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

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National foreword

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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,

auto tune or manual tune, to optimize the instrument. The ICP-MS may be tuned to optimize the response for a particular m/z value.

Special attention should be paid that the sample introduction system of the ICP-MS is clean. Analysis of nanoparticle suspensions with high particle concentrations may lead to contamination of the ICP-MS instrument, especially the instrument tubing, resulting in continuous background levels. On the other hand, if high concentrations of other type of samples have passed through the tubing, this can cause adsorptions giving erroneous results when determining the transport efficiency and measuring true samples. If unsure, change the tubing of the sample introduction system. Because spICP-MS normally uses diluted samples suspensions, a set of tubing may be reserved for this method only.

6.6 Determination of the transport efficiency

Since only a part of the introduced sample reaches the plasma, knowledge of the transport efficiency is required for the calculation of results. It is determined using a known nanoparticle standard; in this method, the 60 nm gold reference particle (6.6.1). If not available, any other well-characterized nanoparticle suspension can be used; however, some dilutions and concentrations should be recalculated. If nanoparticles of known size are available but no concentration is known, an alternative method can be used (6.6.2).

6.6.1 Determination of transport efficiency based on measured particle frequency

Calculate the particle number concentration in the working standard (6.3.3.2) using Formulae (1) and (2):

$$q_{\mathrm{p}} = \frac{C_{\mathrm{p}} \eta_{\mathrm{n}} V}{60} \tag{1}$$

$$C_{\mathrm{p}} = \frac{C_{\mathrm{m}}}{m_{\mathrm{p}}} \tag{2}$$

where

C_{p} is the particle number concentration (particles/L);

C_{m} is the mass concentration of the particle suspension (g/mL);

m_{p} is the mass per particle (g).

The mass of a 60 nm gold nanoparticle is $2{,}2 \times 10^{-15}$ g and with a mass concentration of 50 ng/L; this results in a particle concentration $C_{\mathrm{p}} = 2{,}3 \times 10^{7}$ particles/L.

Analyse the working standard (6.3.3.2) using the settings according to the procedure (6.5.1) and determine the particle flux in the plasma, i.e. the number of particle pulses per second in the time scan. Calculate the transport efficiency using Formula (3):

$$\eta_{\mathrm{n}} = \frac{6 \cdot 10^{4} q_{\mathrm{p}}}{C_{\mathrm{p}} V} \times 100\ \% \tag{3}$$

where

η_n is the transport efficiency (%);

q_p is the particle flux in the plasma (particles/s);

C_p is the particle number concentration (particles/L);

V is the sample flow (mL/min);

$6 \cdot 10^4$ is the conversion factor from min to s and from mL to L.

With a standard type of nebulizer, η_n is expected to be in the order of 2 % to 5 %; however, nowadays, more efficient nebulizers are available and may be used.

6.6.2 Determination of transport efficiency based on measured particle size

If a nanoparticle standard is available of which only the size is known, the transport efficiency can be determined if a series of ionic standards (Table 1) of the same element as the nanoparticle is analysed in the same series.

Analyse the working standard of the particle suspension (6.3.3.2) and the working standards of the ionic solutions (6.3.3.4) using the settings according to the procedure (6.5.1). Using linear regression, determine the correlation coefficient of the calibration line. The correlation coefficient should be >0,99. Calculate the transport efficiency using Formula (4):

$$\eta_n = \frac{R_{ionic}}{R_{NP}} \times 100\ \% \tag{4}$$

where R_{ionic} = ICP-MS response for ions (cps/µg) and calculated as:

$$R_{ionic} = \frac{RF_{ion} \times 6 \cdot 10^7}{V \times t_d}$$

where

RF_{ion} is the ICP-MS response factor for the ion standard [cps/(µg/L)];

V is the sample flow (mL/min);

t_d is the the dwell time (ms);

$6 \cdot 10^7$ is the conversion factor from min to ms and from L to mL.

and R_{NP} = ICP-MS response for nanoparticles (cps/µg) and calculated as:

$$R_{NP} = \frac{\bar{I}_{NP}}{m_{NP}}$$

where

$\bar{I}_{NP}$ is the average nanoparticle intensity minus the background intensity measured for nanoparticles in the working standard suspension (cps);

m_{NP} is the the mass of the nanoparticle (µg).

6.7 Determination of the linearity of response

Analyse the working standards of the ionic solutions (6.3.3.4) using the settings according to the procedure (6.5.1). Determine the correlation coefficient of the calibration line using linear regression. The correlation coefficient should be ≥0,99.

6.8 Determination of the blank level

If the detection limit is defined as three times the standard deviation of the blank, then, using counting statistics, it can be shown that the number of particles observed in the measuring period should not exceed 10. Analyse three blank samples, purified water or the water used for sample dilution using the settings according to the procedure (6.5.1) and determine the number of detected particles in the measuring period. The number of observed particles in the blank should not exceed 10.

6.9 Analysis of aqueous suspension

Prepare the instrument for analysis and set up an injection list including blanks, ionic calibration standards and/or nanoparticle standards and sample suspensions. Blanks, ionic calibration standards and/or nanoparticle standards are included in the analyses sequence at the start, after every 10 samples, and at the end of the sample sequence to verify instrument performance over the course of the run. The calibration curve of the ionic standards is included only at the start of the sequence and at the end of the sequence if no more than 5 series of 10 samples are analysed. A typical sample sequence looks as follows:

1 Purified water

2 Ionic standard 0,2 µg/L

3 Ionic standard 0,5 µg/L

4 Ionic standard 1 µg/L

5 Ionic standard 2 µg/L

6 Ionic standard 5 µg/L

7 Purified water

8 Nanoparticle standard 2×10^7 particles/L (for 60 nm gold, this is ~50 ng/L; for 60 nm silver, ~25 ng/L)

9 Purified water

10 Sample 1

11 Sample 2

12 Sample 3

13 Sample 4

14 Sample 5

15 Sample 6

16 Sample 7

17 Sample 8

18 Sample 9

19 Sample 10

20 Purified water

21 Ionic standard 2 µg/L

22 Purified water

23 Nanoparticle standard 2 × 10^7 particles/L (for 60 nm gold, this is ~50 ng/L; for 60 nm silver, ~25 ng/L)

24 Purified water

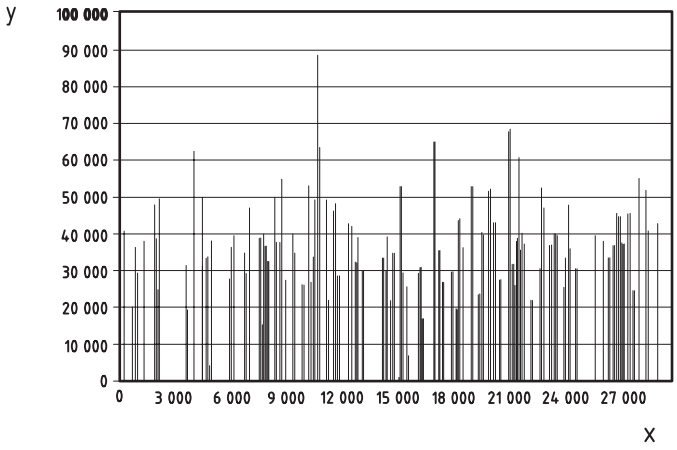
25 Sample 11

26 Etc.

If uncertain about the quality or concentration of the samples, each sample may be followed by blank purified water to check for memory effects or blank development.

6.10 Data conversion

The spICP-MS data has the form of a "time scan", an intensity signal as function of time as shown in Figure 1. Currently, a limited number of ICP-MS systems are equipped with dedicated software that can calculate nanoparticle size and concentration from spICP-MS data. However, most ICP-MS systems have the ability to convert data directly into a spreadsheet program or export data as a CSV file which can be imported into a spreadsheet program for data processing.



Key

x time/ms

y signal/cps

NOTE The number of pulses is directly proportional to the particle concentration in the sample suspension. Pulse height or area corresponds to the particle mass and spherical equivalent diameter to the third power.

Figure 1 — Time scan showing pulses for the individual particles that are detected

7 Results

7.1 Calculations

Automated calculations from a commercially available software package may be available. If not, calculations may be performed by importing the spICP-MS data and the required information in a

dedicated spreadsheet (see Annex A). Alternatively, the calculation of particle size and particle- and mass-based concentrations can be performed using the formulae in this clause.

NOTE A dedicated spreadsheet can be downloaded from the Internet[4].

7.1.1 Calculation of the transport efficiency

The transport efficiency η_n is calculated using the information on the nanoparticle working standard (6.3.3.2) and the formula presented in 6.6.1. If no suitable nanoparticle standard of known concentration is available, the transport efficiency may be determined as described in 6.6.2.

7.1.2 Calculation of the ICP-MS response

The ICP-MS response is calculated from the calibration line of the ionic working standards (6.3.3.4) using linear regression. The ICP-MS response is the slope of the calibration function expressed as cps/(µg/L).

7.1.3 Calculation of particle concentration and size

The particle number concentration in the aqueous sample is calculated as Formula (5):

$$C_p = \frac{N_p}{\eta_n} \times \frac{1\,000}{V} \tag{5}$$

where

C_p is the particle number concentration (L^{-1});

N_p is the number of particles detected in the time scan (min^{-1});

η_n is the transport efficiency;

V is the sample input flow (mL/min).

The particle mass of the individual particles in the aqueous sample is calculated as Formula (6):

$$m_p = \frac{I_p \times t_d}{RF_{ion}} \times \frac{V \times \eta_n}{60} \times \frac{M_p}{M_a} \tag{6}$$

where

m_p is the particle mass (ng);

I_p is the particle signal intensity minus baseline intensity in the sample (cps);

RF_{ion} is the ICP-MS response for ion standard [cps/(µg/L)];

t_d is the dwell time (s);

V is the sample flow (mL/min);

η_n is the transport efficiency;

M_p is the molar mass nanoparticle material;

M_a is the molar mass analyte measured.

To calculate the particle mass concentration in the aqueous sample, the masses of all individual particles are summed in Formula (7):

$$C_{\mathrm{m}} = \frac{\sum m_{\mathrm{p}} \times 1\,000}{\eta_{\mathrm{n}} \times V \times t_{\mathrm{a}}} \tag{7}$$

where

C_{m} is the particle mass concentration (ng/L);

$\sum m_{\mathrm{p}}$ is the summed particle masses (ng) of particles detected during time scan;

t_{a} is the duration of the time scan (min);

η_{n} is the transport efficiency;

V is the sample flow (mL/min).

The particle size, expressed as the particle's diameter (and assuming a spherical particle shape), is calculated as Formula (8):

$$d_{\mathrm{p}} = \sqrt[3]{\frac{6\,m_{\mathrm{p}}}{\pi\,\rho_{\mathrm{p}}}} \times 10^{4} \tag{8}$$

where

d_{p} is the particle diameter in the sample (nm);

m_{p} is the particle mass (ng);

ρ_{p} is the particle density (g/mL).

7.1.4 Calculation of the particle concentration detection limit

The number-based concentration detection limit is determined from the number of particles in the blank control samples and calculated as Formula (9):

$$\mathrm{LOD}_{\mathrm{NP}} = \frac{\left(\bar{N}_{\mathrm{p}} + 3 \times SD_{\mathrm{p}}\right) \times 1\,000}{\eta_{\mathrm{n}} \times V \times t_{\mathrm{a}}} \tag{9}$$

where

$\mathrm{LOD}_{\mathrm{NP}}$ is the number-based concentration detection limit (particles/L);

$\bar{N}_{\mathrm{p}}$ is the average number of particle pulses observed in the blank control samples;

SD_{P} is the standard deviation of the number of particle pulses observed in the blank control samples;

η_{n} is the transport efficiency;

V is the sample flow (mL/min);

t_{a} is the duration of the time scan (min).

The mass-based concentration detection limit is calculated as Formula (10):

$$\mathrm{LOD_{MP}} = \mathrm{LOD_{NP}} \times \bar{m}_{\mathrm{p}} \tag{10}$$

where

$\mathrm{LOD_{MP}}$ is the mass-based concentration detection limit (ng/L);

$\mathrm{LOD_{NP}}$ is the number-based concentration detection limit (particles/L);

$\bar{m}_{\mathrm{p}}$ is the average particle mass (ng).

In the absence of nanoparticle contamination and memory effects, the detection limit for a typical counting process as spICP-MS is 10 pulses during the total acquisition time.

7.1.5 Calculation of the particle size detection limit

The size detection limit is determined by the signal intensity of a pulse that can just be distinguished from the background. To qualify a given intensity as a pulse, an iterative approach can be used in which the 3·SD value of all the intensity signals (background and pulses) is calculated first and added to the average[2]. Pulses having values greater than this value are considered to be due to nanoparticles and are consequently removed. This process is repeated with the remaining intensity signals until no more pulses can be differentiated.

$$I_{\mathrm{n+1}} = \bar{I}_{\mathrm{n}} + 3 \times I_{\mathrm{SD}} \tag{11}$$

where

$I_{\mathrm{n+1}}$ is the signal intensity of a pulse that can just be distinguished from the background in the n+1 iterative run;

$\bar{I}_{\mathrm{n}}$ is the average signal intensity of the data in the n iterative run;

I_{SD} is the standard deviation of the signal intensity of the data in the n iterative run.

When no more pulses can be differentiated, i.e. $I_{\mathrm{n+1}} = I_{\mathrm{n}}$, the value of $I_{\mathrm{n+1}}$ is entered as I_{p} in Formula (6) and the particle size detection limit is calculated as Formula (12):

$$\mathrm{LOD_S} = d_{\mathrm{p}} \tag{12}$$

where

$\mathrm{LOD_S}$ is the size detection limit (nm);

d_{p} is the particle size calculated according to 7.1.3.

Alternatively, $\mathrm{LOD_S}$ can be determined graphically from a frequency distribution in the dedicated spreadsheet in Annex A.

7.1.6 Calculation of ionic concentration

Apart from calculating nanoparticle size and concentration, the ionic concentration in the sample may be estimated from the continuous baseline signal generated by the ions. The ionic concentration in the aqueous sample is calculated as Formula (13):

$$C_{\text{ion}} = \frac{\bar{I}_{\text{ion}}}{RF_{\text{ion}}} \tag{13}$$

where

C_{ion} is the ionic concentration (µg/L);

$\bar{I}_{\text{ion}}$ is the average baseline intensity in the sample corrected for the background intensity in a blank sample (cps);

RF_{ion} is the ICP-MS response for ion standard [cps/(µg/L)].

If small nanoparticles are not recognized and isolated during data processing, these will be part of the baseline intensity and will be unjustly quantified as ionic material. The spreadsheet will produce a limit of detection value for the ionic concentration if the average baseline intensity is smaller than three times the standard deviation of the baseline intensity.

7.2 Performance criteria

7.2.1 Transport efficiency

The transport efficiency (6.6) should be ≥1,0 %. If the transport efficiency is <1,0 %, check the nebulizer, its position and the nebulization gas flow to increase the transport efficiency.

7.2.2 Linearity of the calibration curve

The correlation coefficient of the calibration curve (6.7) should be ≥0,99. If the correlation coefficient is <0,99, check the instrument and the ionic working standards for calibration (6.3.3.4) and repeat the calibration.

7.2.3 Blank samples

The number of particles detected in the blank samples (6.8) shall not exceed 10 particles. If the number of observed particles is >10, check the instrument, especially the tubing in the sample introduction system. Repeat the blank analysis.

7.2.4 Number of detected particles

To produce a first estimate of an otherwise unknown size distribution, a minimum of 100 pulses should be detected. Depending on the polydispersity of this first estimate, (many) more particles need to be measured. Guidelines for estimating the required number of particles are found in ISO 14488. In general, the maximum number of detected particles in the time scan of a nanoparticle standard or sample extract shall not exceed 10 % of the maximum number that can be detected. If this number is exceeded, the aqueous sample extract should be diluted and re-analysed.

8 Test report

The test report shall specify the following:

a) all information necessary for the complete identification of the sample;

b) a reference to this ISO method (i.e. ISO/TS 19590);

c) the sample preparation method used;

d) the test result(s) obtained;

e) all operating details not specified in this ISO method, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

f) any unusual features (anomalies) observed during the test;

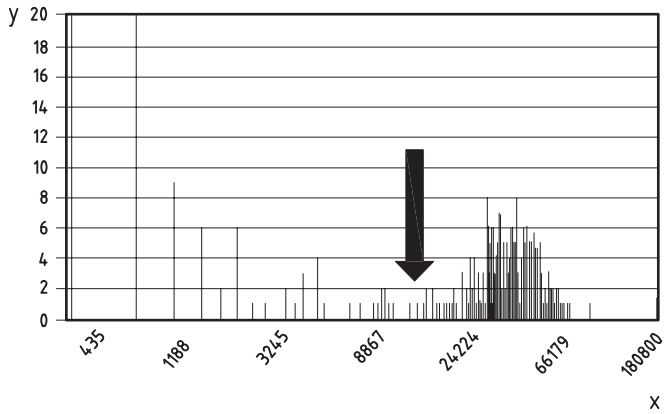
g) the date of the test.

Annex A
(informative)

Calculation spreadsheet

A.1 General

Calculations are performed by importing the spICP-MS data and the required information in a dedicated spreadsheet. Such a spreadsheet and the procedure on how to use it have been published and can be downloaded[4][11]. The spICP-MS data has the form of a "time scan" (Figure 1, signal height as function of time) and is imported from the spreadsheet where it is converted into a "signal distribution" (Figure A.1, frequency as function of signal height) to differentiate the particles from the background (instrument noise and ions). The ICP-MS response that separates the particles from the background is found as a minimum in the graph (see Figure A.1). This minimum is calculated by the spreadsheet, or alternatively, visually determined from the signal distribution graph and entered in the spreadsheet as the "limit for particle detection".



Key

x signal/cps

y frequency

NOTE ICP-MS responses left from the minimum correspond with background signals and ions, those right from the minimum correspond with particles.

Figure A.1 — Signal distribution graph plotting the frequency of the ICP-MS response in data points as a function of the ICP-MS response

The calculation spreadsheet consists of a "Calibration" and a "Sample" worksheet. The "Calibration" worksheet requires the following information:

— administrative data (dates, file names and operator);

— the converted ICP-MS data of the nanoparticle working standard (6.3.3.2) to determine the transport efficiency (use the "paste special" "values" option to enter these data);

— the instrument settings (sample inlet flow and dwell time);

— the composition of the nanoparticle working standard to determine the transport efficiency (element, element density, particle mass concentration, particle diameter, limit for particle detection);

— the ICP-MS response of the ionic working standards (6.3.3.4) for calibration (element and ICP-MS response from linear regression).

The "Sample" worksheet requires the following information:

— administrative data (dates, file names and operator);

— the converted ICP-MS data of the sample (use the "paste special" "values" option to enter these data);

— the composition information on the target particle (particle composition, molar mass ratio and density);

— the calibration data (limit for particle detection, other information is collected from the "Calibration" worksheet).

A.2 Calculation of the transport efficiency

The transport efficiency η_n is calculated in the "Calibration" worksheet using the entered information on the nanoparticle working standard (6.3.3.2) and the formula presented in 6.6.1. Alternatively, the transport efficiency may be determined using the method described in 6.6.2 and entered directly in the "Calibration" worksheet.

A.3 Calculation of the ICP-MS response

Calculate the ICP-MS response from the calibration line of the ionic working standards (6.3.3.4) using linear regression. The ICP-MS response is the slope of the calibration function expressed as cps/(µg/L) and is entered in the "Calibration" worksheet.

A.4 Calculation of particle concentration and size

Following the input of the required data in the "Calibration" and "Sample" worksheets, the particle concentration and size are calculated and presented in the "Results" section and the "Particle size distribution" graph in the "Sample" worksheet. The formulae presented in 7.1.3 are used for these calculations in the spreadsheet. The "Results" section gives the mean particle diameter while the diameters of the individual particles are plotted in the "Particle size distribution" graph.

A.5 Calculation of ionic concentration

In addition to the particle concentration and size, the ionic element concentration can be calculated from the baseline intensity in the time scan in the "Sample" worksheet. The formula presented in 7.1.4 is used for that calculation in the spreadsheet. The spreadsheet will produce a limit of detection value for the ionic concentration if the average baseline intensity is smaller than three times the standard deviation in the baseline intensity.

NOTE Particles with particle sizes below the size detection limit cannot be differentiated from ions.

Figure A.2 — Calculation spreadsheet — Calibration worksheet

Calibration File					
ADMINISTRATION			**INSTRUMENT SETTINGS AND CALIBRATION DATA**		
Date	A		**Instrument settings**		
Project	B		ICPMS sample inlet flow	0,5	ml/min
Particle standard	C		ICPMS dwell time	3	ms
Data file	D				
Ion standard	E		**Properties of target particle**		
Data file	F		Elemental composition of target particle	Au	
Operator	G		Molar mass particle/molar mass analyte	1	
			Particle density	19,3	g/ml
			Calibration data		
			Transport efficiency	0,024	
			ICPMS response ion standard	10 500	cps/(µg/l)
			Limit for particle detection	15 000	cps
			Number of particles detected	275	
			Results		
			Particle concentration (number-based)	2,3E+07	particles/l
			Particle concentration (mass-based)	50	ng/l
			Particle size	60	nm
			Ionic concentration	<100	ng/l

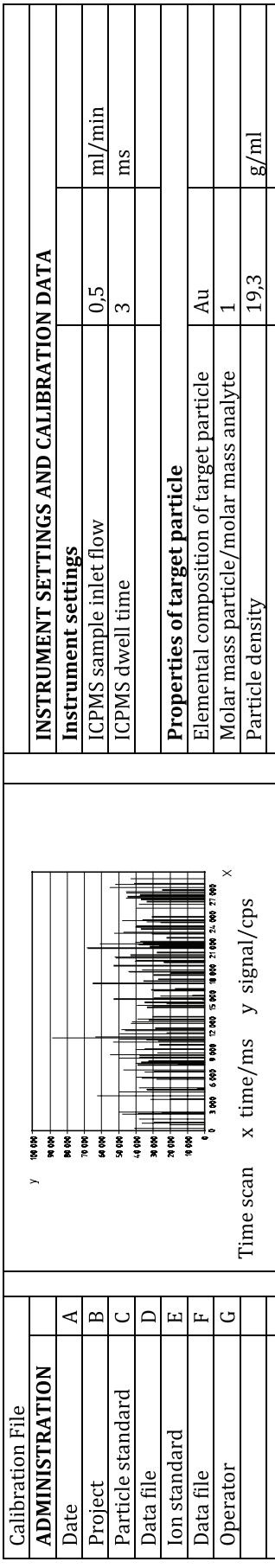


Figure A.3 — Calculation spreadsheet — Sample worksheet

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