Technical Specification

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Nanotechnologies — Characterization of nano-objects using single particle inductively coupled plasma mass spectrometry

Nanotechnologies — Caractérisation des nano-objets par spectrométrie de masse à plasma induit en mode particule unique

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 352, *Nanotechnologies,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO/TS 19590:2017), which has been technically revised.

The main changes are as follows:

— general restructuring;

— expansion of text on the test method;

— inclusion of considerations regarding method precision and measurement uncertainty;

— updates to normative and bibliographical references.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Following the introduction of single particle inductively coupled plasma mass spectrometry (spICP-MS) by Degueldre in 2003,[1] the technique has increasingly been used for nano-object characterization due to its high sensitivity, elemental specificity, the fact that often minimal sample preparation is needed and the development of much improved instrumentation, along with user-friendly data analysis software.

In spICP-MS, a very diluted suspension containing nano-objects is introduced continuously into an ICP-MS system with the intent that the ion cloud from one particle at a time arrives at the detector, set to acquire data with a high time resolution (i.e. dwell time). Following the nebulization, a fraction of the nano-objects enter the plasma where they are atomized, and the individual atoms ionized. Every atomized particle results in a cloud of ions which is then sampled by the mass spectrometer. The mass spectrometer can be tuned to measure any specific element. Typically, only one mass-to-charge value per single particle will be monitored with a quadrupole-based MS instrumentation. However, the technique can also be used with time-of-flight (TOF) mass spectrometers, allowing simultaneous multi-element and multi-isotope detection.

The number of events detected in each run (time scan) is directly proportional to the number of nano-objects in the suspension introduced but necessitates calibration of the sample transport efficiency to calculate the particle number concentration. Several available approaches to measure the transport efficiency are described in detail in this document. The intensity of the measured signal is directly proportional to the mass of the measured element in the nano-object, which can be derived following appropriate calibration of the instrument's response factor, also described in this document. For particles of known geometry, composition and density, the mass can be related to particle size. Most of the currently available, commercial data analysis software assumes spherical geometry; particle diameter is proportional to the cubic root of the mass of element(s) in a spherical nano-object. In addition to nano-object characterization with spICP-MS, mass concentrations of dissolved element present in the same sample can also be determined from the same data, if a good separation between the dissolved and particulate fraction is achieved. This represents one of the key advantages of the technique.

spICP-MS was once predominantly the domain of specialist laboratories, but with recent developments in commercially available hardware and software, the technique is now more commonly used and increasingly popular for high-throughput analysis as well as high accuracy reference measurements.

Further information on spICP-MS can be found in ISO/TS 24672, and References [1], [2], [3], [4] and [5].

Nanotechnologies — Characterization of nano-objects using single particle inductively coupled plasma mass spectrometry

1 Scope

This document specifies parameters, conditions and considerations for the reliable detection, characterization and quantification of nano-objects in aqueous suspension by spICP-MS.

Particle number concentration, particle mass, particle mass concentration, particle spherical equivalent diameter, and number-based size distribution are considered the main measurands, but the technique also allows for determination of the dissolved element mass fraction in the sample. This document provides general guidelines and procedures related to spICP-MS application, and specifies minimal reporting requirements.

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 18115-1, *Surface chemical analysis — Vocabulary — Part 1: General terms and terms used in spectroscopy*

ISO/TS 80004-6, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

ISO/TS 80004-8, *Nanotechnologies — Vocabulary — Part 8: Nanomanufacturing processes*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-6, ISO/TS 80004-8, ISO 18115-1 and the following apply.

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
nano-object
discrete piece of material with one, two or three external dimensions in the nanoscale

[SOURCE: ISO 80004-1:2023, 3.1.5]

3.2
nanoscale
length range approximately from 1 nm to 100 nm

[SOURCE: ISO 80004-1:2023, 3.1.1]

3.3
particle
minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: This general particle definition also applies to nano-objects.

[SOURCE: ISO 80004-1:2023, 3.2.1]

3.4
nanoparticle
NP
nano-object with all external dimensions in the nanoscale

Note 1 to entry: If the dimensions differ significantly (typically by more than three times), terms such as "nanofibre" or "nanoplate" are preferable to the term nanoparticle.

[SOURCE: ISO 80004-1:2023, 3.3.4]

3.5
agglomerate
collection of weakly or medium strongly bound particles where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces, for example, van der Waals forces or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO 26824:2022, 3.1.2]

3.6
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces, for example, covalent or ionic bonds, or those resulting from sintering or complex physical entanglement.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO 26824:2022, 3.1.3, modified — Note 1 to entry has been adapted.]

3.7
spICP-MS
single particle inductively coupled plasma mass spectrometry
method using inductively coupled plasma mass spectrometry whereby a dilute suspension of nano-objects is analyzed, and the ICP-MS signals collected at high-time resolution, allowing particle-by-particle element detection at specific mass peaks and number concentration, size and size distribution to be determined

3.8
dwell time
time during which the ICP-MS detector accumulates signal corresponding to an individual reading along the time scan

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

3.9
transport efficiency
ratio of detected particle events to particles introduced

Note 1 to entry: Depending on the solvent and analyte combination used, transport efficiency can be considered equal to nebulization efficiency.

3.10
nebulization efficiency
ratio of the amount of nebulized sample reaching the plasma to the amount of the sample introduced

Note 1 to entry: It is often used interchangeable with "transport efficiency".

3.11
time scan
total acquisition time
duration of one replicate measurement

Note 1 to entry: This is typically set as 1 min, but can be extended to few minutes in order to increase the number of registered particle events.

3.12
event
signal intensity registered by mass spectrometer caused by the ion cloud from a single particle, aggregate or agglomerate

3.13
BED
background equivalent diameter
spherical equivalent diameter of the smallest particle that can be detected with spICP-MS

Note 1 to entry: Assuming spherical geometry, for particles of known chemical composition and density, the corresponding background equivalent diameter can be calculated (see 5.3) from the mass of the smallest particle that can be detected with spICP-MS, which in turn is determined by the instrument sensitivity along with the background signal, for the given dwell time.

3.14
particle number concentration
number of particles in the specific mass of a suspension

Note 1 to entry: Particle number concentration is typically expressed as g^{-1} or kg^{-1}.

Note 2 to entry: It can also be expressed per volume, e.g. L^{-1}.

Note 3 to entry: To convert between units, the density of the suspension must be determined.

3.15
m/z
mass-to-charge ratio
positive absolute value of the quantity formed by dividing the mass of an ion by the unified atomic mass unit and by its charge number

[SOURCE: ISO 18115-1:2023, 20.1]

4 Abbreviated terms

For the purposes of this document, the following symbols and abbreviations apply.

BIPM CCQM	Bureau International des Poids et Mesures Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
DI	delegated institutes
DMF	dynamic mass flow
EM	electron-multiplier
ICP-MS	inductively coupled plasma mass spectrometry

ILC	interlaboratory comparison
IS	internal standardization
LOD	limit of detection
LOQ	limit of quantification
NMI	national measurement institute
PTA	particle tracking analysis
PHD	pulse-height distribution
QCM	quality control materials
Q-MS	quadrupole mass spectrometers
RM	reference materials
RTM	representative test material
SF-MS	sector-field mass spectrometers
TE	transport efficiency
TEM	transmission electron microscopy
TOF	time-of-flight
TRA	time resolved analysis
ULOQ	upper limit of quantification
ULOQsize	upper size limit of quantification
VAMAS	Versailles Project on Advanced Materials and Standards

5 Principles of operation

5.1 Introduction to spICP-MS

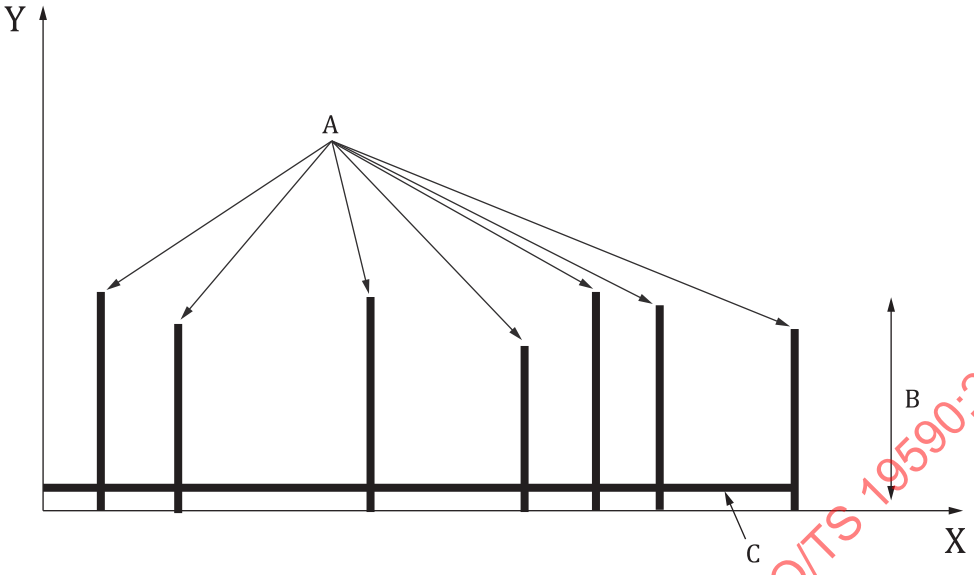
Since the introduction of spICP-MS by Degueldre in 2003,[1] the technique has increasingly gained popularity for nanoparticle analysis due to its high sensitivity, elemental specificity, often minimal sample preparation and the development of much improved instrumentation with fast, continuous data acquisition and software able to handle the large amount of data produced during spICP-MS experiments.[2]

In spICP-MS, a very dilute particle suspension is introduced into the instrument to minimize the possibility of more than one particle being detected in a single event (e.g. 2 or 3 particles). The inductively coupled plasma atomizes and ionizes the constituent analyte, generating discrete pulses of ions above the continuous background signal at a corresponding mass-to-charge ratio (m/z), lasting a few hundred microseconds. If the MS detector is set to acquire data with dwell times in the range from microsecond to low millisecond, individual or so called "single" particle events (signal intensity spikes) can be detected.

The number of events detected in each analysis window (time scan) is directly proportional to the number of nano-objects in suspension introduced into the ICP-MS, whilst the intensity of the measured signal is directly proportional to the mass of the measured element within the nano-object.

NOTE 1 Constituent particles as well as aggregates are counted as single objects, and in some cases, it can be challenging to resolve the two.

In addition, the mass concentrations of dissolved element fraction present in the sample alongside nano-objects can be determined from the same data, as illustrated in Figure 1.



Key

X time
Y event intensity, counts
A particle number-based concentration
B element mass per particle (~size)
C dissolved fraction

Figure 1 — Measurement principle of spICP-MS

The number of detected events in the time scan is related to the particle number concentration, whilst their intensity is related to the mass of element in the particles, which in turn can be converted to particle size. A dissolved element appears as constant background signal.

However, to obtain accurate particle number concentration and size values with spICP-MS, it is necessary to establish what portion of the acquired nano-objects is actually detected as particle events. This is a key parameter in spICP-MS analysis, called the transport efficiency ($\eta_{transport}$). The terms "transport efficiency" and "nebulization efficiency" are often used interchangeably and are related to sample introduction and nebulization processes in atomic spectrometry.

NOTE 2 Reference [3] suggested transport efficiency is a combination of nebulization and transmission efficiencies. In this regard, nebulization efficiency is the amount of introduced liquid actually converted into a spray and reaching the plasma (i.e., 100 % in case of a total consumption nebulizer). While the transmission efficiency is the extent of this spray effectively reaching the detector, i.e. after being desolvated, vaporized, atomized, ionized, passed into the mass spectrometer, and then collected on the ion-detector after mass separation. However, the issue of the different ionization, extraction, transmission and detection of particles versus dissolved elements can also be addressed alternatively from a calibration approach,[4] where the efficiency of these processes is included as a detection efficiency ($K_{\text{ICP-MS}}$) in Formula (1):

$$Y_{\text{R}} = K_{\text{R}}\, X^{\text{M}} = K_{\text{intro}}\, K_{\text{ICP-MS}} K_{\text{M}} X^{\text{M}} \quad (1)$$

where

K_R	is the analytical sensitivity obtained from a conventional calibration of Y_R vs. X^M (signal intensity in cps vs. element mass concentration);
K_{intro} $(= \eta Q_{sam})$	is a factor related to the sample introduction;
η	is the analyte nebulization efficiency;
Q_{sam}	is the sample introduction flow rate;
K_{ICP-MS}	is the detection efficiency, which represents the ratio of the number of ions detected versus the number of analyte atoms of the measured isotope introduced into the ICP;
K_M	is a factor related to the element measured, including the atomic abundance of the isotope considered, the Avogadro number, and the atomic mass of the element.

It is important to highlight that spICP-MS methods are not different from any other quantitative analytical methods based on calibration and the use of standards. More importantly, both particle number concentration and size can be derived by direct calibration using reference materials (RMs) characterized for particle number concentration or size, respectively.

With classic sample introduction systems (i.e. pneumatic nebulizer and cyclonic or double pass spray chamber), depending on the instrument manufacturer, the $\eta_{transport}$ is expected to be between 1 % to 15 %. However, more efficient introduction systems are now available, such as total consumption sample introduction systems, direct injection nebulizers, demountable direct injection high efficiency nebulizers and single-cell introduction systems. These can be used to reach transport efficiencies of up to 100 %. However, transport efficiency parameters should be determined for both standard and high consumption sample introduction systems, in order to ensure reliable nano-object characterization.

Two of the most popular approaches used for calculation of the transport efficiency include the particle frequency and the particle size methods. Both approaches rely on the use of nanoparticle RMs. Since the number of RMs available commercially is limited (see Table 2), recent research efforts have focused on the development of RM-free approaches to transport efficiency determination, such as dynamic mass flow (DMF) and microdroplet methods. These methods can be used to characterize RM, representative test materials (RTMs) or quality control materials (QCMs) in house for particle number concentration, amongst other techniques, such as electron microscopy which can be used to characterize particle size.

5.2 Reference material dependent calibration methods

5.2.1 Particle frequency method

In the particle frequency method, one of the following is introduced into the ICP-MS:

a) a monodisperse nanoparticle RM with known particle characteristics, such as elemental composition and density and with certified spherical equivalent diameter and elemental mass concentration (see Formula 2);

b) a particle-number concentration (see Formula 3).

The number of particles is then measured over duration of time scan.

In practical terms, for sample uptake rate in the range of (0,2 – 0,4) g min^{-1}, and RM concentration in the range of (50 000 – 200 000) g^{-1} for (25 – 100) µs dwell time or (10 000 – 30 000) g^{-1} for (3 – 10) ms dwell time, a typical particle flux into the plasma in the range of (1 000 – 3 000) min^{-1} is observed for (25 – 100) µs dwell time and (150 - 1 000) min^{-1} range for (3 – 10) ms dwell time.

Depending on the known characteristics of the RM, the transport efficiency ('$\eta_{transport}$') is then calculated from Formula (2): [5]

$$\eta_{\text{transport}} = \frac{N_{\text{NP}} \cdot d_{\text{ref}}^3 \cdot \rho_{NP} \cdot \pi \cdot 10^{-9}}{6 \cdot C_{\text{m}} \cdot Q_{\text{sam}} \cdot t_{\text{i}}} \cdot 100\,\% \tag{2}$$

where

N_{NP} is the number of events detected per time scan (a.u./no unit);

t_{i} is time scan (min);

d_{ref} is the mean spherical-volume-equivalent particle core diameter (nm);

ρ_{NP} is the particle density (g cm^{-3});

C_{m} is the elemental mass concentration of particle suspension (pg g^{-1});

Q_{sam} is the average sample uptake rate (g min^{-1}).

or Formula (3):

$$\eta_{\text{transport}} = \frac{N_{\text{NP}}}{C_{\text{NP}} \cdot Q_{\text{sam}} \cdot t_{\text{i}}} \cdot 100\,\% \tag{3}$$

where

N_{NP} is the number of particles detected per time scan (a.u./no unit);

t_{i} is time scan (min);

C_{NP} is the number concentration (g^{-1});

Q_{sam} is the average sample uptake rate (g min^{-1}).

It is important to note that the implementation of Formula (2) must include characterization of all input particle characteristics, including the particle density (ρ_{NP}). This is because, particle density has been shown to be close to bulk density only for a limited number of materials, e.g., gold. For other materials, e.g. silicon dioxide, particles can have densities ranging from below 1,9 g cm^{-3} (in the hydrated amorphous form of Stöber silica) to above 2,6 g cm^{-3} for quartz. In case of silver particles in the size range from 30 nm to 100 nm, measured density was found to be 18 % to 24 lower than nominal density of metallic silver.[6]

Because all parameters of Formula (2) and Formula (3) come with their associated uncertainty, the combined uncertainty of $\eta_{\text{transport}}$, estimated following the particle frequency method, is relatively high. It represents the main contributing factor to the overall uncertainty associated with the particle number-based concentration measurements by spICP-MS in this case (assuming particle population is well-separated from the background signal).

As an example, using 60 nm spherical gold particles (e.g. NIST RM 8013) and Formula (2), the uncertainty associated with $\eta_{\text{transport}}$ is approximately 12 % relative expanded uncertainty (see Table 8 in Reference [7]). In case of Formula (3) and materials with a given number concentration value (e.g. LGCQC5050, 30 nm colloidal gold nanoparticles), the uncertainty associated with the calculated transport efficiency will be

mostly impacted by the uncertainty associated with the C_{NP} parameter given on the certificate of analysis. For example, 19 % relative expanded uncertainty for LGCQC5050.

NOTE NIST RM 8013 and LGCQC5050 are used as examples and are possibly not available commercially.

5.2.2 Particle size method

In the particle size method, an RM suspension of particles certified for particle spherical equivalent diameter is used for the calculation of transport efficiency. Moreover, an elemental standard solution with a known mass concentration of the same element is measured. Transport efficiency is then calculated from Formula (4).

$$\eta_{\text{transport}} = \frac{R_{\text{ionic}}}{R_{\text{NP}}} \cdot 100\,\% \tag{4}$$

where

R_{ionic} is the instrument's response to ions (cps μg^{-1});

R_{NP} is the instrument's response to the particle suspension (cps μg^{-1}).

R_{ionic} and R_{NP} can be calculated as follows from Formulas (5) and (6), respectively:

$$R_{\text{ionic}} = \frac{RF_{\text{ion}} \cdot 6 \cdot 10^{7}}{Q_{\text{sam}} \cdot t_{\text{d}}} \tag{5}$$

where

RF_{ion} is the instrument's response factor to elemental standard, derived from regression analysis of the calibration curve (cps μg^{-1} kg);

t_{d} is the dwell time used (ms);

Q_{sam} is the sample uptake rate (g min^{-1}).

$$R_{\text{NP}} = \frac{I_{\text{NP}} - I_{\text{diss}}}{m_{\text{NP}}} \tag{6}$$

where

I_{NP} is the average particle intensity (cps);

I_{diss} is the average intensity of the dissolved background (cps);

m_{NP} is the mass of element in a single particle (µg).

m_{NP} can be calculated from Formula (7):

$$m_{\text{NP}} = \frac{d_{\text{ref}}^{3} \cdot \rho_{NP} \cdot \pi}{6 \cdot 10^{-15}} \tag{7}$$

where

d_{ref} is the spherical equivalent diameter of particle core (nm);

ρ_{NP} is the particle density (g cm^{-3}).

For the purpose of transport efficiency determination with the particle size method, a suspension of nanoparticle RM of known size is analyzed in combination with an ionic calibration standard of the same element of interest. The ionic calibration standard is typically prepared from commercially available ICP-MS elemental standard solution and measured using the same ICP-MS settings (e.g., dwell time, flow rate, etc.) as the nanoparticle RM. The detector response should be linear in the range of intensities measured, since larger particles can lead to detector saturation, resulting in an underestimation of the ICP-MS response and therefore overestimation of the transport efficiency.[8] As such, the following options are currently available:

a) to analyze nanoparticle RMs or RTMs of different sizes and to check the linearity of the regression line for R_{NP};

b) to analyze several ionic calibration standards of different concentrations to encompass the intensities (in cps) reached during the analysis of nanoparticle RMs or RTMs.

The particle size method is based on several assumptions, such as particle sphericity, and that dissolved and NP analyte elements have equal ionization efficiencies in the plasma, as well as equal collection efficiencies through the sampler cone into the MS. The equivalent spherical diameter of particle, chemical composition and particle density are all required input parameters into the equations that come with their associated uncertainty values, meaning that the uncertainty associated with $\eta_{\text{transport}}$ estimated following the particle size method is comparable with the particle frequency method. However, there are several literature reports highlighting differences in the mean $\eta_{\text{transport}}$ values obtained with the two approaches. Some authors demonstrate that the frequency method systematically underestimates $\eta_{\text{transport}}$ compared to the size method by a factor as large as 25 %[9], while others report much smaller differences between the two methods.[10],[11] The reasons for this are still unknown, although an explanation can be found in the differences in the transmission of atoms coming from dissolved material versus atoms coming from nanoparticles, a phenomenon dependent on both the uptake rate and the mass spectrometer behaviour.[3] Other factors, such as the impact of inadequate sample storage can also be considered.

5.3 Reference material free calibration methods

5.3.1 Dynamic mass flow method

A methodology based on the DMF approach has been developed,[12] which does not require an RM for determination of the nebulization efficiency and transport efficiency. The DMF approach is performed by continuously measuring the mass of sample uptake and the mass of sample reaching the plasma on-line over time (sample mass flow), whilst the ICP-MS system is in equilibrium. The sample nebulization efficiency value is then calculated as the ratio between the mass flow of sample reaching the plasma and the mass flow of sample uptake using the Formula (8).

$$\eta_{\text{transport}} = \frac{Mf_{\text{pl}}}{Mf_{\text{up}}} \tag{8}$$

where

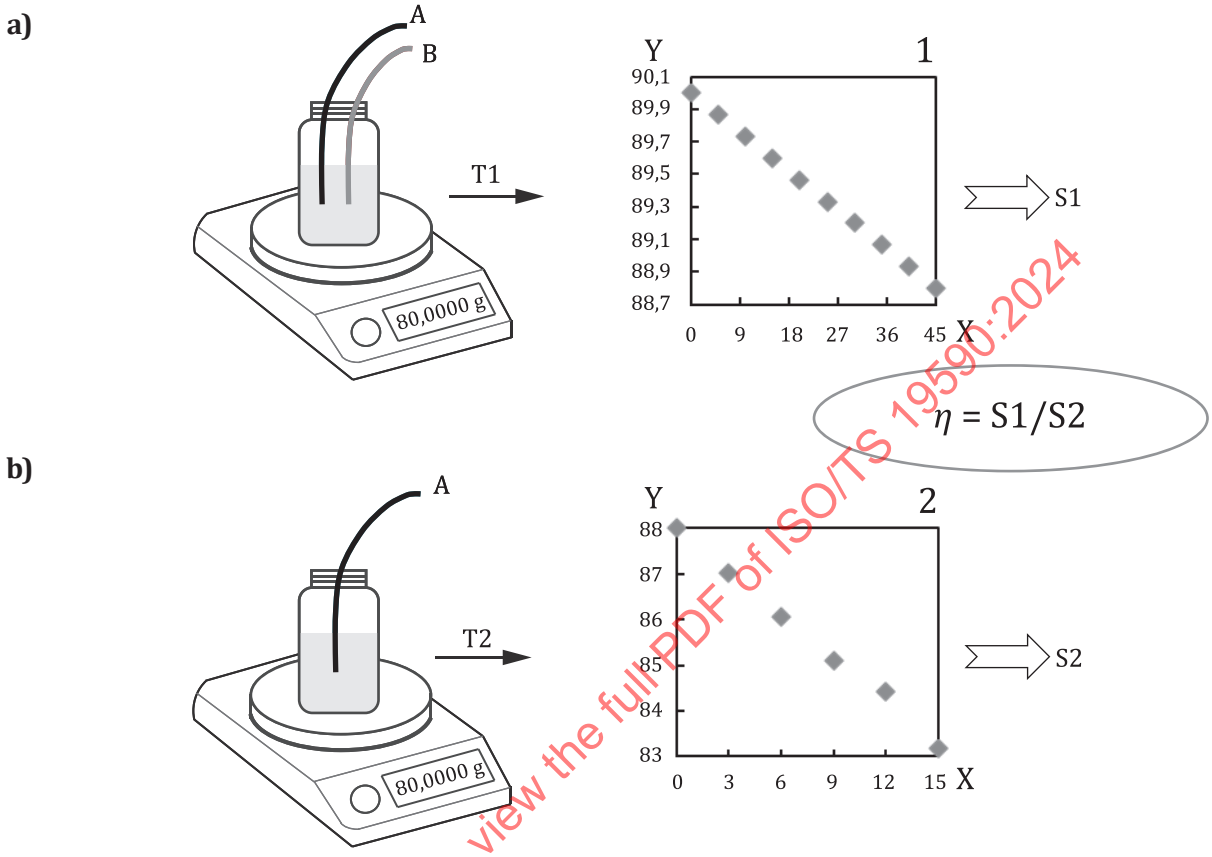
Mf_{pl} is the slope from the regression analysis representing mass flow reaching plasma (g min^{-1});

Mf_{up} is the slope from the regression analysis representing mass flow of sample uptake (g min^{-1}).

In this case, the $\eta_{\text{transport}}$ determination relies on weighing of the suspension over time, therefore its associated uncertainty has been demonstrated to be mostly based on mass measurements. These measurements can be accomplished with high accuracy and precision and a relative expanded measurement uncertainty of ~2,5 %, under the working conditions specified in the literature.[12]

So far, the precision and accuracy of the method have been demonstrated for sample introduction systems comprising double pass spray chamber cooled down to ~2 °C.[12] Under these specific conditions, the

solvent and sample nebulization efficiency has been shown to be equal to the analyte nebulization and transportation efficiency. Working with a cooled spray chamber helps to reduce the amount of water vapor (produced from evaporation of water from aerosol in the spray chamber) entering the plasma, thus minimizing the contribution of this source of error to the uncertainty of the mass-based $\eta_{transport}$.



Key

X	time (min)	1	mass flow of sample reaching the plasma
Y	mass (g)	2	mass flow of sample uptake
A	sample uptake		
B	waste tube		
S1	slope 1		
S2	slope 2		
T1	45 minutes		
T2	15 minutes		

Figure 2 — Schematic representation of transport efficiency determination using the DMF approach

This procedure is typically performed at the beginning and at the end of each analysis day. Due to possible fluctuations in the mass flow of sample uptake caused by the pump during the analysis, and to improve the accuracy of measurements, the mass flow of acquired sample should also be monitored throughout the analysis.[12]

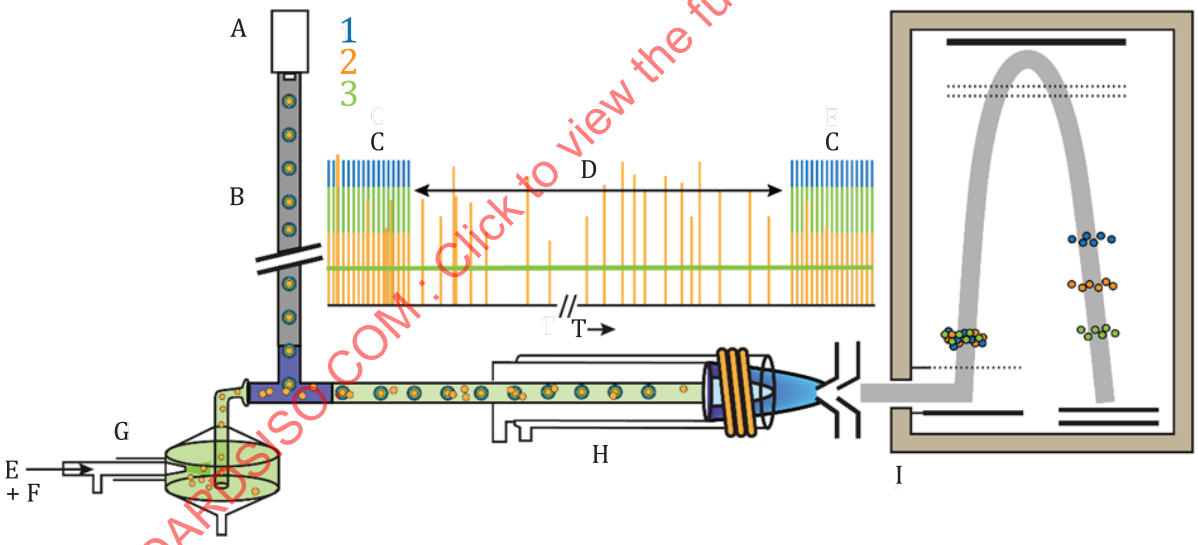
Nebulization efficiency or transport efficiency determined by DMF, implemented correctly (e.g. using double pass spray chamber cooled down to 2 °C), agrees within the associated measurement uncertainty with RM-dependent approaches.[11],[12] Such methodology, although more laborious than RM-dependent approaches due to the time required to measure the mass flows (Figure 2), shows promise for the validation of other laboratory techniques for particle number concentration, as demonstrated in an interlaboratory comparison(ILC) study organized under VAMAS TWA 34[13] and BIPM CCQM-P194.[14] So far, the applicability

of this method has been demonstrated for metal (i.e. gold and silver) and metal(loid) oxide (i.e. titania and silica) NPs.

Accurate number concentration values can be determined using an ICP interface and sample introduction system conventionally used by many ICP practitioners. This highlights the benefits of the DMF approach in assigning directly reliable number concentration values (with a combined relative expanded uncertainty of approximately 10 %, compared to approximately 25 % for the RM dependent methods[7],[12]) to RTM with physicochemical characteristics similar to particles present in commercial products, biological or environmental samples. This enables the use of such RTMs by testing laboratories to determine transport efficiency (e.g. value assignment of SiO_2 NPs in RTM for number concentration analysis of SiO_2 NPs in commercial product) using RM-dependent methods (i.e., frequency or size methods) with other ICP interfaces (e.g., total consumption) or in scenarios requiring high throughput analysis. This expands current possibilities for metrologically relevant analysis of materials for which RM similar to analyte are not available commercially and for which behaviour in the ICP-MS system is possibly not the same as that of the available RM of different chemical composition or surface chemistry. Considering production of a range of new RM covering all possible chemical compositions of nano-objects which can be found in commercial products, would involve a considerable long-term effort for RM producers worldwide.

5.3.2 Microdroplet calibration method

An alternative RM-free method based on the use of an online-microdroplet calibration strategy has been proposed.[15]-[17] In this approach, microdroplets with known elemental concentrations are introduced into the plasma along with analyte nano-object suspensions, which are introduced with a conventional pneumatic nebulizer and spray chamber. A schematic of the online-microdroplet setup is provided in Figure 3. With online-microdroplet calibration, absolute sensitivities for all elements of interest, plasma uptake rates, and spICP-MS signals are measured in one run.



Key

A microdroplet dispenser (multi-element standards in droplets)
B falling tube with He/Ar gas mix for desolvation
C microdroplet burst region
D sp-region
E NP-containing sample
F plasma-uptake standard
G pneumatic nebulizer
H inductively coupled plasma
I TOF mass spectrometer
T time
1 microdroplet signals, identification by tracer

2 analyte signals, NPs in sample and standard in droplets

3 plasma-uptake standard, spiked into sample and in droplets

[SOURCE: Reference [16], reproduced with the permission of the authors.]

Figure 3 — Schematic diagram of the online-microdroplet calibration setup

The setup is shown with ICP-TOF-MS, however, online microdroplet calibration may also be used with ICP-Q-MS instruments. Only one *m/z* can be monitored at a time.

The setup consists of a microdroplet generator used to create monodisperse microdroplets of roughly (50 – 75) µm in diameter or (65 – 220) pL in volume at a constant sample introduction frequency of ~50-100 Hz. Monodisperse microdroplets may be produced with a generator of the user's choosing. Concentration of elemental solution should be in the range of (10-300) ng g^{-1}, depending on droplet size and ICP-MS instrument used. An average signal of between 100 and 1 000 counts should be registered per analyte isotope per droplet. The average size of the monodisperse droplets is measured online with microscopic stroboscopic imaging. Droplet size combined with known multi-element concentrations, allow calculation of the exact mass of elements introduced into the plasma in each droplet. Droplets are dried in a helium-filled "falling tube"[18] and are transported with 100 % efficiency into the ICP.[19],[20] Because microdroplets are introduced concurrently with nanoparticle-containing samples, any matrix-dependent signal attenuation or enhancement will affect the analyte NPs and microdroplet calibration standards to similar extents. Additionally, a plasma uptake standard (e.g. Cs) is spiked into all nanoparticle-containing samples; this element is also in the microdroplet standard solution. By taking the ratio of sensitivities of the plasma uptake standard in the droplets versus that in the sample, the sample uptake rate (q_{plasma}) can be determined using Formulas (9), (10) and (11) and the absolute sensitivity "$S_{drop,i}$" of element *i* (counts g^{-1}) can be determined from Formula (12). The calculation for q_{plasma} is analogous to the calculation reported in Formula (4) for the particle size method, except that droplet standards are used in lieu of RMs (i.e. $S_{drop,i}$ instead of R_{NP}) and q_{plamsa} is the transport efficiency ($\eta_{transport}$) multiplied by the sample uptake rate (Q_{sam}). Additionally, in this method q_{plamsa} is recorded online for each sample.

$$S_{neb,UpStd} = \frac{\lambda_{neb,UpStd} / t_{acq}}{C_{neb,UpStd}} \quad (9)$$

$$S_{drop,UpStd} = \frac{\left(I_{drop,UpStd} - \lambda_{Neb,UpStd}\right)}{C_{drop,UpStd} \cdot V_{drop}} \quad (10)$$

$$q_{plasma} = \frac{S_{Neb,UpStd}}{S_{drop,UpStd}} \quad (11)$$

$$S_{drop,i} = \frac{\left(I_{drop,i} - \lambda_{diss,i}\right)}{C_{drop,i} \cdot V_{drop}} \quad (12)$$

For Formulas (9) to (12):

$S_{neb,UpStd}$ is the average sensitivity of uptake standard in the sample (counts s^{-1}/g mL^{-1});

$\lambda_{neb,UpStd}$ is average signal in counts of the uptake standard in sample;

t_{acq} is the acquisition time per mass spectrum or MS data point (s);

$C_{neb,UpStd}'$ is the concentration of uptake standard spiked into sample (g mL^{-1});

S_{drop}' is absolute sensitivity of the uptake standard or analyte element (*i*) from droplets (counts g^{-1});

I_{drop} is average intensity of signal from droplets in counts;

$\lambda_{diss,i}'$ is average dissolved signal for analyte, *i*, in counts;

C_{drop}' is concentration of the uptake standard or analyte element *i* in droplet solution (g mL^{-1});

V_{drop} is volume of droplets (mL);

q_{plasma} is volumetric flow rate of sample into plasma (mL s^{-1});

Compared to conventional spICP-MS calibration, online microdroplet calibration offers improved throughput achieved by the elimination of multiple calibration steps, allowing for matrix-matched calibration, and compensating for drift. Because sensitivities from the plasma-uptake standard in the sample and in the microdroplets are matrix-matched, online determination of q_{plasma} corrects for sample-introduction related matrix effects, which can correct for changing nebulization efficiency throughout an analysis. The influence of matrix on both particle mass and C_{NP} determinations is documented in References [21] to [23]. A limitation of online-microdroplet calibration is that it uses a single-point calibration (not unlike laser-ablation ICP-MS analysis) to calibrate elemental sensitivities. However, for samples with variable matrices, the benefit of matrix-matched calibration outweighs potential drawbacks from single-point calibration. To date, online microdroplet calibration has been demonstrated for accurate quantification of Ag, Au, and Pt NPs in the following:

— variable acid-concentrations at simulated ICP-MS drift conditions;[15]

— milk and fruit juice;[17]

— phosphate-buffered saline;[15], [16]

— Triton-X surfactant;[16]

— wastewater treatment plant effluent;[16]

— in organic solvents.[24]

Online microdroplet calibration is well suited to multi-elemental spICP-TOF-MS analysis. The method is also adaptable to measurement with single-channel mass analyzers; however, each measured element requires a separate run.[25]

5.4 Particle number concentration determination

spICP-MS can be used to determine number concentration C_{NP} (g^{-1}) using Formula (13).

$$C_{NP} = \frac{N_{NP}}{\eta_{transport} \cdot Q_{sam} \cdot t_i} \quad (13)$$

where

N_{NP} is the number of events detected during acquisition time t_i (a.u./no unit);

$\eta_{transport}$ is the transport efficiency (a.u./no unit);

Q_{sam} is the sample uptake mass flow (g min^{-1});

t_i is the time scan (min).

or Formula (14) in the case of the microdroplet method (see 5.3.2)

$$C_{NP} = \frac{Nf_{NP,i}}{q_{plasma} \cdot t_i} \quad (14)$$

where

$N_{NP,i}$ is the number of events detected during acquisition time for each element *i*;

q_{plasma} is the flow rate of sample into plasma (g s^{-1});

t_i is the time scan (s).

In spICP-MS analysis, the number of events registered is proportional to particle number concentration [Formula (13) and Formula (14)], provided the concentration regime guarantees the detection of one NP per reading. As such, it is possible to derive a multiple-point calibration curve by plotting the total number of events counted for one or a series of RMs against its particle number concentration over a range of concentrations (see References [26] and [27]) Thus, the particle number concentration of an unknown sample can be derived from regression analysis of such calibration curve (g^{-1}), avoiding the need for the calculation of transport efficiency.

In Formula (13) and (14), the acquisition time and the sample uptake mass flow can be measured with high accuracy. $\eta_{transport}$ is discussed in 5.2 and 5.3. The one other critical parameter that requires careful optimization is the number of events detected in time scan. Several factors should be taken under consideration, including ICP conditions, dwell time and the sample dilution factor applied (see 5.2 and 5.4), but the most critical one is the threshold selection between the background signal and the particle events. For particles whose size is close to the background, the position of the detection threshold has higher impact on the accuracy of particle counting. The most popular approach for discrimination of the particle events over the baseline is the application of n-σ criteria, where σ is the standard deviation of the baseline. Commonly, coefficients of 3 and 5 are applied, although 7σ and 8σ criteria have also been used by some authors. Depending on the criterion adopted, the significance of the number of false positives varies, however, with 5σ (or above) criterion, the number of false positives is negligible in a broad range of conditions.[28]

Particle number concentration determined with spICP-MS should be expressed in g^{-1} since mass of a substance is recognized by the SI. For this reason and to minimize the associated errors, it is recommended to prepare all necessary sample and standards dilutions gravimetrically.

5.5 Particle mass and corresponding spherical equivalent diameter determination

In contrast to particle number-based concentration, particle size cannot be determined directly using spICP-MS, despite the calibration strategy used, whether calibration with RMs, with ionic standards, DMF or microdroplets. This is because measured particle signal intensity, in spICP-MS, is converted to particle mass using a calibration curve, whilst the resultant particle mass can only be translated to particle size if particle chemical composition and density are known, since they are possibly not the same as bulk (see 5.2.1). Moreover, spICP-MS does not provide information on the exact geometry of the analyzed nano-objects and either relies on complementary microscopy techniques (e.g. transmission electron microscopy) for such information or it assumes spherical geometry and delivers output particle size as spherical equivalent diameter.

The mass of element *i* in individual particles in the aqueous sample "$m_{i,NP}$" (ng), is calculated as shown in Formula (15):

$$m_{i,NP} = \frac{I_{p,i} I \cdot t_d \cdot Q_{sam} \cdot \eta_{transport}}{RF_{ion} \cdot 60} \quad (15)$$

where

$I_{p,i}$ is the signal intensity of analyte element *i* (particle minus baseline) (cps);

t_d is the dwell time (s);

Q_{sam} is the average sample uptake rate (g min^{-1});

$\eta_{transport}$ is the transport efficiency (a.u./no unit);

RF_{ion} is the ICP-MS response to elemental standards (cps/µg kg^{-1}).

or as shown in Formula (16), in the case of microdroplet method (see 5.3.2):

$$m_{i,NP} = \left(\frac{I_{p,i} \cdot t_{acq}}{S_{drop,i}} \right) \quad (16)$$

where

$I_{p,i}$ is the same as in Formula 14;

t_{acq} is the spectral acquisition period (s);

$S_{drop,i}$ is the absolute sensitivity for element *i* from droplets (counts ng^{-1}).

The spherical equivalent particle diameter "*d*" (nm) is calculated as shown in Formula (17):

$$d = 10^4 \left(\frac{6 \cdot m_{i,NP}}{k_{i,NP} \cdot \pi \cdot \rho_{NP}} \right)^{1/3} \quad (17)$$

where

$m_{i,NP}$ is the mass of element *i* in the particle (ng);

ρ_{NP} is particle density (g cm^{-3});

Q_{sam} is the average sample uptake rate (g min^{-1});

$k_{i,NP}$ is the mass fraction of element *i* in a particle with known stoichiometry (a.u./no unit).

Likewise, to convert from mass of element *i* in a particle to mass of a particle with known stoichiometry, Formula (18) may be used:

$$m_{NP} = \frac{m_{i,NP}}{k_{i,NP} \cdot} \quad (18)$$

where

m_{NP} is the total mass of the particle (ng);

$m_{i,NP}$ is the mass of element *i* in the particle (ng);

$k_{i,NP}$ is mass fraction of element *i* in particle with known stoichiometry (a.u./no unit).

To calculate the particle mass concentration in the aqueous sample "C_m" (ng g^{-1}), the masses of all individual particles are summed in Formula (19):

$$C_{\mathrm{m}} = \frac{\sum m_{\mathrm{NP}}}{n_{\mathrm{transport}} \cdot t_{\mathrm{i}} \cdot Q_{\mathrm{sam}}} \tag{19}$$

where

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

t_{i} is the acquisition time (min);

Q_{sam} is the average sample uptake rate (g min^{-1});

$\eta_{\mathrm{transport}}$ is the transport efficiency (a.u./no unit).

For online microdroplet calibration, $q_{\mathrm{plasma}} = Q_{\mathrm{Sam}} \cdot \eta_{\mathrm{transport}}$

When a series of RMs of different sizes of the same elemental composition are available, an alternative to the calculation of transport efficiency is to derive a calibration curve by relating the third power of the diameter with the net intensities of the NP pulses (i.e. the median of the lognormal distribution of the fitted net histograms), as described in References [26] and [27]. Such calibration can then be used to derive the diameter (nm) and size distribution of an unknown sample of the same chemical composition as RMs for solid spheres or the mass of analyte (ng) per particle for heterogeneous particles. Similarly, Reference [29] reported the use of NIST RM 8013 as a single calibration standard to establish a response factor, expressed in counts per ng of Au, for the validation of spICP-MS for routine measurement of particle size and size distribution of commercial gold nanoparticle suspensions of varying size and surface charge.

Another issue to consider when performing mass calibration is potential matrix effects if the particle sample is suspended in a different diluent or matrix than the mass calibration standards.[23] This can lead to signal enhancement or reduction and often considerable biases in the estimated particle diameters. Indeed, the presence of salts or high amounts of dissolved solids typically results in signal suppression, while the presence of organic compounds on the other hand can lead to an increase in signal intensity. It is therefore recommended to check if such effects are present and if so, to use matrix-matched approaches. However, it is important to note that matrix-matching works only if the signal is enhanced and quenched by the same factor between particulates and dissolved elements/ions. When matrix matching is not possible, the use of either standard addition or internal standardization (IS) approaches, or both, have been shown to help minimize the matrix effects.[30]

As an alternative to external calibration or standard addition strategies, internal calibration (such as isotope dilution) can also be used to minimize matrix effects.[31] These strategies are associated with better accuracy and lower uncertainty, in comparison to those based on external calibration but are possibly not practical in high throughput routine work. These strategies are expensive (as they require isotopically enriched standards) and time consuming. In cases where the signal suppression is too pronounced and the low-end tail of the particle signal intensity distribution is lost, it cannot be retrieved through internal standard correction and will lead to truncated mass and size distributions. Furthermore, in case of sequential mass analyzers, due to the non-simultaneous detection, IS should be used with caution. When using IS, two consecutive pulses in the data do not relate to two subsequent time periods during the analysis, thereby preventing the application of split event correction.[32] It is important to note that such measurements can be achieved simultaneously with non-sequential detectors, i.e. TOF. Although the use of IS allows to account for signal intensity changes, it cannot be used to correct for particle number concentration fluctuations dependent on sample delivery rate and matrix composition.

In recent years, efforts have been made to derive information about particle shape from spICP-MS data, more specifically from differences in signal time profiles and transit times.[33] In case of spherical particles, the shortest and longest transit times are very similar, whereas in case of elongated shapes, such as nanorods, the shortest and longest transit times are correlated with the characteristic width and length of the particles, respectively.

5.6 Dissolved element fraction

spICP-MS is capable of measuring the dissolved fraction of the element within the same run, provided that a fair distinction between either the background or dissolved signal, or both, and the nano-object signal is achieved and that the amount of dissolved element is measurable (above typical limits of quantification). This distinction is based on the different behaviour of the analyte inside the plasma in its dissolved and particulate forms. This difference comes from the different distribution of the dissolved and particulate species among the aerosol droplets.[26] The dissolved element mass fraction in the sample can be calculated from Formula (20):

$$C_{\text{ion}} = \frac{\bar{I}_{\text{ion}}}{RF_{\text{ion}}} \tag{20}$$

where

C_{ion} is the ionic concentration (ng g^{-1});

$\bar{I}_{\text{ion}}$ 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 factor to elemental standard (cps/ng g^{-1}).

It is important to note that particles with sizes below the lower size limits of detection are detected as part of the background signal intensity and quantified as "dissolved" element mass fraction.

5.7 Multi-isotope and multi-elemental analysis

Recent developments in ICP instrumentation, such as the following:

— micro-second dwell times;

— quasi-simultaneous multi-isotopic TOF detectors allowing multi-element;

— multi-isotope detection in spICP-MS mode.

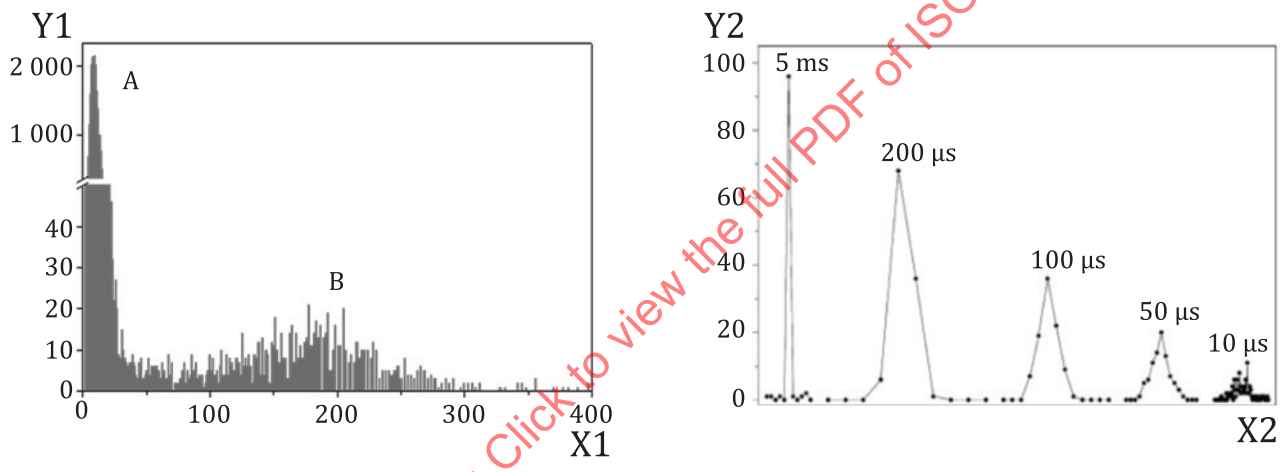
are of particular interest in the characterization of complex materials, e.g. core and shell nanocomposites, as well as multicomponent biological or environmental samples.

spICP-MS analyzes performed with quadrupole mass spectrometers (Q-MS) and sector-field mass spectrometers (SF-MS), which analyze ions of different *m/z* sequentially, allow only one or two isotopes at most to be monitored over the short transient signal produced by a nanoparticle (approximately 200 µs to 1 000 µs). For this reason, for applications requiring simultaneous monitoring of a larger number of either isotopes or elements, or both, different types of mass analyzers, such as TOF mass spectrometers (TOF-MS) are gaining popularity, despite typically having lower sensitivity than Q-MS and SF-MS instruments.[34] The fast and simultaneous detection capabilities of TOF-MS instruments enable the capture of the full atomic mass range (*m/z* 6 to *m/z* 238) at times scales appropriate for single particle analysis, although *m/z* coverage can be limited in some TOF instruments at the low *m/z*. Although spICP-TOF-MS holds great promise, it also brings its own challenges regarding thresholding, data evaluation and particle detection. The underlying principle in multi-element particle analysis is that if multiple mass-channels present coincident signals, these are assumed to stem from the same particle. However, although unlikely, it is possible that these signals originate from independent particles, that reach the plasma at the same time. Consequently, the particles will not be viewed as independent particles but instead as a multi-element particle. The probability of such events can be determined by coincidence analysis and limited using adequate dilution.[35]

To date, the capabilities of ICP-TOF-MS have been demonstrated through various case studies including: bimetallic and multi-element nanoparticle compositions,[34],[36] isotopic ratios[37] and distinction between naturally occurring and engineered nanoparticles.[38]

5.8 Data treatment

The spICP-MS data obtained with ICP-Q-MS and ICP-SF-MS instruments has the form of a "time scan", an intensity signal as a function of time and the output is a sequential chart of individual particle events superimposed above a continuous baseline, as shown in Figure 1. Raw time scans can be processed by plotting the event intensity against their frequency, obtaining histograms as shown in Figure 4 (left), where the first peak arises from either the background or the presence of dissolved element fraction, or both, and the second one represents the nano-objects population. Owing to the duration of nanoparticle events being in the range of (200 µs to 1 000 µs),[8],[39] when using dwell times in the millisecond range (3 ms to 10 ms) particle events are recorded as single points per peaks, whereas for dwell times in the microsecond range (10 µs to 200 µs), they are recorded as multiple points per peaks [Figure 4b)]. When particle events are recorded as multiple points per peaks, approaches similar to those applied to the detection of chromatographic peaks in transient signals are required for peak integration.[40],[41] Following the peak integration, further data treatment (i.e thresholding between particle population and dissolved background) is the same as in case of data obtained with millisecond dwell times. Raw data can be processed by the users, using programs and spreadsheets developed in-house, freely available on-line, or more recently, using proprietary software provided by the ICP-MS instruments manufacturers. For example, a dedicated spreadsheet for treating data acquired using milliseconds dwell times (or following integration of peaks acquired using microsecond dwell times) and TE determined using the frequency approach is available from Reference [59]. For peaks detection and integration acquired using microsecond dwell times, see Reference [60].



Key

X1 pulse intensity (counts)
Y1 number of pulses
A dissolved fraction
B NP population
X2 time
Y2 reading intensity (counts)

[SOURCE: Reference [42], reproduced with the permission of the authors.]

NOTE This figure shows the selective detection of 40 nm Ag particles from dissolved Ag (background) using spICP-MS with 0,1 ms dwell time (left) and profiles of particle events recorded at different dwell times for 50 nm gold nanoparticles (averaged total intensity per particle event: 96 counts).

Figure 4 — Selective detection of 40 nm Ag particles from dissolved Ag (background)

Unlike ICP-Q-MS and ICP-SF-MS instruments, ICP-TOF-MS instruments provide complete mass spectra at time resolutions amenable to single-particle analysis. In TOF-MS, there is no "dwell time", but rather a mass-spectral acquisition time (t_{acq}). The t_{acq} is typically between (1-3) ms in duration and the measurement is continuous, i.e. there is no "dead time" between TOF-MS spectral acquisition periods. spICP-TOF-MS data analysis requires a unique data treatment approach because of the multiplexed nature of the measurement and the signal distribution of the data. In ICP-TOF-MS, electron-multiplier (EM) based detection combined

with fast analog-to-digital conversion results in low-count data being compound-Poisson distributed,[15],[43] unlike Poisson behaviour seen in secondary electron multipliers currently used in quadrupole and double focusing spectrometers. This compound-Poisson distribution is a function of the pulse-height distribution (PHD) of the EM detector and Poisson-distributed arrival of ions. Through Monte Carlo methods and measurement of the PHD of the EM detector, compound-Poisson-based expressions for critical values are developed and can be used to threshold spICP-TOF-MS data in a manner similar to spICP-Q-MS data.

6 Method development

6.1 Sample specification

spICP-MS is compatible with aqueous suspensions of most metal and metal(loid) oxide particles and, to some extent, with other types of particles, which are capped or stained with tags visible to ICP-MS in quantities allowing detection over the background signal in the sp mode. These include, but are not limited to: silver, gold, silica, titania, lead, ceria, platinum, palladium, alumina, selenium, iron oxide, zinc oxide, copper, molybdenum and carbon particles tagged with yttrium and cobalt amongst others. With the recent advances in hardware and software available commercially, analysis of carbon-based particles is also possible, using isotope ^{13}C.[44]-[46] The accessible lower particle size limit of detection (LOD_{size}) will vary depending on the particle composition and the type of the element monitored, but also depends on the instrument set-up. However, working close to the LOD_{size} can impact both accuracy and precision of the measurements. See References [47] to [49] for an overview of the LOD_{size} possible to achieve with certain type of instrumental set-up for different materials. Overall, the lowest LOD_{size} (in the order of a few nanometers) is typically achieved for particles composed of single elements (e.g. gold) that are stable in dilute aqueous suspension (i.e. do not dissolve) and do not suffer from contribution of procedural blanks. For ICP-QQQ-MS type instruments with microseconds detection capability, the LOD_{size} for metal oxides (e.g. titania or zinc oxide) is typically in the order of 20 nm to 40 nm. For metalloid oxides (e.g. silica), the LOD_{size} is typically around 50 nm to 80 nm. Whilst for carbon based materials, it is in the micron size range.

6.2 Sample preparation

spICP-MS is considered a suitable technique for the analysis of aqueous particle suspensions. However, sample dilution is usually needed before analysis to avoid violation of the single particle rule. To minimize the occurrence of multiple events, the sample dilution factor should be carefully optimized to achieve an optimal particle flux and therefore, reduce impact of particle counting on the overall measurement uncertainty with minimal formation of double and triple events.[39]

Choosing an appropriate strategy for dispersing nano-objects in suspension depends on several factors, the most influential being the chemical nature and concentration of the particles and the matrix components. As a general rule, sample preparation should be kept as simple as possible and limited to the minimum needed to achieve stable suspension without altering the nano-objects properties. Dispersion should be carried out in purified water or with the addition of one of the following:

a) stabilizing agent (e.g., salts, buffers, surfactants or proteins);

b) pH adjustment and ultrasound assistance (e.g., bath or probe sonication).[50]

However, some materials are difficult to fully disperse, even using probe sonication, and have a tendency to sediment over relatively short time periods. It can possibly also be necessary to sonicate samples after dilution for spICP-MS, in order to obtain a well-dispersed suspension. In addition to this, for materials that are soluble, variability in analyte dissolution kinetics upon dilution should also be considered, particularly as dissolved element mass fraction is one of the measurands in spICP-MS.

If filtration steps are needed, they should be thoroughly evaluated in terms of particle recovery to ensure particle loss due to chemical affinity for the filtering membrane does not occur.

If centrifugation steps are needed, they should be thoroughly evaluated in terms of the particle recovery and potential changes in their morphology. Since particle settling time is function of its density and size, the viscosity and density of the dispersion medium and the centrifugal force applied, centrifugation step can be designed to:

i) isolate the particles in a pellet so that the unwanted low molecular matrix components remaining in the supernatant can be removed (usually requires long centrifugation time and high centrifugal force);

ii) isolate large particle agglomerates and aggregates and high molecular matrix components in the pellet, whilst keeping the desired particle fraction in the supernatant (usually requires short centrifugation time and low centrifugal force).

Table 1 provides a general overview with regards to the typically used dispersion approach for the various types of matrices and particles.

Table 1 — Typically used dispersion approaches for different types of matrices and particles.

Matrix	Particle	Typically used dispersion approach
water-soluble	hydrophilic	with eventual stabilizing and dispersing agent (see 6.2.1)
water-soluble	hydrophobic	with stabilizing and dispersing agent such as a surfactant (see 6.2.2)
fatty	hydrophilic	with extracting agent and eventual digestion step (see 6.2.2)
fatty	hydrophobic	with extracting agent and eventual digestion step, as well as stabilizing and dispersing agent in the final aqueous phase (see 6.2.2)
jellified	hydrophilic	with digestion step, as well as eventual stabilizing and dispersing agent in the final aqueous phase (see 6.2.2)
jellified	hydrophobic	with digestion step, as well as stabilizing and dispersing agent in the final aqueous phase (see 6.2.2)

6.2.1 Aqueous suspensions and paste

Aqueous suspensions and paste can be dispersed in purified water or with the addition of a stabilizing and dispersing agent, pH adjustment and ultrasound assistance. Typically used stabilizing agents include the following:

— Salts and buffers such as trisodium citrate (concentration in the mM range).

— Surfactants such as Triton X-100 and SDS (used either above or below critical micellar concentration, or both).

— Proteins such as BSA (concentration in the mg/mL range).

Some particles can require pH adjustment to remain stable in suspension. As an example, anatase phase of titanium dioxide tend to agglomerate with acidic pH. The zeta-potential diagram of the particle as function of pH can be of help in defining the acceptable pH range for a suspension.

The use of ultrasonic bath or probe sonicator can be required to initiate either particle dispersion or deagglomeration of the particles, or both. If sonication is required, the effect of sonication time and power on the particle integrity should be thoroughly studied.

6.2.2 Non-aqueous suspensions and creams

Non-aqueous suspensions and creams can be treated as described in 6.2.1 with the addition of a stabilizing and dispersing agent, extracting agent, pH adjustment, digestion step, heating and ultrasound assistance. Typically used extracting agents can include but are not limited to:

— Polar organic solvents, such as isopropanol and ethanol.

— Non-polar organic solvents, such as hexane followed by liquid-liquid extraction in aqueous phase.

— Surfactant, such as Triton X-100, Triton X-114 or SDS used in cloud point extraction (CPE) mode.

The digestion step can include:

— Alkaline digestion using tetramethyl ammonium hydroxide (TMAH) or sodium hydroxide (NaOH) assisted by heat.

— Enzymatic digestion using proteinase K, macerozyme R-10 or other enzymes.[51]

6.2.3 Powders

Powders can be treated as described in 6.2.1 with the eventual addition of a stabilizing agent, pH adjustment and ultrasound assistance.

Powders composed of pristine particulate material often need more energy for the initial dispersion. The use of a sonication probe can therefore be more useful than bath sonication in order to provide more energy. The effect of sonication time and power on the particle integrity should also be thoroughly studied in this case.

Hydrophobic particles and particles coated with a hydrophobic material (e.g. titania coated with stearic acid) can require the addition of a stabilizing and dispersing agent as described in 6.2.2.

6.2.4 Larger pieces of solids

Dispersion of solid blocks or large pieces of materials can require a combination of the approaches described in (6.2.1, 6.2.2 and 6.2.3) depending on matrix and particle types (e.g., hydrophilic, hydrophobic, water-soluble matrix, fatty matrix, jellified matrix etc.). A sonication step is usually required to ensure a good dispersion of the particles. Additionally, a grinding step can be included prior to dispersion in a liquid medium. The effect of grinding step on the particle size distribution should be thoroughly studied.

6.3 Selection of reference materials, quality control materials and representative test materials

To obtain reliable and meaningful number concentration and spherical equivalent diameter data with spICP-MS methodology following approaches to transport efficiency determination described in 6.4, selection of the RM used for calibration purposes shall be appropriate for calibration purposes. Elemental standards are readily available from multiple commercial suppliers, but the nanomaterial standards characterized for either particle number concentration or size and elemental mass fraction, or both, are very scarce. See Table 2 for selected examples of materials relevant to spICP-MS. As shown in the table, most of the nanoparticle-based QCMs and RMs available from RM/QCM producers contain information on particle size and total element concentration, with only one material characterized for nanoparticle number concentrations (LGCQC5050). Since the transport efficiency is likely to be material dependent for certain sample introduction systems used in spICP-MS, it is generally recommended to use material of the same chemical composition for calibration purposes. For this reason, the appropriate (similar to analyte) nano-objects suspension shall be sourced and characterized in-house. This shall be performed either with the RM-free approaches described in 5.3 or other orthogonal techniques available in the laboratory, before use as calibrant for the frequency or size methods, unless empirical data exist to support the use of commercially available RMs or QCMs in that specific case. Nanomaterials used as calibrants, as well as elemental standards should be dispersed or diluted in the same media or matrix as the analyte of interest if possible, in order to avoid biases arising from unwanted matrix effects. Alternatively, a calibration strategy, which compensates for such effects (e.g. isotope dilution, microdroplet method, etc.) should be followed. This is because, although samples often must be diluted to a large extent to prevent coincident particle events, the sample matrix can still interfere with the nebulization process because of differences in droplet surface tension and particle surface properties. Changes in the nebulization efficiency can lead to bias on the particle number concentration, whereas changes in the ionization affect the particle mass and the equivalent spherical diameter.

Table 2 shows examples of non-certified, nanoparticle reference and quality control materials relevant to spICP-MS, which are available commercially.

Table 2 — Examples of non-certified, nanoparticle reference and quality control materials

Product no./Producer[a]	Material type	Size (nm)	Number concentration (kg^{-1})	Mass concentration (mg kg^{-1})	Mass (mg)
RM8012/NIST[b]	gold particles	27,6 ± 2,1[c]	not given	48,17 ± 0,33	N/A
RM8013/NIST[b]	gold particles	56,0 ± 0,5[c]	not given	51,86 ± 0,64	N/A
LGCQC5050/LGC	gold particles	32,7 ± 2,0[d]	(1,47 ± 0,28) E14	45,1 ± 1,5	N/A
RM8017/NIST[e]	silver particles	74,6 ± 3,8[c]	N/A	N/A	2,162 ± 0,020

[a] The product numbers given in this table are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.
[b] Temporarily out of stock at the time of publication, replacement products are likely to have different characteristics.
[c] Size given by transmission electron microscopy (TEM).
[d] Size given by particle tracking analysis (PTA).
[e] Sold as powder.

6.4 Optimization of ICP-MS operating conditions

The ICP-MS instrument configuration needed for analysis in the single particle mode is not different from the standard set-up, meaning that the instrument's performance should be optimized for maximal signal-to-noise ratio at the particular *m/z* of interest. In case of isotopes that suffer from polyatomic interferences, signal-to-noise ratio is typically improved by using a collision reaction cell or sector-field based instrumentation. Recent developments in ICP instrumentation featuring dwell time as short as 0,05 ms, opened up the potential for multi-isotope and multi-element analysis in the sp mode. Most instrument manufacturers nowadays also offer designated software for spICP-MS analysis allowing easy processing of the acquired data. Typically, spICP-MS analyzes are performed using time resolved analysis (TRA) with dwell times in the range of 0,05 ms to 10 ms. However, it is important to note that the probability of detecting a single particle pulse split between two adjacent measurement windows increases as the dwell time is decreased from 10 ms to 1 ms. For longer dwell times, on the other hand, distinguishing particles from the background become more difficult, also the probability of registering more than one particle per dwell time increases. Dwell times below 1 ms result in the particle signal being spread over multiple data points, which significantly improves the performance of the technique, but adds complexity to data processing. Also, with dwell times of ≤100 µs, wider linear ranges have been obtained due to lower occurrence of multiple events counted as a single event. Finally, a reduced impact of background and dissolved fraction contribution on the selectivity of NP detection has been achieved when using micro-second dwell times in comparison with those in the millisecond range, resulting in smaller background equivalent diameters (BED).[39]

Special attention should be paid to the sample introduction system of the ICP-MS and the one that is the most appropriate for the type of sample being analyzed should be chosen. Choice of the sample introduction system used has implications with regards to the transport efficiency determination strategy that is compatible with it (see 5). Typical transport efficiency values obtained for commercially available interfaces, other than pneumatic nebulizer and cyclonic or double pass spray chamber combination specified in 5, especially for high efficiency sample introduction systems, typically exceed 15 %.[11][41]

All glassware and tubes shall be clean before the analysis commences, in order to avoid analyte carry over or loses.