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Nanotechnologies — Size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectrometry

Nanotechnologies - Distribution de taille et concentration de nanoparticules inorganiques en milieu aqueux par spectrométrie de masse à plasma induit en mode particule unique

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by ISO/TC 229, *Nanotechnologies*.

Introduction

This document was developed in response to the worldwide demand of suitable methods for the detection and characterization of nanoparticles in food and consumer products. Products based on nanotechnology or containing engineered nanoparticles are already in use and beginning to impact the food-associated industries and markets. As a consequence, direct and indirect consumer exposure to engineered nanoparticles (in addition to natural nanoparticles) becomes more likely. The detection of engineered nanoparticles in food, in samples from toxicology and in exposure studies therefore becomes an essential part in understanding the potential benefits, as well as the potential risks, of the application of nanoparticles.

Single particle inductively coupled plasma mass spectrometry (spICP-MS) is a method capable of detecting single nanoparticles at very low concentrations. The aqueous sample is introduced continuously into a standard ICP-MS system that is set to acquire data with a high time resolution (i.e. a short dwell time). Following nebulization, a fraction of the nanoparticles enters the plasma where they are atomized and the individual atoms ionized. For every particle atomized, a cloud of ions results. This cloud of ions is sampled by the mass spectrometer and since the ion density in this cloud is high, the signal pulse is high compared to the background (or baseline) signal if a high time resolution is used. A typical run time is 30 s to 200 s and is called a “time scan.” The mass spectrometer can be tuned to measure any specific element, but due to the high time resolution, typically only one m/z value will be monitored during a run (with the current instruments).

The number of pulses detected per second is directly proportional to the number of nanoparticles in the aqueous suspension that is being measured. To calculate concentrations, the transport efficiency has to be determined first using a reference nanoparticle. The intensity of the pulse and the pulse area are directly proportional to the mass of the measured element in a nanoparticle, and thereby to the nanoparticle’s diameter to the third power (i.e. assuming a spherical geometry for the nanoparticle). This means that for any increase of a particle’s diameter, the response will increase to the third power and therefore a proper validation of the response for each size range of each composition of nanoparticle is required. Calibration is best performed using a reference nanoparticle material; however, such materials are often not available. Therefore, calibration in this procedure is performed using ionic standard solutions of the measured element under the same analytical condition.

The data can be processed by commercially available software or it can be imported in a custom spreadsheet program to calculate the number and mass concentration, the size (the spherical equivalent diameter) and the corresponding number-based size distribution of the nanoparticles. In addition, mass concentrations of ions present in the same sample can be determined from the same data.

The interested reader can consult References [1] to [4] for further information.

Nanotechnologies — Size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectrometry

1 Scope

This document specifies a method for the detection of nanoparticles in aqueous suspensions and characterization of the particle number and particle mass concentration and the number-based size distribution using ICP-MS in a time-resolved mode to determine the mass of individual nanoparticles and ionic concentrations.

The method is applicable for the determination of the size of inorganic nanoparticles (e.g. metal and metal oxides like Au, Ag, TiO_2, BVO_4, etc.), with size ranges of 10 nm to 100 nm (and larger particles up to 1 000 nm to 2 000 nm) in aqueous suspensions. Metal compounds other than oxides (e.g. sulfides, etc.), metal composites or coated particles with a metal core can be determined if the chemical composition and density are known. Particle number concentrations that can be determined in aqueous suspensions range from 10^6 particles/L to 10^9 particles/L which corresponds to mass concentrations in the range of approximately 1 ng/L to 1 000 ng/L (for 60 nm Au particles). Actual numbers depend on the type of mass spectrometer used and the type of nanoparticle analysed.

In addition to the particle concentrations, ionic concentrations in the suspension can also be determined. Limits of detection are comparable with standard ICP-MS measurements. Note that nanoparticles with sizes smaller than the particle size detection limit of the spICP-MS method may be quantified as ionic.

The method proposed in this document is not applicable for the detection and characterization of organic or carbon-based nanoparticles like encapsulates, fullerenes and carbon nanotubes (CNT). In addition, it is not applicable for elements other than carbon and that are difficult to determine with ICP-MS. Reference [5] gives an overview of elements that can be detected and the minimum particle sizes that can be determined with spICP-MS.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-1 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

3.1

nanoparticle
nano-object with all three external dimensions in the nanoscale

[SOURCE: ISO/TS 80004-2:2015, modified]

3.2
aqueous suspension
particle suspension whose suspending phase is composed of water

3.3
inductively coupled plasma mass spectrometry
ICP-MS
analytical technique comprising a sample introduction system, an inductively coupled plasma source for ionization of the analytes, a plasma/vacuum interface and a mass spectrometer comprising an ion focusing, separation and detection system

3.4
dwell time
time during which the ICP-MS detector collects and integrates pulses

Note 1 to entry: Following integration, the total count number per dwell time is registered as one data point, expressed in counts, or counts per second.

3.5
transport efficiency
particle transport efficiency
nebulization efficiency
ratio of the number of particles or mass of solution entering the plasma to the number of particles or mass of solution aspirated to the nebulizer

3.6
particle number concentration
number of particles divided by the volume of a suspension, e.g. particles/L

3.7
particle mass concentration
total mass of the particles divided by the volume of a sample, e.g. ng/L

3.8
number-based particle size distribution
list of values that defines the relative amount by numbers of particles present according to size

4 Abbreviated terms

spICP-MS	single particle inductively coupled plasma mass spectrometry (for the definition of ICP-MS, see 3.3 or ISO/TS 80004-6:2013, 4.22)

5 Conformance

This method is restricted to aqueous suspensions of pure nanoparticles, aqueous extracts of materials or consumer products, aqueous digests of food or tissue samples, aqueous toxicological samples or environmental water samples. The applicability of the method for such samples should be evaluated by the user. Information about sample processing of non-aqueous samples can be found in the literature. Aqueous environmental samples are filtrated and diluted[6], food and toxicological samples are chemically or enzymatically digested and diluted[7][8]. However, to relate particle number or mass concentrations in aqueous suspensions to the concentrations in the original sample information on extraction, efficiency and matrix effects are required. Additional validation by the user is required.

6 Procedure

6.1 Principle

When nanoparticles are introduced into an ICP-MS system, they produce a plume of analyte ions. The plumes corresponding to individual nanoparticles can be detected as a signal spike in the mass spectrometer if a high time resolution is used. Using dwell times of ≤10 ms and an appropriate dilution of the nanoparticle suspension allows the detection of individual nanoparticles, hence the name “single particle”-ICP-MS. Dilution is often required to avoid violation of the “single particle rule” (i.e. more than one particle arriving at the detector in one dwell time). As an example, using a dwell time of 3 ms, a maximum of 20 000 particles can be registered per minute. However, to satisfy the “single particle rule”, the number of pulses in the time scan should not exceed ca. 1 200 per minute[9] (as a guidance, a suspension of 60 nm gold particles with a mass concentration of 200 ng/L at an ICP-MS input flow of 0,5 mL/min and a transport efficiency of 3 % will result in this number of pulses).

6.2 Apparatus and equipment

6.2.1 Inductively coupled plasma mass spectrometer, capable of handling dwell times ≤10 ms.

6.2.2 Vortex mixer.

6.2.3 Analytical balance, capable of weighing to the nearest 1 mg.

6.2.4 Ultrasonic bath.

6.2.5 Standard laboratory glassware.

6.3 Chemicals, reference materials and reagents

6.3.1 Chemicals

6.3.1.1 Sodium dodecyl sulfate (SDS); $C_{12}H_{25}NaO_4S$.

6.3.1.2 Sodium citrate; $C_6H_5Na_3O_7 \cdot 2H_2O$.

6.3.1.3 Nitric acid, 70 %.

6.3.1.4 Purified water, typically, water with a >18 MΩ·cm resistivity and <5 µg/L of dissolved salts.

6.3.1.5 Rinsing fluid for the ICP-MS sampling system, consisting of 3 % nitric acid prepared by diluting 40 mL of concentrated nitric acid (6.3.1.3) to 760 mL purified water in a 1 L plastic container.

6.3.2 Reference materials

6.3.2.1 For the determination of the transport efficiency, a nanoparticle reference material is used, for example a suspension of gold nanoparticles, nominal particle size 60 nm, with a nominal mass concentration of 50 mg/L stabilized in a citrate buffer. As an alternative, a suspension of silver nanoparticles, nominal particle size 60 nm stabilized in a citrate buffer can be used provided the materials are sufficiently homogeneous and stable[10]. Since the nanoparticle reference materials are used only to determine the transport efficiency, having the same chemical composition as the nanoparticle analyte is not required.

6.3.2.2 For the size determination single element, ionic standard solutions are used, namely certified reference materials intended for use as a primary calibration standard for the quantitative determination of an element.

6.3.3 Reagents

6.3.3.1 Stock standard of nominal 60 nm gold nanoparticles (50 µg/L). Pipet 50 µL of the gold nanoparticles (6.3.2.1) to 25 mL purified water in a calibrated 50 mL glass measuring flask and fill to the mark with purified water, resulting in a final mass concentration of 50 µg/L. Mix thoroughly and store at room temperature in amber glass screw necked vials or in the dark. This intermediate standard is expected to be stable at room temperature for at least two weeks. This stability shall be checked. Prior to use, place the standard in an ultrasonic bath for 10 min.

NOTE Recalculate for particle standard suspensions having different compositions or concentrations.

6.3.3.2 Working standard of nominal 60 nm gold nanoparticles (50 ng/L). Prepare the working standard by pipetting 50 µL of the stock standard (6.3.3.1) to 25 mL of purified water in a 50 mL glass measuring flask and fill to the mark with purified water resulting in a final mass concentration of 50 ng/L. Mix thoroughly and store at room temperature in amber glass screw necked vials. Although this standard is stable for several days, it is prepared daily.

6.3.3.3 Stock standards of ionic solutions of the particle's elemental composition (100 µg/L). Assuming the supplied ionic standard solution (6.3.2.2) has a concentration of 100 mg/L, pipet 50 µL of the standard to 25 mL purified water in a 50 mL glass measuring flask and fill to the mark with purified water resulting in a concentration of 100 µg/L. Mix thoroughly and store this intermediate standard in amber glass screw necked vials. Protected from light, this intermediate standard is expected to be stable at room temperature for at least two weeks. This stability shall be checked.

NOTE Recalculate for ionic standard solutions having different concentrations.

6.3.3.4 Working standards of ionic solutions of the nanoparticle analytes elemental composition (a range of 0,2 to 5,0 µg/L can be used as a starting point). According to Table 1, pipet the volumes of the stock standard (6.3.3.3) to ca. 25 mL of purified water in a 50 mL glass measuring flask and fill to the mark with purified water. Mix thoroughly. A calibration curve is constructed from the resulting working standards in Table 1. Store the working standards at room temperature in glass bottles. Protected from light, these intermediate standards are stable at room temperature for the period indicated in Table 1.

Table 1 — Volumes for the preparation of the working standards of the ionic stock solution

Volume of the stock standard (6.3.3.3) diluted to 50 mL purified water in mL	Ionic concentration of the working standard (6.3.3.4) in µg/L	Stability of the ionic working standard in glass
2,5	5,0	2 weeks
1,0	2,0	2 weeks
0,50	1,0	2 weeks
0,25	0,5	1 week
0,10	0,2	1 week

6.4 Samples

6.4.1 Amount of sample

The minimal required sample volume depends on the ICP-MS instrument used, but generally a volume of 5 mL is sufficient.

6.4.2 Sample dilution

In general, the number of pulses detected in a time scan shall not exceed a maximum number of pulses based on the dwell time (6.1). For the instrumental settings used in this procedure (6.5.1), a particle number concentration in the range of 2 × 10^6 particles/L to 2 × 10^8 particles/L results in useful measurement data. Table 2 gives the corresponding mass concentrations for different types and sizes of particles as guidance.

Table 2 — Mass concentration ranges of different types of nanoparticles at number concentrations of 2 × 10^6 particles/L to 2 × 10^8 particles/L

Particle composition	Nominal particle size (spherical equivalent diameter)		
	30 nm	60 nm	100 nm
Gold (Au)	1 ng/L to 100 ng/L	5 ng/L to 500 ng/L	20 ng/L to 2 000 ng/L
Silver (Ag) Cerium oxide (CeO_2)	0,5 ng/L to 50 ng/L	2 ng/L to 200 ng/L	10 ng/L to 1 000 ng/L
Titanium dioxide (TiO_2) Iron oxide (Fe_2O_3) Zinc oxide (ZnO)	0,2 ng/L to 20 ng/L	1 ng/L to 100 ng/L	5 ng/L to 500 ng/L

If no information on the nanoparticle concentration in a sample or aqueous suspension is available, a 10 000 times dilution is recommended as a starting point. Based on the observed number of pulses in the analysis of the diluted sample, the dilution can then be adapted. Dilutions are made in purified water or, if stabilization is required, in 5 mM sodium citrate or sodium dodecyl sulphate (SDS) in purified water.

6.5 Instrumental settings and performance check

6.5.1 Settings of the ICP-MS system

The instrument configuration for spICP-MS is not different from standard ICP-MS. Therefore, the optimal instrument settings as provided by the supplier are used.

A 3 % nitric acid solution is used to rinse sampling system, tubing, etc. of the ICP-MS before and in-between runs.

In general, dwell times in the range of 1 ms to 10 ms are compatible with most commercial ICP-MS systems and can be used, though the probability of detecting a single nanoparticle pulse split between two adjacent measurement windows increases as the dwell time is decreased. If longer dwell times (>10 ms) are used, it is more difficult to isolate the particles from the background in the data and more than one nanoparticle may be registered by the detector in one dwell time event. Shorter dwell times (<1 ms) may be used, however, the ion plume generated by the nanoparticle in the plasma (typical width, 0,1 ms to 0,3 ms) may be divided over multiple dwell time events and dedicated software is required to reconstruct and quantify the particle pulse.

In the case of low m/z values, as for Ti (48) and Fe (56), interferences by polyatomic ions such as SO and ArO may cause high background levels, rendering small particles invisible. In that case, the results may be improved by monitoring an alternative (secondary) m/z pulse for the element of interest or by using a collision/reaction cell or other technique to remove polyatomic ions. While in both cases, the absolute sensitivity for the element of interest will be lower, the signal-to-noise ratio (important for differentiating nanoparticles from the background) may be higher.

6.5.2 Checking the performance of the ICP-MS system

ICP-MS systems have a performance check and an auto tune or manual tune function. Carry out the performance check. If the criteria of the performance check are not met, perform an instrument tuning,