ICP-MS: a promising tool in the detection and analysis of nanoparticles

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Introduction

In October 2011 the European Commission published its recommendation on a common definition of the term "nanomaterial" for regulatory purposes. According to this recommendation, a nanomaterial is a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external diameters is in the size range 1 nm – 100 nm. In this definition, a particle, aggregate and agglomerate are defined as follows:
1)   "particle" means a minute piece of matter with defined physical boundaries
2)   "aggregate" means a particle comprising of strongly bound or fused particles
3)   "agglomerate" means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%. Furthermore, fullerenes, grapheme flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

This definition was developed specifically for use in the regulatory field. Hence, through measurements it should be verified whether a material meets the definition of a nanomaterial. Especially the type of the constituent particles (their chemical composition), their external size and the median value of the particle size distribution have to be determined. Methods for measuring the size and/or number of nanoparticles can be grouped as follows:
1)   "ensemble methods" like dynamic light scattering (DLS), measure large numbers of particles simultaneously and report intensity-weighted particle sizes
2)   "counting methods" like particle tracking analysis (PTA), electron microscopy (EM) and atomic force microscopy (AFM), study particle by particle
3)   "fractionation methods" like field-flow fractionation (FFF), hydrodynamic chromatography (HDC) and centrifugal liquid sedimentation (CLS), separate samples into monodisperse fractions prior to quantifying the particles. Fractionation methods often have to be coupled to a suitable detector system.

There is currently not a single technique able to measure satisfactorily and routinely the chemical composition, the size and the particle size distribution simultaneously. Furthermore, most of the available techniques are inadequate for the study of nanoparticles in complex systems such as food. One particular challenge is distinguishing nanoparticles from other constituents of the matrix such as carbon-rich substances and debris. Another problem is that method detection limits are for many techniques higher than the expected exposure concentrations.

In practice the only method that is technically capable to count and size particles in both free and agglomerated states is transmission electron microscopy. However, sample preparation artifacts, the high particle count required, and unsuitability for high-throughput applications limit the applicability of this technique for numerous routine measurements.

Because of its elemental specificity, excellent resolution and low detection limit, inductively coupled plasma – mass spectrometry (ICP-MS) is becoming a very promising detection method for inorganic nanomaterials. ICP-MS can be coupled to fractionation methods such as FFF and HDC, or used as a stand-alone technique when operated in single particle mode (SP-ICP-MS).

Field flow fractionation

Field flow fractionation is a fractionation or separation technique: it separates particles based on their hydrodynamic size. The separation process is similar to chromatography, except that the separation is based on physical forces as opposed to chemical interaction. The sample, which has to be a suspension of particles, is pumped through a narrow channel in a laminar flow, which means that the fluid in the center moves faster than the fluid at the edges of the channel (Figure 1). A 'field' is applied perpendicular to this flow, which is in most cases a second flow (flow field flow fractionation – F[4]), but this field can also be electric, magnetic, thermal, etc., which pushes the particles to the edge of the channel, the accumulation wall, where they move slower. Diffusion associated with Brownian motion tends to counteract this motion. Particles with a smaller mean hydrodynamic diameter, which have higher diffusion rates, tend to reach an equilibrium position closer to the center of the channel, where they move faster. The two effects result in a separation between big and small particles. It is possible to transform the retention time to a hydrodynamic diameter, requiring either calibration with particle size standards or theoretical calculations using the physical properties of the medium, the particles and the channel parameters. Calibration with particle size standards is only reliable if the particles that have to be measured, have the same properties as the calibration standards. Quantification of the amount of particles depends on the sort of detector used and its calibration. In the case of inorganic particles, the method can be combined on-line with ICP-MS. The resultant hyphenation of FFF-ICP-MS provides nanoparticle sizing, detection and compositional analysis capabilities at the parts per billion level, which is critical to environmental and toxicological investigations of nanomaterials.

FFF exploits a rather complex system, where interactions between particles, the carrier liquid and the channel membrane must be considered. Highlights of the FFF-ICP-MS technique are the capability to detect very small particles (circa 1 nm) over a wide size range (10- to 20-fold) with superb resolution (10 nm), and its multi-element capabilities (i.e. it is suitable for use with mixed nanoparticle systems). The size range and separation capability can be altered by varying flow rates and operation conditions. However, significant experience is required to develop methods for FFF. As a separation technique, FFF is well suited to deal with polydispersity (i.e. the presence of particles of different sizes in one sample), but it does not distinguish between primary particles, aggregates and agglomerates. These need to be broken up to obtain information on the primary particles. Presuming all particles

in the sample are of regular (spherical) shape and have equal interaction with the membrane, the interpretation of the elution profile is rather straightforward. On the other hand, particles build up at the lower channel wall in the course of an analysis, resulting in poor recoveries and limiting the number of samples that can be analysed in one run. Furthermore, FFF is prone to interference of large particles (> 1 µm), hence sample preparation is required for most samples. The preparation methods depend on both the character of the matrix and the properties of the nanoparticles, so development of suitable sample preparation techniques plays a major role in FFF analysis. With more studies being published, researchers will have a better understanding of how to minimize the problems associated with FFF, and it will only be a matter of time before FFF-ICP-MS becomes a routine analytical technique for the measurement of inorganic nanoparticles in a variety of samples.
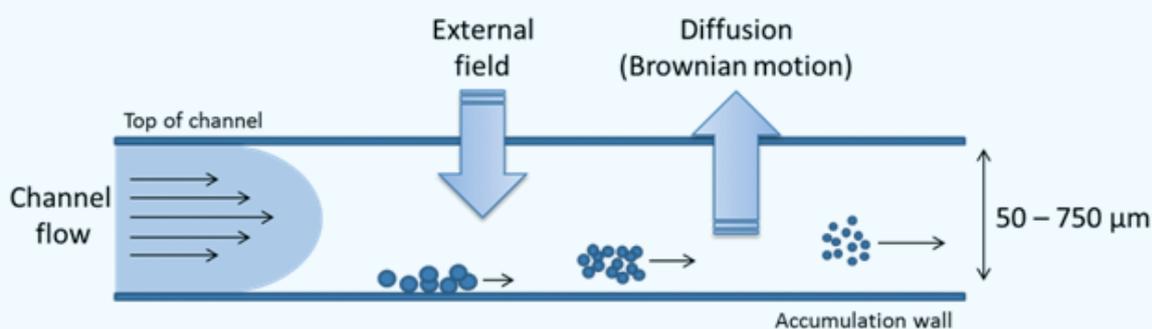

Figure 1. Schematic view of a field flow fractionation channel showing smaller particles moving closer to the center of the channel in a higher velocity zone and thus eluting before the larger ones.

Hydrodynamic chromatography

Hydrodynamic chromatography is not a sizing method as such but also a separation method. The nanoparticle suspension flows along a column packed with non-porous beads, which build up flow channels or capillaries. In the narrow conduits, larger nanoparticles are transported faster than the smaller ones, as they cannot get as close to the slow-flow regions near the non-porous beads, resulting in separation between particles according to size. Similar to FFF, the time from sample introduction to arrival at the detector can be calibrated for apparent (equivalent spherical) particle size, and coupled to ICP-MS it allows the simultaneous analysis of most of the commonly used inorganic nanoparticles in a single run.

In general, the method has rather poor separation power, i.e. unless particle sizes vary widely, they will leave the instrument as one broad 'peak'. Furthermore, currently only one column is available for HDC. On the other hand, investment costs are much lower than for FFF, sample analysis time is less than 10 minutes per sample, and separation over a wide range (5-300 nm) is possible. As hydrodynamic chromatography separates particles independent of their density, gold nanoparticles can function as universal size calibration standards. Another attractive feature is its minimum requirement for sample pretreatment.

Given the poor separation power, HDC is not suitable for measuring nanoparticles according to the definition recommendation. However, the method is useful to separate the nanoparticles in question from other material components and can therefore play an important role in the determination of nanoparticles in finalized products.

The quantitative aspects of both FFF-ICP-MS and HDC-ICP-MS are not yet fully addressed, as quantitative ionic standards are not suitable for use with FFF or the HDC column. This might be achieved using on-channel/column and post-channel/column calibration with standards of different sizes and concentrations of the nanoparticle of interest, but for many nanoparticles these standards are not yet available. Even though hyphenated methods like HDC-ICP-MS and FFF-ICP-MS are suitable for providing both metal content and size distribution information, they cannot provide particle number concentration information, neither can they distinguish between a high concentration of nanoparticles of a certain size and containing a small fraction of a given metal, from a low concentration of nanoparticles of the same size with a large fraction of the metal. To address this drawback the use of SP-ICP-MS online with HDC or FFF is currently being investigated.

Single particle ICP-MS

Single particle ICP-MS is a new technique that relies on the extremely sensitive elemental detection capability of ICP-MS. The liquid sample is transformed into an aerosol, which is then transported into a plasma of very high temperature (10000 K), where the particles are atomized and to some extent ionized. Each plume of ions enters the mass spectrometer over a period of approximately 0.5 ms. Single particle ICP-MS hence splits the total observation time into thousands of very small time windows, called "dwell times" of 10 ms and below. By injecting sufficiently diluted samples, each discrete ion plume is originating from a single particle and gives rise to a single signal peak, the intensity of which is proportional to the mass of the particle. Assuming a certain particle shape, the particle size can be calculated. Ideally the method is combined with imaging methods to gain information on the shape to assume during size calculation. As the technique measures the total mass during a time window, it cannot distinguish between particles, aggregates and agglomerates. On the other hand the intensity readings can be collected as a function of time, which makes it both a counting and a sizing technique and allows particle size distribution calculations. The technique requires little sample preparation, even for complex matrices, and little additional method development for a given matrix and/or analyte. It can differentiate the particle of interest of other incidental particles of the same size, but different composition, by its elemental specificity. The size resolution is about 10 nm. Distinguishing dissolved from nanoparticulate constituents of a given metal is another distinct advantage of SP-ICP-MS, as is the low sample analysis time (1-3 min per sample). Furthermore, as SP-ICP-MS uses a relatively standard laboratory instrument and no additional equipment, laboratories would not incur extra costs in performing this type of analysis.

However, the required dilution might change particle properties (e.g. due to dissolution). Furthermore, the technique is highly dependent on the signal-to-noise ratio of a given ICP-MS, which may significantly hinder analysis of smaller sized nanoparticles. The lowest particle sizes detectable are between 10 and 20 nm and depending on the chemical composition of the particle, which may exclude it as a viable option to be used for measurements in the regulatory field. However, efforts are underway to deconvolute smaller sized nanoparticles from background intensities.
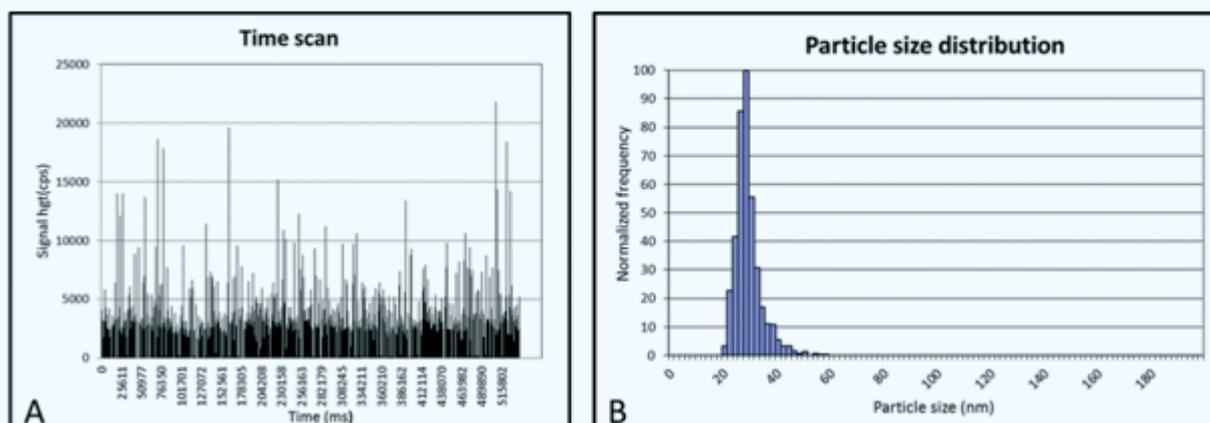
Figure 2. A) Time scan of intensity readings during single particle ICP-MS; B) Histogram of the particle size distribution after single particle ICP-MS analysis.

Conclusions

The problem of the detection and analysis of nanomaterials in complex matrices is only starting to be addressed. The analysis is challenging because appropriate and fit-for-purpose methods, i.e. suitable, robust, standardized and of reasonable cost, are not yet available. The use of ICP-MS, either hyphenated or stand-alone, provides a number of nanoparticle properties that are specifically relevant to environmental and toxicological studies and in the regulatory field, such as size, concentration and associated dissolved constituents. However, each technique has specific strengths, which prove valuable for defining a given set of nanoparticle characteristics, as well as limitations that are inherent to each technique.

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