube carries an auxiliary flow that positions the plasma so that it does not melt the torch. The inner tube contains the argon flow from the nebulizer/spray chamber, carrying the fine aerosol droplets. The sample aerosol is carried through the central region of the plasma, where it is almost instantaneously dried, dissociated, and broken down into its component atoms, which are then ionized.

The plasma converts the elements in the sample into positively charged ions, which are extracted into the low-pressure region of the mass spectrometer via a vacuum interface. The interface consists of several (usually two) metal plates called cones, with small circular apertures through which the ions can pass while maintaining the vacuum in the chamber beyond. The extracted ions are focused by electrostatic lenses into a collision/reaction cell (CRC), where spectral interferences can be reduced or removed using collision or reaction processes. After passing through the CRC, the ions enter the mass spectrometer, which separates the ions such that only the selected mass can pass through and reach the detector at a given time. Most commercial ICP-MS systems use a quadrupole mass filter, a type of scanning mass spectrometer that separates ions according to their mass-to-charge (m/z) ratios. The argon ICP forms mostly singly charged ions, so the m/z of an ion is equivalent to its atomic mass (u).

The quadrupole mass filter comprises a set of four conducting rods arranged parallel to one another around a central axis. Direct current (DC) and RF voltages applied to opposite pairs of rods set up an electric field in the central axis of the quadrupole. This field causes ions traveling through the quadrupole to oscillate as they travel along the central axis. Ions with a certain m/z value (resonant ions) enter stable trajectories and can make it all the way through the quadrupole to the detector. Other ions – with higher or lower m/z values – enter unstable trajectories, causing them to collide with the rods before they can reach the detector. In operation, the quadrupole can be set to sequentially allow ions with different m/z values through to the detector by automatically changing the DC and RF voltages. When the spICP-MS mode of operation is selected, DC and RF voltages applied to the quadrupole are fixed according to the

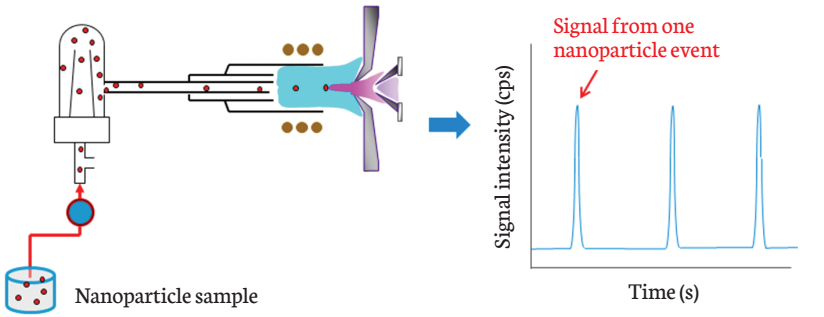

Figure 2. Schematic diagram of particle transport from sample to ICP-MS interface and resulting time-resolved signal generated by the mass spectrometer.

Source: Image Courtesy of Agilent Technologies Inc.

m/z value of the target element in the nanoparticles for continuous data acquisition (Figure 2).

While CRCs have made an enormous contribution to the control of spectral interferences, recent advances have seen the development of "triple quadrupole" ICP-MS systems (ICP-QQQ), which possess an additional quadrupole mass filter (Q1) before the CRC. This first mass filter controls the mass of ions that can enter the CRC, providing significantly improved control of reaction chemistry and therefore interference removal. The CRC is followed by a second quadrupole (Q2) to provide a second mass section (tandem mass spectrometry, MS/MS), which ensures that only the selected ions (or reaction product ions) are passed to the detector. The ICP-QQQ configuration can provide higher ion transmission efficiency, making it four to five times more sensitive, and lower background signal, particularly for elements that suffer spectral overlaps (see Section "Problems and Solutions"). As a consequence, ICP-QQQ can detect smaller nanoparticles of any composition (down to around 5 nm in diameter, depending on the element of interest, compared with a limit of around 20 nm for a single quadrupole system), while offering far superior performance for nanoparticles that contain difficult elements such as iron, sulfur, titanium, and silicon.

ICP-MS is particularly suited to nanoparticle analysis because of its high sensitivity and selectivity. The technique also provides more information on particle number and composition than other nanoparticle

characterization techniques, such as multi-angle light scattering (MALS) or dynamic light scattering (DLS). These optical techniques can, however, provide an absolute (standardless) value for a particle's hydrodynamic size. This information is not available from ICP-MS, so the techniques are complementary and are sometimes used in combination. ICP-MS is also fast and requires little sample preparation, giving it advantages over microscopy techniques such as scanning electron microscopy (SEM) and atomic force microscopy (AFM).

There are two main ways to use ICP-MS for nanoparticle analysis: single particle mode (spICP-MS) or combined with a separation technique.

First developed in 2003, spICP-MS can detect and analyze individual nanoparticles as they pass through the plasma in the solution aerosol. The technique is able to determine the number, size, and size distribution of the nanoparticles in a sample, as well as the particle concentration and the concentration of the element of interest dissolved in the sample.

In spICP-MS, a solution containing suspended nanoparticles is introduced via a conventional nebulizer and spray chamber. The aerosol containing the suspended nanoparticles is carried to the ICP, where individual nanoparticles are dissociated, atomized, and ionized to produce a discrete signal pulse or peak. These pulses can be seen above the baseline signal, which comes from the instrument background plus the continuous signal from the dissolved component – the portion of the target element dissolved in the solution. The height of each signal pulse is proportional to the mass (and therefore the size) of that nanoparticle, and the frequency of the pulses indicates the number of nanoparticles in the sample solution. As long as the sample is diluted appropriately, individual nanoparticles will generate separate signal pulses. These nanoparticle signal pulses are measured by setting the quadrupole to the analyte mass of interest and using a transient signal measuring approach called time-resolved analysis (TRA) (Figure 3).

By measuring the signal intensity at each measured data point and counting the number of particle signal peaks, both the size and number of the nanoparticles can be determined, allowing a size distribution to be calculated. It should be noted that the calculation used to determine particle size from signal intensity assumes that the nanoparticles are hard spheres.

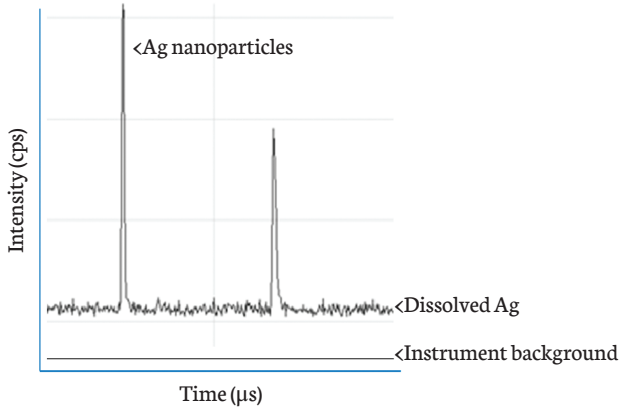

Figure 3. Expanded view of signal from two silver nanoparticle events.
Source: Image Courtesy of Agilent Technologies Inc.

Because ICP-MS is a destructive technique, it is unable to identify the true shape and morphology of the nanoparticles; such information can, however, be provided by other complementary techniques.

Single nanoparticle data analysis at the fastest TRA sampling rates can be challenging, as the technique generates a very large number of data points, and managing and processing such large data files with reasonable speed is critical. For spICP-MS data processing, data are first acquired and stored in a TRA data file. The average background signal is determined by evaluating the signal between nanoparticle peaks and subtracting this from the raw data. In this way, the total signal generated for each nanoparticle is due to the particle alone, and not to any dissolved element content or instrument background signals.

spICP-MS is ideal for characterizing liquid-based samples containing one type of nanoparticle of known composition. However, when characterizing mixtures of different-sized nanoparticles in complex samples such as natural waters or wastewaters, soil, biological matrices, food, or cosmetics, ICP-MS is occasionally combined with a separation technique such as FFF or CE. These techniques can separate the different-sized nanoparticles from one another and from any interfering compounds in the sample.

FFF, also known as asymmetric flow field-flow fractionation (AF4), can separate macromolecules, colloids, and nano- and microparticles according to size, chemical composition, or density from a few nanometers up to several micrometers. FFF works by pumping the sample through a narrow ribbon-like channel while applying a separation force at right angles to the sample flow. The separation force can be gravity, an electrical potential, a centrifugal force, or a thermal gradient. In AF4, the separation force is a crossflow solution, which pushes the nanoparticles toward the bottom of the channel. Brownian motion causes smaller particles to diffuse upward against this separation force to a greater extent than larger particles, so smaller particles tend to move back toward the middle of the channel where the flow is fastest. This results in different-sized particles separating into different flow layers that travel at different velocities, causing the particles to emerge from the channel separated according to size, with the smallest particles first (Figure 4).

CE separates charged molecules and nanoparticles using an electric field applied along a capillary. The electric field causes the charged molecules and nanoparticles to migrate along the capillary: positively charged nanoparticles move toward the cathode and negatively charged nanoparticles move toward the anode. The speed with which different nanoparticles migrate through the capillary depends on their size and charge state, allowing them to be separated. CE benefits from much shorter run times

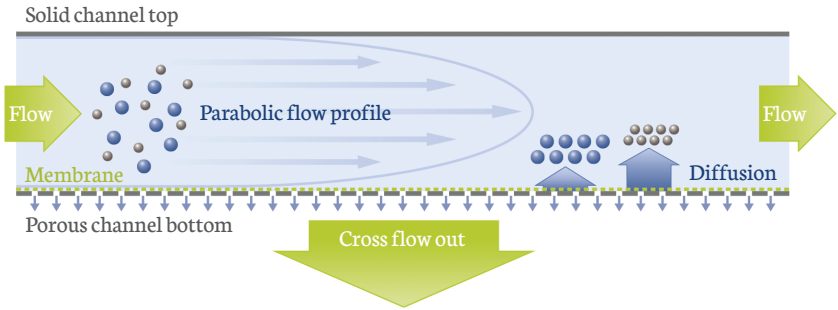

Figure 4. Schematic diagram of a field-flow fractionation channel.

Source: Image Courtesy of PostNova Analytics.

than FFF, as well as higher resolution and smaller sample size requirements, but can obviously only work with charged particles.

As long as sufficient numbers of nanoparticles are present, with appropriate separation resolution, there is no practical limit to the minimum size of nanoparticles that can be determined by combining ICP-MS with FFF or CE. Additionally, there is no practical limit on the number of elements that can be determined within a particle population.

ICP-MS is increasingly becoming a standard technique for determining the distribution and fate of nanoparticles in the environment and biological systems. This is necessary since nanoparticles have become more widely used in manufactured goods and consumer products, thereby becoming more widespread in the environment.

Nanoparticles composed of gold and silver provide good sensitivity and low backgrounds and can be detected at low concentrations because they are typically unaffected by polyatomic interferences. But conventional single quadrupole ICP-MS does not perform as well for nanoparticles composed of elements such as iron, sulfur, titanium, and silicon, which suffer from more or less severe spectral interferences. These problems are largely addressed with the latest ICP-QQQ systems. This means that ICP-QQQ is now being applied to the detection and characterization of a wider range of metal-based nanoparticles in more complex natural samples.

IN PRACTICE

Several factors can influence the performance of ICP-MS when characterizing nanoparticles, with sensitivity being perhaps the most important. It is especially critical when measuring small nanoparticles with spICP-MS, as without sufficient sensitivity, small nanoparticles cannot be clearly detected above the background signal. Sensitivity, expressed as counts per second per unit concentration (cps/ppb), is determined by many things including the ion transmission efficiency of the ICP-MS instrument and the detector gain. Instrument design also strongly influences the level of the background, which includes contributions from random noise and spectral background or interference.

The sensitivity of an ICP-MS instrument dictates the mass of the smallest particle that can be measured by spICP-MS, known as the particle size detection limit. From this, assuming a particle of spherical shape and known stoichiometry, the corresponding minimum detectable particle diameter – known as the background equivalent diameter (BED) – can be determined. Higher sensitivity also provides better precision, which allows the use of shorter TRA acquisition times, also known as the dwell time or integration time.

Some spICP-MS systems are now capable of detecting nanoparticles with diameters smaller than 10 nm, but further improvement in the nanoparticle size detection limit requires a significant increase in sensitivity. This is because the diameter of a spherical particle is a function of the cube root of its mass, so a small reduction in particle diameter leads to a significant decrease in mass (and therefore signal).

Other factors that influence performance in spICP-MS are the ability to scan fast enough to capture the signal from a single nanoparticle and to keep the time between measurement scans, known as the settling time, to a minimum (or, ideally, zero). The plasma must be robust enough to ensure effective decomposition of the sample matrix, in order to reduce the effects of differences in sample matrices between samples and standards, and to provide complete atomization and ionization of the nanoparticles. The speed of the nanoparticle data analysis software is also important, as it must

be able to perform complex calculations quickly and automatically while providing accurate results.

The best results with spICP-MS are obtained when the sample dilution and integration time are such that around 1 in 10 of the measured data points contains a nanoparticle signal. The remaining measured points provide accurate measurement of the background, which is vital to allow signals for small particles to be distinguished from the dissolved element and the random instrumental background.

Determining the optimum sample dilution to give this 1 in 10 ratio of particle signal to background often requires a pre-analysis screening measurement to determine the nanoparticle number in the original sample. Target nanoparticle numbers are typically in the region of 10^7 particles per liter; the applied dilution leads to elemental (particle) concentrations between single or sub-nanograms per liter and several hundred nanograms per liter, depending upon the size of the nanoparticles.

Traditionally, the integration time for spICP-MS was set to be just long enough to collect the entire signal from one nanoparticle. If the integration time was too short, the signal for a single particle would be split between two data points, which leads to underestimating the size of the nanoparticles and overestimating their number. On the other hand, if the integration time was too long, two nanoparticles would be measured in a single integration period, which leads to overestimating the size of the nanoparticles and underestimating their number. Appropriate integration times are also important for ensuring that the nanoparticle signal can be accurately discriminated from the background signal. In general, integration times used for this type of analysis range from 1 to 10 ms per point, with an integration time of 3 ms being typically reported (Figures 5 and 6).

The latest generation of ICP-MS systems can now support much shorter integration times (below 1 ms), while the settling time has been eliminated completely, making the TRA measurement truly continuous. In these systems, the acquisition rate is fast enough for several individual measurements to be collected during the signal peak from one particle. This allows integration of the signal from a single nanoparticle, similar to the measurement of a very short-lived chromatographic peak.

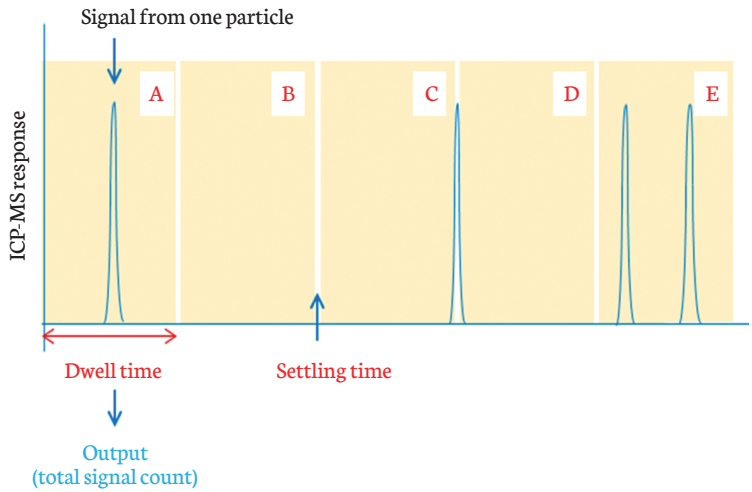

Figure 5. Dwell time is much longer than particle plume duration.

Source: Image Courtesy of Agilent Technologies Inc.

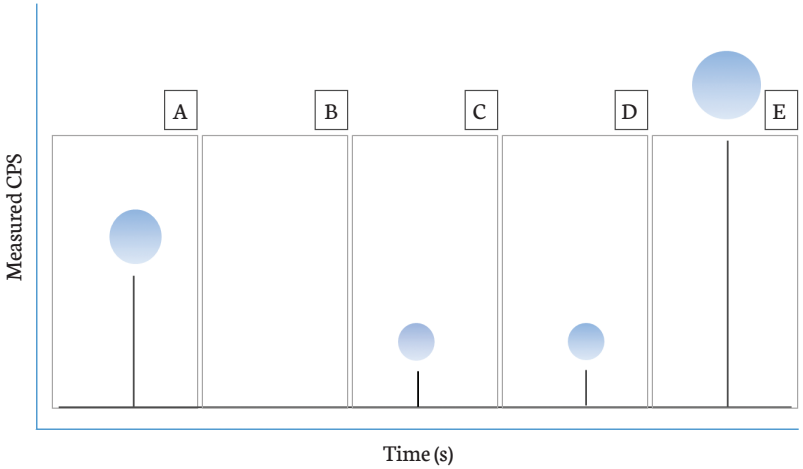

Figure 6. Resulting mass spectrum (integrated signal over each dwell time) and approximate particle sizes, for example, in Figure 5.

Source: Image Courtesy of Agilent Technologies Inc.

One of the advantages of ICP-MS over other techniques for characterizing nanoparticles is that preparing samples is typically straightforward and relatively fast. Environmental water samples usually require filtering (to remove larger particulates) and the addition of a surfactant (to ensure

nanoparticles remain suspended). Biological and organic samples can be solubilized using alkaline reagents or enzymatic digestion to remove the organic matrix, but care must be taken not to alter the number or size distribution of the nanoparticles.

Even the simplest sample preservation approach can potentially lead to nanoparticle dissolution, a process that also happens naturally after nanoparticles are released into the environment. As a result, acidification or acid digestion is not commonly used for sample preparation when analyzing nanoparticles, and internal standards are also not typically added. Finally, to ensure that samples are fully homogenized, they can be placed in an ultrasonic bath for 10 minutes before final dilution and again immediately prior to analysis.

CASE STUDY 1

When investigating the various processes by which metal nanoparticles find their way into the environment, researchers are increasingly turning to ICP-MS. In a recent study, a team of Swedish and Belgian researchers used the technique to detect and analyze platinum nanoparticles in samples of road dust.

High platinum concentrations are found in road dust as a result of their emission from catalytic converters in vehicles, which has led to high levels of platinum appearing in lakes and river sediments, transported there by rainwater runoffs. Recent research suggests that some of the platinum present in the environment appears as nanoparticles, offering a potential risk to wildlife, but little is known about how these nanoparticles form. This is in part because characterizing platinum nanoparticles in the environment is challenging, as it requires a technique that can handle a broad range of sizes at low concentrations.

In this study, a team from Chalmers University of Technology and Uppsala University in Sweden and Ghent University in Belgium used a quadrupole ICP-MS to characterize platinum in road dust samples

from Gothenburg, Sweden and Ghent, Belgium, respectively. According to the researchers, this technique provides the sensitivity required for the analysis of platinum at environmental concentrations and the fast scanning capability required for the detection of small particles in a sample stream. ICP-MS is widely used for the determination of total platinum concentrations in environmental samples, but this is the first time this technique is used for the identification of platinum nanoparticles in environmental samples.

ICP-MS revealed that the samples contained, on average, 19 ng of platinum per gram of dust, similar to concentrations found in other medium-sized cities around the world. Next, the team placed the dust samples in stormwater (to simulate real-world weather conditions) and used spICP-MS to show that the resultant leachate contained both soluble, ionic platinum and platinum nanoparticles, with the nanoparticles varying in size between 9 and 21 nm.

The team was able to show that only around 3% (on average) of the platinum in the samples became nanoparticles within the leachate. However, the researchers reported that this is still significant because the soluble and nanoparticulate platinum leachate is most susceptible to uptake by biological systems and so is the most relevant in terms of bioavailability.

The form in which platinum occurs in the environment is key to understanding emission mechanisms and environmental pathways, as well as assessing the potential risks of the contamination caused by catalytic converters.

The researchers also found that larger platinum nanoparticles (18 nm) leached from road dust collected in Gothenburg than from road dust collected in Ghent (10 nm). According to them, this is possibly because fewer cars have diesel engines in Sweden compared with those in Belgium.

Folens, K., Van Acker, T., Bolea-Fernandez, E. et al. (2018). Identification of platinum nanoparticles in road dust leachate by single particle inductively coupled plasma-mass spectrometry. Science of the Total Environment *615: 849–856. doi: https://doi.org/10.1016/j.scitotenv.2017.09.285.*

CASE STUDY 2

Researchers in the United States have, for the first time, used ICP-MS to characterize metal-containing nanoparticles in asphaltenes, a group of complex molecules found in crude oil that can cause problems for oil production and refining. This work demonstrates according to the researchers, how ICP-MS could be used for routinely characterizing metals in crude oils and petroleum-derived materials.

Metals are well known for accumulating in the heavy fractions of crude oil, particularly in asphaltenes, where they can poison refining catalysts and promote corrosion. Asphaltenes can also precipitate from crude oil during production, accumulating on internal surfaces of equipment and passing into fuel products. So understanding the origin and nature of trace metals in crude oils and bitumens is of substantial practical interest to the petroleum industry.

Among the metals present in crude oils, vanadium and nickel are found in the largest concentrations and can become incorporated into asphaltene aggregates. Iron and molybdenum also naturally occur in crude oils in trace amounts, but little is known about the forms they take.

Researchers led by Estrella Rogel of Chevron Energy Technology Company in Richmond, CA, used spICP-MS to investigate the nature of vanadium, nickel, iron, and molybdenum in three samples of asphaltenes.

This revealed that nickel and vanadium form metal complexes such as porphyrins rather than nanoparticles, whereas molybdenum and iron can both become incorporated as nanoparticles.

By considering the relative concentrations of these metals in the nanoparticles, the team found that most molybdenum is actually present as soluble compounds in the organic medium, while iron predominantly forms nanoparticles. They also discovered that the distributions of molybdenum nanoparticles were very similar in all three samples, whereas the size of the iron nanoparticles increased as the total iron concentration increased.

Knowing the relative sizes of the metal-containing entities and their concentrations is important for designing new refining catalysts and for understanding the geochemical origin of petroleum.

Nelson, J., Yamanaka, M., Lopez-Linares, F. et al. (2017). Characterization of dissolved metals and metallic nanoparticles in asphaltene solutions by single-particle inductively coupled plasma mass spectrometry. Energy & Fuels *31: 11971–11976. doi: https://doi.org/10.1021/acs.energyfuels.7b02380.*

PROBLEMS AND SOLUTIONS

Single Particle ICP-MS (spICP-MS)

Detecting very small nanoparticles (<20 nm) can be challenging for spICP-MS because such a small particle mass generates a very low signal. The ICP-MS signal depends on the number of atoms present in the individual nanoparticle, and the number of atoms is proportional to the mass of the nanoparticle, which decreases as the cube of the diameter. The cube relationship between diameter and mass means that a 15 nm particle will generate only 1/64 the signal of a 60 nm particle. The challenge for an accurate spICP-MS analysis of smaller nanoparticles is that the ICP-MS instrument must have a very high sensitivity that can obtain a very low background signal.

For single particle analysis, the background signal is composed of instrument noise, spectral interferences, and the dissolved ion concentration. For example, when titanium dioxide nanoparticles are measured in a water sample, the sample may also contain traces of titanium dissolved in the water matrix. Instrumental design and optimization can minimize instrument noise and spectral interferences, with ICP-QQQ being particularly effective (see below). But this still leaves the dissolved element contribution, which must be identified and corrected for to allow the signal (and thereby mass) of the particles to be determined accurately. In many applications, determining the concentration of dissolved ionic metals in the sample can allow greater insight into the dynamics of the nanoparticles in the system under investigation.

Separation Techniques

The combination of FFF and ICP-MS can provide information on chemical composition for a range of nanoparticle sizes and compositions, reveal element distributions and associations in different nanoparticles, and provide atomic ratio distributions for elements. Because FFF delivers the separated particles to the ICP at a flow rate and resolution that allows for multi-element screening, this combination is ideal for characterizing samples where the elemental composition of the particles is not known in advance. FFF-ICP-MS also boasts good sensitivity for small particles when the concentration is high enough.

However, FFF-ICP-MS does present certain challenges. There can be problems with method development, as standard reference materials may not be readily available for nanoparticles in real-world samples. What is more, run times are relatively long and it is not possible to directly determine ionic (dissolved) concentrations.

Coupling ICP-MS with CE also comes with advantages and disadvantages. CE has relatively short analysis times, suffers from minimal matrix interferences, can handle multiple elements, and may provide ionic concentration information. But sample injection volume is limited, which may constrain sensitivity. CE is also not as well characterized as the other separation techniques and only works with charged particles.

Physically coupling CE with ICP-MS also requires the addition of a precise flow rate of a makeup solution, which is added to the sample flow between the CE and the ICP-MS. At the same time, an effective electrical contact must be maintained in order to provide stable and reproducible electrophoretic separations.

Interference

One of the main challenges involved in measuring nanoparticles accurately by conventional single quadrupole ICP-MS is distinguishing the signal generated by a small particle from the background. Elements such as silver and gold are relatively easy to measure by ICP-MS as they have high sensitivity, do not usually occur in natural samples, and are not typically affected by interference from spectral overlap. However, many natural and ENPs are based on elements such as iron, sulfur, titanium, and silicon, which are much more problematic to measure by ICP-MS. For these elements, potentially intense interferences can arise from the sample matrix or plasma gases. In some cases, the sensitivity can also be affected by poor ionization efficiencies or the need to measure a minor isotope.

There are three main types of spectral interference in ICP-MS: isobaric, doubly charged, and polyatomic overlaps. Isobaric interferences occur when two different elements have an isotope that share a common atomic mass, such as calcium and argon, which both have their major isotope at mass 40. Almost all elements have an isotope that is free from

isobaric overlap, so these interferences tend to be an issue only when minor isotopes or secondary (qualifier) ions must be measured.

Doubly charged interferences occur as a result of quadrupole mass spectrometers separating ions based on m/z rather than mass. If an atom has a weakly bound second electron, it may lose two electrons as it passes through the plasma rather than the usual one electron. As a result, the doubly charged ion will appear at an apparent mass that is half its true mass. For example, doubly charged barium-138 will have a m/z of 138/2, causing it to appear in the mass spectrum at m/z 69, where it overlaps with the preferred isotope of gallium. Doubly charged interferences can be a problem in some specific cases, but most elements do not form doubly charged ions at a significant level. Also, in natural samples, the major elements are mostly lighter elements (below mass 40), and so their doubly charged ions do not overlap with critical trace analytes at higher masses.

Polyatomic interferences occur when atomic ions are incompletely dissociated (such as refractory oxides) or when they recombine while passing through the interface into the mass spectrometer. These effects can produce a molecular ion with a near-identical mass to a target element. Common sources of polyatomic ions are argon from the plasma gas, elements contained in the sample solvent (oxygen, hydrogen, nitrogen, carbon, etc.), and matrix ions or other analyte ions from the sample.

For example, silicon dioxide (SiO_2; silica) nanoparticles in the form of synthetic amorphous silica (SAS) are found in a variety of consumer products, including cosmetics, food additives, toothpaste, and pharmaceuticals. They act as a thickening agent in pastes and provide flow control and anticaking properties in powders. But measuring low levels of silicon by ICP-MS is not easy, as the major isotope of silicon at m/z 28 is affected by intense spectral interferences from a nitrogen polyatomic ion (two nitrogen atoms with a single positive charge – $^{14}N^{14}N^+$) and from a carbon–oxygen polyatomic ion ($^{12}C^{16}O^+$). Nitrogen polyatomic ions are derived from the air or from the addition of nitric acid, while carbon–oxygen polyatomic ions are derived from carbonate, organic solvents, or trace organic compounds in the sample.

One solution is to make use of a reaction chemistry approach in the CRC of an ICP-MS. ICP-QQQ provides tandem mass separation capability (MS/MS), where the target ion's mass is selected by an additional mass filter (Q1) positioned before the cell. This means that potential overlaps on the target ion can be eliminated more completely and more consistently, allowing ICP-QQQ to overcome even the most challenging spectral overlaps. Complex samples can contain carbon matrices that greatly increase the intensity of the $^{12}C^{16}O^{+}$ polyatomic ion overlap on $^{28}Si^{+}$, but an ICP-QQQ in MS/MS mode with hydrogen cell gas can eliminate this overlap effectively.

Sulfur is another element that is difficult to measure at low levels by conventional single quadrupole ICP-MS. This is because there are significant overlaps from polyatomic ions derived from oxygen, nitrogen, and hydrogen that affect all of the sulfur isotopes (at m/z 32, 33, and 34). Again, an ICP-QQQ method using MS/MS and oxygen cell gas can completely eliminate these interferences.

Multi-element Nanoparticle Analysis

Some nanoparticles contain more than one element, and characterizing these particles presents a challenge for analytical instruments that use a single detector – as is the case for quadrupole ICP-MS instruments. There is, however, growing interest in analyzing such multi-element particles and in detecting multiple particles of different compositions in a mixed sample.

Quadrupole ICP-MS in single particle mode can fully characterize one element in each particle or provide qualitative information (but not accurate particle sizes or particle numbers) on up to two elements. But the measurement of more than one element is severely compromised, as some of the signal is inevitably missed when the quadrupole switches between measuring the masses of the different elements.

Some newer advances, however, provide a novel approach to multi-element nanoparticle analysis. One new approach in spICP-MS involves using sophisticated data acquisition and data analysis software to collect data for up to 16 elements in a single sample, without compromising the measurement of each individual element. Multi-element data is collected from a single

sample acquisition and combined into a table that provides comprehensive information about the nanoparticles containing each of the measured elements. This saves time, as sample uptake times are reduced and there is no need to rinse out the system between each sample. Data quality is also better, as the risk of sample contamination is significantly reduced and element associations can be inferred from the multi-element size distributions.

Another approach that can provide information on multi-element particles is coupling ICP-MS with a separation technique such as FFF. Using FFF to separate the different particles into size fractions prior to ICP-MS allows the ICP-MS measurement to be performed using conventional TRA acquisition, rather than the fast TRA required for spICP-MS. Conventional TRA allows the collection of multiple masses (40 or more) during each TRA measurement, so that element associations can be clearly identified.

ICP-MS systems can utilize a different type of mass spectrometer, which can allow multiple mass signals to be detected simultaneously. Some ICP-MS instruments use time-of-flight (TOF) MS, which, unlike a scanning quadrupole spectrometer, can measure several elements simultaneously. TOF MS works by using a pulsed electric field to accelerate packets of ions into a flight tube. For a given accelerating force, the kinetic energy – and therefore velocity – of singly charged ions depends on their mass. The different velocities of the ions leads to separation of the masses, with lighter ions (and those with a higher charge) traveling faster and therefore reaching the end of the flight tube sooner. The mass of an ion can thus be determined from the length of time it takes to reach the detector, allowing construction of a mass spectrum from the ions in the original ion packet, all of which were sampled from the plasma at the same time. All isotopes of all elements can be measured and stored for the entire spectrum.

While theoretically very appealing for the measurement of multiple elements in fast time-resolved signals (such as those generated by nanoparticles passing through an ICP), the performance of current TOF MS instruments is compromised in terms of sensitivity, dynamic range, control of interferences, and matrix tolerance. Nevertheless, ICP-TOF-MS may become an interesting alternative to quadrupole ICP-MS for nanoparticle characterization.

WHAT'S NEXT?

ICP-MS is rapidly becoming the technique of choice for detecting and characterizing nanoparticles in complex samples such as food and biological tissues, providing a growing understanding of their potential benefits and risks. The main drivers for development in the field are likely to be the need to detect even smaller nanoparticles and to characterize nanoparticles containing a wider range of elements in more diverse and complex natural samples.

The size of nanoparticles is critical when it comes to their usefulness and fate. Recent research indicates that some metal nanoparticles can accumulate in biological tissues and the environment and that their toxicity may be related to their size. At the nanoscale, particles are more chemically reactive and bioactive, allowing them to cross cell membranes and easily penetrate organs and cells. Hence there is a growing need to develop and validate techniques that are sensitive enough to detect and characterize these nanoparticles.

Current detection limits for spICP-MS are around 5–20 nm for individual nanoparticles, but are lower when using ICP-MS to analyze collections of nanoparticles. Researchers are working to enhance the sensitivity of spICP-MS and to remove background interferences even more efficiently. ICP-QQQ will become an important tool for characterizing more challenging nanoparticles such as titanium dioxide, silicon dioxide, and zinc oxide, where spectral interferences can cause high background signals in single quadrupole ICP-MS.

Researchers are also trying to enhance sensitivity by determining optimal values for various ICP-MS instrument parameters. For example, a team of Hungarian researchers recently showed that the signal generated by gold and silver nanoparticles could be significantly improved by optimizing the plasma sampling depth, meaning the distance between the tip of the sampling cone and the load coil. By decreasing this depth from a standard 8–10 to 4 mm on an Agilent 7700x ICP-MS instrument, they found that the signal could be nearly doubled.

For ICP-MS to emerge from academic laboratories and become part of routine nanoparticle analyses, it must be able to combine high performance with the ability to cope with a variety of samples.

To this end, new and innovative features are being added all the time, including refinements to automated data acquisition software (see Section "Problems and Solutions"). This means researchers can study nanoparticles at very low concentrations in samples such as environmental surface waters without the need for any offline data processing. The technique also allows the effective and selective measurement of changing particle size, aggregation, and dissolution over time at low concentrations. Other new features should allow multi-element spICP-MS to become more effective in the future, as scanning quadrupoles become faster and TOF systems become more sensitive.

Such advances are leading researchers to use spICP-MS to rapidly and accurately detect and measure nanoparticles in biological fluids such as blood, both at low concentrations and in mixtures. In the future, this approach could be applied to other types of metal and metal oxide nanoparticles in a variety of complex matrices including wastewater, effluents, culture media, and other biological tissue and fluids.

Researchers are even applying spICP-MS to the analysis of single cells, producing a technique known as single cell inductively coupled plasma mass spectrometry (SC-ICP-MS). This involves embedding single cells in droplets generated by conventional nebulization and then introducing them directly into the plasma. Each cell generates an ion cloud that is detected as an individual signal peak, revealing information about the cell's elemental composition. In a recent study, a team of German researchers used SC-ICP-MS with an Agilent 8800 ICP-QQQ to assess, for the first time, the bioavailability of arsenite in individual cells.

spICP-MS is also proving useful in the semiconductor industry, where production efficiencies and increased yield are critical. It has the potential to help maintain consistent conditions during processing and fabrication of devices by detecting impurities in the manufacturing environment and in chemical reagents. Metallic contaminants on semiconductor products can impact production yields and device performance,

and spICP-MS can be used to detect, count, and size individual nanoparticles at very low concentrations.

Another way to enhance the ability of ICP-MS to cope with a range of samples is to combine it with other separation techniques, such as high-performance liquid chromatography (HPLC) and size-exclusion chromatography (SEC), to overcome the limitations of FFF and CE. The main problem with separating nanoparticles with chromatography-based separation techniques is that the nanoparticles have a tendency to become trapped on the chromatography column.

Recently, however, several research groups have found that adding surfactants to the mobile phase can prevent this happening, enhancing recovery and allowing these techniques to be combined with ICP-MS. For example, a team of Chinese researchers used this approach to combine ICP-MS with HPLC for analyzing gold nanoparticles in environmental water samples and commercial antibacterial products.

The adoption of ICP-MS for routine analysis should aid the ongoing development of a sound regulatory framework for nanomaterials. In Europe, nanomaterials are already covered by REACH, the EU's overarching legislation applicable to the manufacture, marketing, and use of chemicals, while other regulations require products to be labeled when they contain nanomaterials that are classed as hazardous.

EU regulations on consumer products such as food additives, cosmetics, and biocides have specific provisions for nanomaterials. For example, cosmetic product manufacturers must not use zinc oxide or titanium dioxide in nanoform as a UV filter at a concentration of more than 25%. However, there is a current lack of approved standard methods to determine whether products meet these regulations.

ICP-MS is already proving itself to be a vital tool for method development and validation in research labs and offers great potential to provide the information that manufacturers and regulators require to support upcoming regulations. In the future, ICP-MS is likely to become even more widely adopted for the statutory monitoring of nanoparticles in food, consumer products, and environmental samples, as well as to support research and fundamental studies.

FURTHER READING

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