
**Nanotechnologies — Guidance on the
measurement of nanoparticle number
concentration**

*Nanotechnologies — Conseils pour la mesure de la concentration en
nombre de nanoparticules*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

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

Nanoparticle number concentration refers to the number of nanoparticles per unit of volume or mass in a sample. It is an important measurand when analysing dispersions containing nanoparticles. Nanoparticle number concentration is also considered a useful metric for supporting materials toxicological assessments. Furthermore, the capability to accurately measure nanoparticle number concentration can help industry to increase product manufacturing quality control and implement quality assurance. Currently, in most applications, nanoparticle number concentration is estimated from indirect mass-balance considerations and validated direct techniques for this measurand are required.

This document provides an overview of commonly used methods for the measurement of nanoparticle number concentration. These are the ensemble measurement techniques of differential centrifugal sedimentation (DCS) (line start incremental disc-type centrifugal liquid sedimentation), multi-angle dynamic light scattering (MDLS), small-angle X-ray scattering (SAXS) and ultraviolet-visible spectroscopy (UV-vis) and the particle counting techniques of particle tracking analysis (PTA), resistive pulse sensing (RPS), single particle inductively coupled plasma mass spectrometry (spICP-MS), condensation particle counter (CPC), and differential mobility analysing system (DMAS).

This document focuses on the analysis of nanoparticles in suspensions (liquid dispersions) but also addresses aerosols measured using a CPC or a DMAS. Particles on surfaces or encapsulated in solid materials are not covered in this document. Nanoparticles rather than nano-objects are discussed as most techniques use the spherical approximation model to measure particle diameter which is more applicable to nanoparticles as opposed to nanofibres and nanoplates. Most of the techniques discussed can also analyse particles of size greater than the nanoscale.

This document provides guidance to help users to select the most appropriate techniques for nanoparticle number concentration measurements suitable for their applications.

Nanotechnologies — Guidance on the measurement of nanoparticle number concentration

1 Scope

This document provides an overview of the methods used to determine the nanoparticle number concentration in liquid dispersions and aerosols. The methods described are the ensemble measurement techniques of differential centrifugal sedimentation (DCS), multi-angle dynamic light scattering (MDLS), small-angle X-ray scattering (SAXS) and ultraviolet-visible spectroscopy (UV-vis) and the particle counting methods of particle tracking analysis (PTA), resistive pulse sensing (RPS), single particle inductively coupled plasma mass spectrometry (spICP-MS), condensation particle counter (CPC), and differential mobility analysing system (DMAS). This document provides information on the use of each technique, along with considerations on sample preparation, advantages and limitations.

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 80004-1, *Nanotechnologies – Vocabulary — Part 1: Core vocabulary*

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 80004-1, ISO/TS 80004-6, ISO/TS 80004-8 and the following apply.

ISO and IEC maintain terminology 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

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: A particle can move as a unit.

Note 3 to entry: This general particle definition applies to nano-objects.

[SOURCE: ISO 26824:2022, 3.1]

**3.2
nanoparticle**

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 preferred to the term nanoparticle.

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

**3.3
primary particle**

original source *particle* (3.1) of *agglomerates* (3.4) or *aggregates* (3.5), or mixtures of the two

Note 1 to entry: Constituent particles of agglomerates or aggregates at a certain actual state may be primary particles, but often the constituents are aggregates.

Note 2 to entry: Agglomerates and aggregates are also termed secondary particles.

[SOURCE: ISO 26824:2022, 3.1.4]

**3.4
agglomerate**

collection of weakly or medium strongly bound *particles* (3.1) 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* (3.3).

[SOURCE: ISO 80004-1:2023, 3.2.4]

**3.5
aggregate**

particle (3.1) 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 80004-1:2023, 3.2.5]

**3.6
differential centrifugal sedimentation
DCS**

analytical centrifugation in which the sample is introduced at a defined position in a rotating disc partially filled with a fluid

Note 1 to entry: Normally the fluid has a density gradient to ensure uniform sedimentation.

Note 2 to entry: Normally there is one detector at a pre-determined position and the times taken for the *particles* (3.1) to reach this detector are recorded.

Note 3 to entry: Depending on the effective density of the particles, the technique can measure particle size and particle size distribution between 2 nm and 10 µm, and can resolve particles differing in size by less than 2 %.

[SOURCE: ISO/TS 80004-6:2021, 4.4.5, modified — the term “line-start incremental disc-type centrifugal liquid sedimentation” has been removed.]

3.7**condensation particle counter
CPC**

instrument that measures the *particle* (3.1) number concentration of an aerosol using a condensation effect to increase the size of the aerosolized particles

Note 1 to entry: The sizes of particles detected are usually smaller than several hundred nanometres and larger than a few nanometres.

Note 2 to entry: A CPC is one possible detector suitable for use with a differential electrical mobility classifier (DEMC).

Note 3 to entry: In some cases, a condensation particle counter may be called a “condensation nucleus counter (CNC)”.

[SOURCE: ISO/TS 80004-6:2021, 4.3.1]

3.8**differential mobility analysing system
DMAS**

system to measure the size distribution of sub-micrometre aerosol *particles* (3.1) consisting of a differential electrical mobility classifier (DEMC), flow meters, a particle detector, interconnecting plumbing, a computer and suitable software

[SOURCE: ISO/TS 80004-6:2021, 4.3.3]

3.9**dynamic light scattering
DLS**

method in which *particles* (3.1) in a liquid suspension are illuminated by a laser and the time dependant change in intensity of the scattered light due to Brownian motion is used to determine particle size

Note 1 to entry: Analysis of the time-dependent intensity of the scattered light can yield the translational diffusion coefficient and hence the particle size as the hydrodynamic diameter via the Stokes-Einstein relationship.

Note 2 to entry: The analysis is applicable to *nanoparticles* (3.2) as the size of particles detected is typically in the range 1 nm to 6 000 nm. The upper limit is due to limited Brownian motion and sedimentation.

Note 3 to entry: DLS is typically used in dilute suspensions where the particles do not interact amongst themselves.

[SOURCE: ISO/TS 80004-6:2021, 4.2.7, modified — the term “photon correlation spectroscopy” has been removed.]

3.10**nanoparticle tracking analysis
NTA****particle tracking analysis
PTA**

method in which *particles* (3.1) undergoing Brownian and/or gravitational motion in a suspension are illuminated by a laser and the change in position of individual particles is used to determine particle size

Note 1 to entry: Analysis of the time-dependent particle position yields the translational diffusion coefficient and hence the particle size as the hydrodynamic diameter using the Stokes-Einstein relationship.

Note 2 to entry: The analysis is applicable to *nanoparticles* (3.2) as the size of particles detected is typically in the range 10 nm to 2 000 nm. The lower limit requires particles with high refractive index and the upper limit is due to limited Brownian motion and sedimentation.

Note 3 to entry: NTA is often used to describe PTA. NTA is a subset of PTA since PTA covers larger range of particle sizes than nanoscale.

[SOURCE: ISO/TS 80004-6:2021, 4.2.8]

3.11 resistive pulse sensing

RPS

method for counting and size measurement of *particles* (3.1) in electrolytes by measuring a drop in electrical current or voltage as a particle passes through an aperture between two chambers

Note 1 to entry: The drop in current or voltage is proportional to the particle volume (Coulter principle).

Note 2 to entry: The particles are driven through the aperture by pressure or an electric field.

Note 3 to entry: The aperture can be nanoscale in size allowing the size measurement of individual nano-objects.

[SOURCE: ISO/TS 80004-6:2021, 4.4.7, modified — the terms “electrical sensing zone method” and “Coulter counter” have been removed.]

3.12 single particle inductively coupled plasma mass spectrometry

spICP-MS

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

[SOURCE: ISO/TS 80004-6:2021, 4.4.8]

3.13 small-angle X-ray scattering

SAXS

method in which the elastically scattered intensity of X-rays is measured for small-angle deflections

Note 1 to entry: The scattering is typically measured in the angular range up to 5°. This provides structural information about inhomogeneities in materials with characteristic lengths typically ranging from 1 nm to 100 nm. Under certain conditions the limit of 100 nm can be significantly extended.

[SOURCE: ISO/TS 80004-6:2021, 4.24, modified — Note 1 to entry has been replaced.]

3.14 ultraviolet-visible spectroscopy

UV-Vis spectroscopy

spectroscopy of radiation that consists of electromagnetic radiation with wavelengths in the ultraviolet and/or visible regions

[SOURCE: ISO/TS 80004-6:2021, 5.6]

4 Abbreviated terms

For the purposes of this document, the following abbreviated terms apply.

BIPM-CCQM	bureau international des poids et mesures consultative committee for amount of substance: metrology in chemistry and biology
CLS	centrifugal liquid sedimentation
CPC	condensation particle counter
DCS	differential centrifugal sedimentation
DLS	dynamic light scattering

DMA	differential mobility analyser
DMAS	differential mobility analysing system
ES	electrospray
MDLS	multi-angle dynamic light scattering
PTA	particle tracking analysis
RPS	resistive pulse sensing
SAXS	small-angle X-ray scattering
spICP-MS	single particle inductively coupled plasma mass spectrometry
TRPS	tunable resistive pulse sensing
UV-vis	ultraviolet-visible spectroscopy
VAMAS	Versailles project on advanced materials and standards

5 Overview

5.1 General

The number concentration of nanoparticles can be measured by techniques that average the number of particles measured over a specific sample volume (henceforth referred to as “ensemble techniques”) or count individual nanoparticles (henceforth referred to as “particle counting” or “particle-by-particle techniques”). The ensemble techniques described in this document are DCS, MDLS, SAXS and UV-vis spectroscopy. In these ensemble techniques, the measured sample volume can have some fractionation, for example in the case of DCS, but an ensemble of particles rather than individual particles are measured at the detector. The particle counting methods described are PTA, RPS, spICP-MS, CPC and DMAS. All the techniques discussed in this document are used for measuring nanoparticles in suspensions except for CPC and for DMAS, which are used to determine the particle number concentration in aerosols, which includes aerosolised suspensions.

The selection of the method of choice is ultimately dictated by the nature of the sample. The measurement of the number concentration of a particle population intrinsically depends on the limits of detection, sensitivity and resolution of the applied technique in terms of particle size. Depending on particle size, some techniques are capable of measuring the relative concentration of particle populations within the same sample. Some techniques measure aggregates or agglomerates as one particle, giving no information on primary particles unless separated by other means. Ensemble techniques generally require the knowledge of other particle characteristics, such as size and refractive index, in order to measure the number concentration.

A summary of VAMAS and BIPM-CCQM P194 international interlaboratory studies on the measurement of the number concentration of colloidal gold nanoparticles with selected techniques is described in [Annex A](#) and a guide on sample preparation for nanoparticles in suspension is described in [Annex B](#).

5.2 Comparison of different techniques

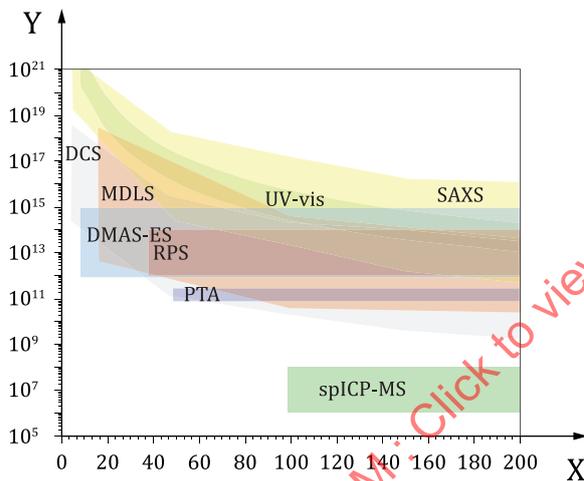
The techniques described in this document are outlined in [Table 1](#). This is not an exhaustive list of methods to measure nanoparticle number concentration measurements; other methods include electron microscopy and asymmetrical flow-field flow fractionation (AF4) coupled to PTA or ICP-MS, but are not discussed in this document. The techniques in [Table 1](#) and [Clauses 6](#) and [7](#) are grouped by ensemble and particle counting, and then listed in alphabetical order. Methods for particles in suspensions (liquid dispersions) are listed first followed by those for aerosols (i.e. CPC and DMAS). Here, instrument footprint refers to the area that the instrument takes up in the laboratory.

Table 1 — Comparison of techniques for measuring nanoparticle number concentration in suspensions (ensemble and particle counting) and airborne

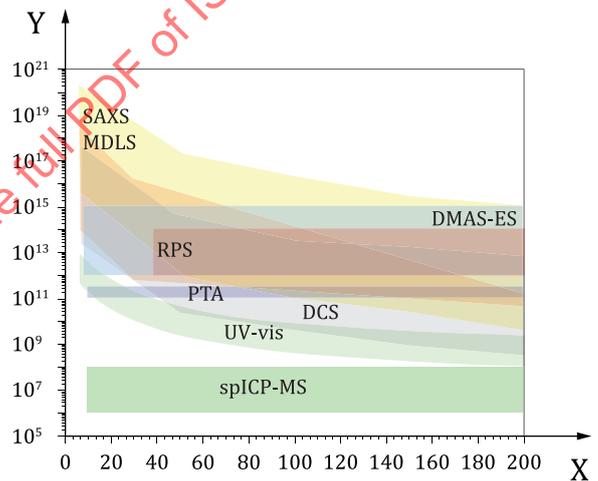
	Technique	Particle type	Critical input parameters	Advantages	Limitations
Ensemble	DCS	Organic and inorganic materials which absorb and/or scatter light or X-rays	Effective density and complex refractive index of the particles, refractive index, average density and viscosity of the density gradient (medium), and some instrument-related parameters. The viscosity of the gradient, as well as the instrument-related parameters, can be replaced by a single method constant based on calibration with spherical reference particles of known effective density and size.	Multiple information (e.g. size and concentration). High resolution of the size distribution. Concentration per size population. Minimal sample preparation.	Longer sedimentation times for smaller nanoparticles or lower density materials. Calibration of particle losses required. Spherical model assumption applied. Spherical calibrant of known size and density required. Data post processing required.
	MDLS	Organic and inorganic materials which scatter light	Complex refractive index and temperature of the medium and the particles, viscosity of the medium	Multiple information (e.g. size and concentration). Rapid measurements. ^a Concentration per size population. Minimal sample preparation.	Spherical model assumption applied. Lower performance for heterogeneous samples.
	SAXS	Organic and inorganic materials which scatter X-rays	Density of the materials (more specifically: effective electron density)	Multiple information (e.g. size, internal structure and concentration). Minimal sample preparation.	Spherical model assumption applied.
	UV-vis	Organic and inorganic material which absorb and/or scatter light	Average particle size and extinction cross-section	Widely available. Rapid measurements. ^a Minimal sample preparation.	Material dependent, particle extinction cross-section is not known for many materials and is also size dependent which limits its applicability.
Particle counting	PTA	Organic and inorganic materials, which scatter light	Effective sensing volume of the instrument	Multiple information (e.g. size and concentration). Concentration per size population. High resolution of the size distribution. Rapid measurements. ^a Low analyte volume.	Sample dilution to optimal concentration. Expert setting of signal thresholds. Calibration of sampling volume required. Dependencies on tracking algorithms.
	RPS	Organic and inorganic materials	Size of the aperture selected	Multiple information (e.g. size and concentration). High resolution of the size distribution. Concentration per size population. Rapid measurements. ^a Low analyte volume.	Concentration calibrant can be required. Stable analyte dispersion in electrolyte solution required. Sample dilution to optimal concentration. Expert setting of signal thresholds.
	spICP-MS	Particles with an element/tag suitable for ICP-MS detection	Transport efficiency of particles	Rapid measurement. ^a Multiple information [e.g. element mass per particle (from which size can be calculated by taking into account density and shape) and number concentration]. Low analyte volume. Very diluted matrix thus minimizing matrix effects. Minimal sample preparation. Information on the dissolved and nanoparticulate fractions simultaneously.	Expert selection of optimal particle concentration Expert setting of signal thresholds. Calculation of transport efficiency required. Limits of detection for sizing limited by procedural blanks, instrumental background and contribution of dissolved fraction.
^a	Rapid measurements refer to those that take approximately 60 s or less per measurement.				
^b	Liquid dispersion.				

Table 1 (continued)

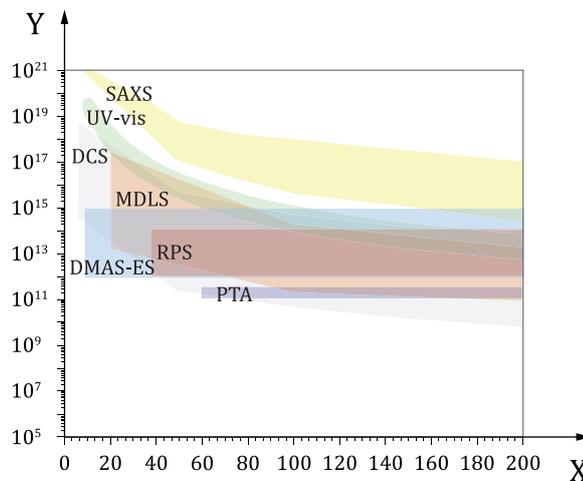
	Technique	Particle type	Critical input parameters	Advantages	Limitations
Airborne particles	CPC	Airborne particles, including aerosolised particles from a suspension ^b	Flow rates of air or gas mixture (aerosol flow and sheath flow)	Rapid measurement (one second resolution). ^a	Sample dilution to optimal concentration. Calibration of transport efficiency required. For dispersions: artefacts derived from solutes in liquid dispersions (electrospray or nebulisation).
	DMAS	Airborne particles, including aerosolised particles from a suspension ^b	The flow rates of air or gas mixture (aerosol flow and sheath flow). Voltage for DMA size discrimination. Efficiency and size distribution preservation of the aerosolization method.	Multiple information (e.g. size distribution and concentration) High resolution of the size distribution. Rapid measurement. ^a	Sample dilution to optimal concentration. Calibration of transport efficiency required. Less direct than CPC for aerosols. For dispersions: artefacts derived from solutes in liquid dispersions (electrospray or nebulisation).
^a Rapid measurements refer to those that take approximately 60 s or less per measurement. ^b Liquid dispersion.					



a) Silica



b) Gold



c) Polystyrene

Key

X particle diameter (nm)

Y particle number concentration (kg⁻¹)

Figure 1 — Comparison of techniques and estimates of related number concentration measurement ranges as a function of particle diameter for various materials types in suspension

Figure 1 shows a summary of the estimated silica, gold and polystyrene particle diameter and number concentration ranges for the different techniques that analyse samples in suspensions and outlined in this document. This includes DMAS with an electrospray (DMAS-ES) for analysis of suspensions. The ranges are different for different types of materials. The technical notes given in Table 2 provide a general description on how the estimated values for various techniques were obtained. For the measurement of aerosol samples using CPC or DMAS, the concentration range for the CPC or DMAS is detailed in 7.4.2.

Table 2 — Technical notes on the calculation of Figure 1

Techniques	Technical notes
DCS	The data was obtained from unpublished experimental work on gold nanoparticles and inferred for the other materials and sizes based on mass equivalence considerations.
MDLS	The data are based on literature values[2].
PTA	The data are based on literature values[1],[3].
RPS	The data are based on unpublished experimental work using typical TRPS measurement parameters.
SAXS	The data are based on experimental measurements. The size range depends on the available q-range and thus the used instrument. For high density particles like gold, large particles will tend to sediment and would need a constant flow-through or a vertical setup for accurate measurement. For low density particles like polystyrene, the concentration determination can be facilitated by increasing the contrast between particles and suspending medium, e.g. by dilution 1:5 in ethanol.
spICP-MS	The data are based on the literature values.[4],[5],[6] The stated diameter and particle-concentration ranges reflect currently available commercial state-of-the art instrumentation (e.g. instruments with microsecond dwell times).
UV-vis	The data are based on the calculations using 405 nm wavelength, Mie theory for extinction cross-sections and an absorbance within the range 0,1 to 2.
DMAS - ES	The data are for DMAS with electrospray analysis of suspensions and is based on literature values[7] and unpublished data for gold nanoparticles. There can be variations as the size becomes very small or very large, but in the current absence of information a flat response as a function of size range is given.

5.3 Considerations when selecting a technique

5.3.1 General

The selection of a suitable technique for the measurement of the number concentration of a nanoparticle sample requires users to consider a number of factors, some of which are discussed in 5.3.2 to 5.3.5.

5.3.2 Type of particles

All the techniques can be applied to a large variety of particle types, including organic, inorganic and biological materials, as summarized in Table 1. Among these, CPC and DMAS are the only techniques that can measure the number concentration of airborne particles whereas the others are suitable for nanoparticle analysis in liquid dispersions (suspensions).

Several of the techniques that operate in suspension (i.e. DCS, MDLS, SAXS, UV-vis and PTA) detect light extinction and thereby require the particles to absorb or scatter light at the wavelength of the light source applied. The analysis of particles and suspending medium with relatively low differential scattering cross-section can be better analysed with other techniques. An example here is measuring

liposomes in aqueous electrolytes. Alternative techniques include RPS and SAXS as these use different detection mechanisms. RPS uses electrical signals, instead of scattered light, to measure particles concentration in conductive liquids. However, care needs to be taken when selecting the dispersant medium since the ionic strength in these electrolytes can affect the sample stability and introduce, for example, agglomeration. spICP-MS is also an alternative for inorganic particles, microparticles or particles which can be capped or stained with elements or tags detected by ICP-MS.

For purely particle counting, the particle shape is largely irrelevant. Most methods for measuring particle size assume that the particles are spherical (equivalent spherical diameter). For some particles, this can deviate from the actual particle dimensions. This assumption is more stringent for ensemble methods than the particle counting methods. It is possible for users to develop mathematical models that support the measurement of non-spherical particles, however, in general, this is not straightforward. An overview of shape considerations for most of the techniques described in this subclause is found in Reference [8].

In the presence of particle agglomeration, some methods, such as SAXS, measure the number concentration of constituent particles, while others, such as PTA, count an agglomerate as a single particle. This is discussed further for each method and also in Reference [9].

The sample volume required for a method can be an important factor in selecting a technique when dealing with samples that are expensive, toxic or available only in limited quantity. The minimum amount required is related to both the minimum sample volume and the number concentration range required by the techniques. Depending on the instrument model, the minimum sample volume of all the listed techniques ranges from 10 µl to a few millilitres. For example, no more than 100 µl of suspension is required for a single measurement using either DCS, SAXS, RPS or spICP-MS methods, although the required particle concentration across these methods can vary significantly.

5.3.3 Number concentration range

Some techniques can measure the number concentration of nanoparticles in a wide concentration range, while others operate within narrow optimal concentration ranges. Typically, particle-by-particle counting methods (e.g. PTA, RPS) fall in the latter category with the exception of the CPC. For these techniques, the sample concentration typically requires adjustment prior to measurement such that they are in the required concentration range. Therefore, ease of sample preparation becomes another important consideration for users to choose an appropriate technique. Detailed guidance and best practice for preparing nanoparticle suspensions with a focus on sample dilution can be found in [Annex B](#).

For techniques whose measurement principles are based on the absorption and scattering properties of the particles (i.e. CLS, MDLS, SAXS, UV-vis and PTA), the accessible number concentration range typically depends upon the instrument sensitivity towards the detected signal intensity and therefore upon type and the size of particles. Related to this is also the choice of the type of light source available for the techniques. In general, instrument sensitivity towards the particle signal is a limiting factor to both size and concentration measurement. [Figure 1](#) provides some examples of these ranges.

5.3.4 Accuracy and precision

The required accuracy with which the number concentration can be known drives significantly the choice of the method to apply. In many practical applications, users' choice is largely dependent upon the aim of the measurement. For example, for product development, it is usually preferable for users to know the sample concentration with high accuracy. For quality control applications, it can be sufficient to only assess the repeatability of a method. In general, techniques that require minimal preparation and no manual setting of signal thresholds tend to be more precise. The uncertainties associated with accuracy and precision of different techniques are currently not fully quantified, but estimation and causes of uncertainty are discussed in each clause. The VAMAS interlaboratory study Project 10 of TWA34 summarized in [Annex A](#) provides a relatively comprehensive example of the accuracy and repeatability expected for selected techniques (i.e. SAXS, DCS, UV-vis, PTA and spICP-MS).

The methods and tools utilized for sample preparation and delivery to the instrument can also have a significant impact on method accuracy and precision. An example is the loss of materials caused by the particle adsorption to the surface of materials such as syringes, pipette tips and vials. The impact of these effects can be mitigated through the use of reference materials with certified particle number concentration, although currently only a quality control material with an “assessed value” of nanoparticle concentration is commercially available for nanoparticles in suspension.^[13] Alternatively, some method optimization can be used^[10].

NOTE The meanings of accuracy and precision are taken from definitions from Reference [11]. Here, the accuracy is taken as the closeness of agreement between a measured quantity value and a true quantity value of a measurand and precision is defined as the closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions.

5.3.5 Other factors

Other factors for users to consider include cost and availability of an analytical instrument plus the instrument lifetime costs (including consumable costs and service contracts), and the requirement of any technical expertise. For example, UV-vis is a widely available and easy to use technique, whereas SAXS is a highly specialized method that requires operators with significant technical expertise. [Table 1](#) and [Figure 1](#) provide further comparative information for the techniques discussed in this document.

5.4 Unit for nanoparticle number concentration

Several different units are commonly used to describe the number concentration of nanoparticles. These units vary from the inverse of a unit volume, expressed in either litres or meters cubed and their submultiples, to the inverse of a mass, expressed in kilograms, to the amount of substance expressed in moles. The specific choice of the unit often depends on the convention adopted by the instrument manufacturers and whether the volume or the mass of the dispersion is measured.

The adoption of a more harmonized system of units would certainly improve measurement reproducibility and result comparability. After extensive consultation, the units of preference for the nanotechnology community to express the number concentration of particles in a liquid medium are those of kg^{-1} and l^{-1} . Both hold advantages depending on the type of sample and the method used and can be used interchangeably when a procedure for conversion has been established.

Expressing the number concentration of nanoparticles in liquid dispersions with the units of kg^{-1} provides the following benefits: the kilogram is an SI base unit and measurement of the mass of a volume of dispersion can be executed with high accuracy with a common laboratory mass balance. Measurements made on a mass balance are metrologically traceable to the SI through calibration masses with defined uncertainty estimates. Gravimetric dilutions of the dispersions, where required, provide dilution factors with accuracy well below 1 %, while accuracy in volumetric dilutions heavily relies on best laboratory practice in terms of operator skills and pipette maintenance and calibration. The unit of kg^{-1} has been adopted for the nanoparticle number concentration in pilot studies by the BIPM.^[12] The only currently commercially available quality control test material with directly value-assigned value for number concentration that is SI traceable uses per mass based units^[13].

Expressing the number concentration of nanoparticles in liquid dispersions with the units of l^{-1} provides the following benefits: the use of the inverse of a volume is widespread and extends to the official unit for the amount of substance concentration, i.e. $\text{mol}\cdot\text{m}^{-3}$, and the number concentration of particles in air, i.e. m^{-3} . The litre is formally accepted as a “Non-SI unit accepted for use with the SI Units”^[14]. Liquid samples are commonly handled with pipettes, whose scale is typically volumetric, although regular calibration is required for accuracy. For concentrated samples, where the mass of the particles contributes significantly to total mass of the sample, it is more practical to use volumetric, rather than gravimetric, dilution factors.

Conversion from units of volume to units of mass is possible and requires knowledge of the density or mass of the dispersion. Where sample availability is not an issue, the preferred approach is that the density of the solution is measured, for example by pycnometry.^[69] Alternatively, the mass of a given volume can be measured using an appropriate scale, with laboratory temperature and humidity being

recorded (see [Annex B](#) for some further considerations). For many practical applications, the mass of the nanoparticles is negligible compared to the dispersant for the current level of accuracy in the measurement of the number concentration. For example, for a sample of gold nanoparticles in water with an average particle diameter of 50 nm, a number particle concentration of $8 \times 10^{15} \text{ kg}^{-1}$ is required for a 1 % relative change in the density of the dispersion compared to that of pure water. In such cases, literature values of the density of the dispersant can be used, as long as the source of information is documented, along with the temperature and humidity of the laboratory.

The measurement of the number concentration of airborne particles by CPC or DMAS is best expressed in units of m^{-3} . CPC and DMAS can also be used to measure the number concentration of nanoparticles in liquids. In this case, particles are aerosolised before the measurements. It is useful to be able to convert the measured concentration in units of m^{-3} into units that relate to the concentration of nanoparticles in the original solute. This task requires careful calibration of the aerosolisation and CPC systems and some more information is provided in [7.4](#).

6 Ensemble techniques

6.1 Differential centrifugal sedimentation

6.1.1 General

Differential centrifugal sedimentation belongs to the centrifugal liquid sedimentation (CLS) family of techniques, also referred to as analytical centrifugation. It is a widely used analytical method that is typically employed to measure the size distribution of particles, but it can also be used to measure the mass concentration of an analyte in a fluid.^[70] DCS is most commonly operated in the so-called line-start incremental method, where all particles are initially contained in a thin band at the liquid-air interface of the sedimentation zone.

The size or effective density of particles in a suspension is measured based on their sedimentation rates in a centrifugal field. The effective (or apparent) density is the ratio of mass to volume for a particle including particulate inclusions, entrapped stagnant liquid and gas in pores, voids and surface fissures as well as surface layers and coatings. As particles with different size, density and shape have different terminal settling velocities, they are effectively separated during sedimentation (or flotation) before detection.

For DCS, the analyte species will need to attenuate light (i.e. by absorption, scattering or a combination of both) at the wavelength of the instrument light source, which typically is in, but not limited to, the visible range. X-rays can also be used.

The determination of particle concentration is performed by measuring the mass of the volume (aliquot) of the particle suspension, M_v , before it is introduced in the DCS instrument and the total mass, M_{tot} , of the particles as measured by DCS. The former can typically be measured with an accuracy of the order of 0.1 % with a properly calibrated laboratory mass balance. M_{tot} is measured by integrating the volume of the particles in each size interval across the volume-based particle size distribution, with knowledge of the particle density, hence, to give mass. To note that the particle density can be unknown even for simplest particle systems. For instruments utilizing visible light sources, the volume-based particle size distribution is derived from the measured particle light extinction intensity according to Mie and Rayleigh's theories. The light extinction is measured as a function of the particle sedimentation time, which is converted to particle size via calibration with spherical reference particles of known average size and effective density. For instruments equipped with X-ray sources, the raw signal is already volume-based and no further computation is required.

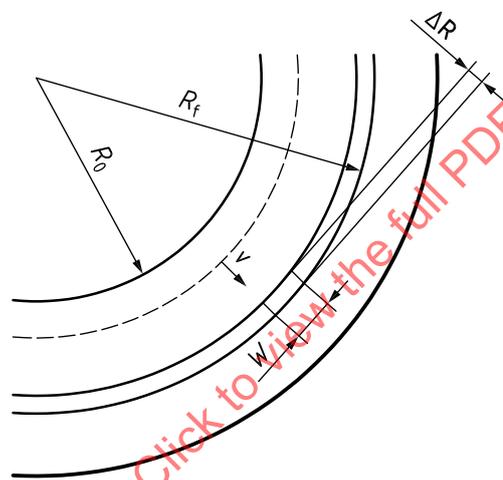
The particle mass concentration is the ratio M_{tot}/M_v . The number-based size distribution is derived by dividing this value by the average mass of a single particle. For this operation, the average diameter and density of the particles needs to be also known or measured.

6.1.2 Sample specifications

Samples for DCS need to be dispersed in a fluid. Most types of nanoparticle material can be analysed by DCS. The nanoparticle concentration can require adjustment via dilution in order for the attenuation of the light to be in a range to match the range detectable by the instrument. Figure 1 shows example ranges for polystyrene, gold and silica particles. Small, translucent particles with refractive indices close to that of the fluid can be difficult to detect. On the other hand, if a suspension is too concentrated, it will require dilution prior to analysis. X-ray sources are preferred for highly concentrated samples. The size and effective density of the particles are important for the selection of the dispersant, and rotational speed of the instrument. The smaller and closer in density to the dispersant the particles, the longer the sedimentation time and the higher the rotational speed required for the experiments. For low density particles (e.g. below the density of the fluid), some in-line instrument models allow the measurement to be undertaken using flotation rather than sedimentation^[15].

6.1.3 Technical aspects

A schematic drawing of the DCS measurement is shown in Figure 2 for the case of line-start photo-sedimentation of spherical particles of mass, M ^[16].



Key

- W width
- ΔR radial distance the particle travel in the time Δt
- R_0 distance from the rotation axis at $t = 0$
- v velocity

NOTE The detection area is defined by W and ΔR .

Figure 2— Schema of a DCS with a line start incremental disc-type CLS instrument

The detector covers an area approximated as a rectangle with width W and length ΔR . The path length of light through the medium is L and the detection volume is the product of the three parameters. The measured light intensity, I , is recorded as a function of time and converted into absorbance, $A = \ln(I_0/I)$ where I_0 is a reference intensity recorded when no particles are in the detection area.

NOTE A factor of $\ln 10 \approx 2,3$ is required if the definition of absorbance is decadic: $A = \log_{10}(I_0/I)$.

Ignoring finer details such as dispersity in the particle population and wall effects, the time taken for particles in the population to cross the detection area, Δt , is given to a good approximation by Formula (1):

$$\Delta t = \frac{\Delta R 18 \eta_f}{R_f \omega^2 (\rho_s - \rho_f) x_{St}^2} \quad (1)$$

where

x_{St} is the Stokes diameter;

η_f is the average liquid viscosity;

R_f is the distance from the rotation axis to the measurement zone;

ΔR is the radial distance the particle travel in the time Δt ;

ρ_s is the effective particle density;

ρ_f is the average liquid density;

ω is the centrifugal angular velocity ($2\pi N/60$) where N is the centrifugal speed in rev/min;

t is the sedimentation time of the particle.

With the same assumptions, the absorbance, A , will be constant during this time and is related to the individual particle extinction cross-section, σ , the number concentration of particles in the analysis volume, $N/(LW\Delta R)$, and the path length L by: $A = \sigma N/W\Delta R$. Thus, the integrated absorbance with time over time interval is Δt is given by [Formula \(2\)](#).

$$A\Delta t = \frac{18\sigma N\eta_f}{WR_f\omega^2(\rho_s - \rho_f)x_{St}^2} \quad (2)$$

Conversion of the time scale to a diameter scale changes the time interval Δt to a diameter interval Δx_{St} and, through differentiation of [Formula \(1\)](#) to obtain $dt/dx_{St} \approx \Delta t/\Delta x_{St}$, the integrated absorbance with diameter is given by [Formula \(3\)](#).

$$A\Delta x_{St} = \frac{\sigma N x_{St}}{2WR_f \ln(R_f/R_0)} \left[\frac{\eta_f(\rho_s - \rho_a)}{\eta_a(\rho_s - \rho_f)} \right] \quad (3)$$

The final ratio in square brackets relates to the difference between the 'average' fluid parameters (denoted with index, a) and those at the measuring zone. These can be combined into a correction parameter, δ , which is a number larger than 1 for sedimentation and depends largely upon viscosity differences, but also upon particle density. [Formula \(4\)](#) relates the number of particles in the detection zone during Δt , N , to the total mass of particles with same size x_{St} , M . It is assumed that the physical diameter of the particle is the same as the sedimentation diameter.

$$N = \frac{6M}{\pi x_{St}^3 \rho_s} \frac{W}{2\pi R_f} \quad (4)$$

Combining [Formula \(3\)](#) and [Formula \(4\)](#) and rearranging to obtain the mass of particles injected divided by the diameter range in which they are detected, [Formula \(5\)](#) is obtained.

$$\frac{M}{\Delta x_{St}} = \frac{1}{\delta} \left[\frac{8\pi R_f^2 \ln(R_f/R_0)}{3} \right] \left\{ \frac{\pi x_{St}^2}{4\sigma} \right\} \rho_s A = \frac{\gamma}{\delta} \rho_s \frac{A}{Q} \quad (5)$$

Here, R_f is the distance from the rotation axis to the measurement zone. The square brackets enclose terms that relate to the instrument, γ . For homogeneous, rigid, spherical particles the terms in curly brackets can be replaced with the reciprocal of the extinction efficiency, Q^{-1} and Q is obtained directly from Mie theory. Assuming, that the physical diameter of the particle is the same as the sedimentation diameter.

Integration of M over x_{St} provides the total mass of the sample, M_{tot} . Critically, the effective density of the sample, ρ_s , needs to be known, along with the size of the particles. For concentration measurements of spherical homogeneous nanoparticles, the size of the particles can be measured by a second independent method, such as PTA or DLS. More generally, methods for determination of particle density are described in the ISO 18747 series. With this knowledge, the sedimentation time measured by DCS is used to infer the particle density. Alternatively, where the particle density is known by independent measurements or information, the DCS method is used to measure the average particle diameter. For some particles, it is also possible to measure both size and density by DCS.^[15] Importantly, the correct value of effective particle density needs to be used for the computation of the mass-based size distribution before its integration. This can be verified by comparing the resulting particle size distribution with that measured by an independent method.

In case of homogeneous, monodispersed samples, the same value of sample effective density, ρ_s , can be used for the computation of the average single particle mass, M_p . The total number of measured particles is then provided by the ratio M_{tot}/M_p . Particle number concentration (in kg^{-1}) can be calculated by taking the total number of particles and dividing by M_v , i.e. the mass of the volume of the measured suspension. A practical approach to the measurement of M_v is to measure the mass of its container, typically a syringe, before and after the dispersion is introduced in the DCS.

6.1.4 Sources of uncertainty and challenges

While the result of [Formula \(5\)](#) can seem to be evident, the analysis is necessary to identify the sources of uncertainty. M is the total mass of particles in the population measured by integration of the converted absorbance versus time data. Note that the conversion to mass-weighted size distribution by using the size dependent extinction efficiency function requires baseline subtraction of the absorbance data, which needs to be treated appropriately to minimize potential biases.^[17] The diameter, x_{St} , is the Stokes or equivalent spherical diameter of the particles and the density, ρ_s , is the effective particle density. Note that the x_{St} term is rarely identical to the measured diameter, which is often different due to shape effects or incorrect densities. The effective density of the particles can significantly differ from that of the same bulk materials and any coating or porosity will contribute to the resulting average effective density of particles. Sample porosity also needs to be considered.^[70] For example, researchers have found that the effective density of 30 nm citrated-stabilized gold nanoparticles is closer to $15 \text{ g}\cdot\text{cm}^{-3}$ rather than $19 \text{ g}\cdot\text{cm}^{-3}$, the density of gold.^[18] For spherical and narrowly distributed particles, both their size and effective density can be measured by DCS in two independent experiments or by isopycnic or multi-velocity based sedimentation methods. The standard method in ISO 18747-2 allows the density determination of particles based on the experimental measurement of particle velocity in gravitational or centrifugal fields based on Stokes law, in different liquids or media, taking into account their dynamic viscosities and densities. Alternatively, an independent method can be used to measure the particle size and DCS can be used to measure the resulting effective density; however, the mismatch of particle size measurands from the different techniques will lead to increased uncertainty. Similarly, the refractive index of the particles at the wavelength of the laser source used by the instrument needs to be known for the determination of the extinction efficiency.

In case of multimodal or wide size distribution samples, the total number of particles in the injected mass (or volume) is often calculated by the conversion of differential mass-based size distribution to differential number-based distribution and by subsequent integration. This conversion requires not only the effective density of particles, but also their volume. In most cases, a simplified approach considering spherical shape is applied during the calculation, adding shape as another source of uncertainty to those ones mentioned above.

For the measurement of particle number concentration, it is also important to note that particle loss inside the instrument and/or consumables (e.g. syringes and vials) is likely to be incurred. For example, particles can stick to the injection port or adhere to the walls of the volume enclosing the dispersant fluid. Thus, it is common for the DCS method to systematically underestimate the number concentration of a particle sample, while exhibiting high precisions for repeatability.^[1] The use of a particle reference material with a known or certified particle number concentration can significantly improve the method accuracy. These reference materials need to have material and shape as close as possible to those of the measured sample.

6.1.5 Outlook

DCS is a well-established technique for the measurement of nanoparticle size distribution and is useful for the measurement of nanoparticle concentration. Its precision makes it particularly useful for quality assurance and control purposes. For accurate concentration measurement, there is a need to develop certified reference materials and ancillary techniques which are required to enable rapid and straightforward interpretation of the DCS data.

6.2 Multi-angle dynamic light scattering

6.2.1 General

Multi-angle dynamic light scattering (MDLS)^{[19],[20]} is an ensemble technique capable of measuring the total concentration of dispersed particle systems, as well as the particle concentration of each size population in suspension. The technique employs DLS^[71] to derive the particle size plus an additional reference scatterer to calibrate the instrument sensitivity. A DLS measurement in its most basic form is conducted as follows^[21]:

- a) the liquid dispersion is illuminated with light that is at least partially, spatially and temporally coherent;
- b) the scattered light is captured at a specific angle or set of angles with respect to the spatially coherent beam in a);
- c) the temporal behaviour of the intensity of the scattered light is analysed.

From the temporal dynamics of the scattered light intensity, the particle hydrodynamic size can be derived and hence the particle size distribution can be determined.

A multi-angle dynamic light scattering measurement is an extension of the DLS technique that detects light scattered at a plurality of angles relative to the incident laser beam. The temporal behaviour of the light scattered into each angle is analysed independently to produce an auto-correlation function that is unique to each angle. In MDLS, the plurality of auto-correlation functions and the time-averaged scattered intensity are collectively analysed to derive the absolute-number particle size distribution^{[19],[20]}.

This technique retains the benefits of DLS; being easy to use (a measurement is possible in several minutes), having a relatively broad dynamic range of particle size and being flexible in material type. Since DLS operates on an ensemble basis and is an inherently light intensity-weighted technique, it is possible to quantify a small number of large particles within a population dominated by smaller particles (i.e. detection of big particles can be achieved at sub-parts per million in number concentration). This is something that would be extremely challenging to do with a particle counting technique, where typically only around 100 particles to 1 000 particles can be sampled. MDLS also has some of the same limitations of DLS.

6.2.2 Sample specifications

For MDLS particle concentration measurements, the particle motion needs to be dominated by Brownian motion and not sedimentation. The consequence of this is that micrometre-size particles are typically only measurable under special conditions – for example: polystyrene, density matched in 13 % sucrose in water. The particle concentration needs to be sufficiently diluted so that photons are singularly scattered by particles. As an approximate rule of thumb, if the sample is ‘water clear’ by eye, multiple scattering is unlikely to occur. Since the differential scattering cross-section is a function of the optical properties of the particle and dispersant, materials with a high refractive index can require additional dilution. Conversely, the sample concentration needs to be not overly diluted such that either particle-scattered photons are not registered above the fluid scattering or too few particles are sampled to statistically represent the population. The number of particles sampled in this case is not stationary which in turn temporally perturbs the scattered light intensity. To determine whether the sample is suitable, the user can inspect the auto-correlation function for acceptable signal-to-noise. However, if

using commercial instrumentation, machine generated data quality guidance to inform whether the sample is suitable for analysis is usually available.

The analysis process (where a plurality of auto-correlation functions is used to derive the absolute-number particle size distribution) requires a model of the scattering to be assumed. Typically, a Mie scattering model is used,^[22] for which the optical properties of the particles and dispersant need to be known, and each particle is assumed to be spherical and homogeneous. Mixtures of particle materials are not suitable for measurement using this technique, although minor deviations can be tolerated (e.g. when measuring mixed vesicles with and without payloads).

6.2.3 Technical aspects

The instrument typically uses a moderate-power laser (approximately 2 mW to 100 mW) to illuminate the sample. The scattered radiation is captured using an avalanche photodiode operating in Geiger mode, typically at three detection angles; e.g. backscatter (typically >173°), side scatter (90°) and forward scatter (typically <20°). An auto-correlator processes the scattered light intensity time signal in real time to produce an auto-correlation function unique to each scattering angle. A non-negative least squares-based process can be used to derive the best-fit particle size distribution from the array of auto-correlation functions. Finally, the particle concentration distribution is derived from the particle size distribution and the ensemble time-averaged scattered intensity according to [Formula \(6\)](#):

$$\rho(d) = \frac{(I_{\text{tot}} - I_{\text{dis}})P(d)R_{\text{tol}}}{I_{\text{ref}} \frac{d\Sigma}{d\Omega}(d, \theta)} \quad (6)$$

where

- $\rho(d)$ is the particle concentration distribution;
- I_{tot} is the backscatter time-averaged photon count rate;
- I_{dis} is the backscatter time-averaged photon count rate measured separately of the dispersant only;
- $P(d)$ is the normalized particle size intensity distribution;
- R_{tol} is the Rayleigh ratio of toluene known from the literature and is equal to $1,35 \times 10^{-5} \text{ cm}^{-1}$ at 632,8 nm and 25 °C;
- I_{ref} is the detected scattered photon count rate of a reference liquid molecular scatterer (such as toluene);
- $\frac{d\Sigma}{d\Omega}(d, \theta)$ is the differential scattering cross-section, $d\Sigma$, per solid angle subtended by the detector, $d\Omega$, per particle of diameter, d , at the scattering angle θ .

The measurement of scattering by toluene serves to reference the sensitivity of the instrument and needs to be performed as part of the routine system verification.

The use of an array of detection angles mitigates one limitation of single-angle DLS, as there is an angular dependence on the scattered light intensity. Different viewing angles will observe certain populations being under-represented in the scattering, limiting the ability of single-angle DLS to quantify the concentration. The absolute-number particle size distribution is independent of scattering angle, unlike the intensity-weighted particle size distribution that is provided with the single-angle DLS result.

A further limitation of single-angle DLS is the resolution. Since the mathematical problem is ill-posed – a small amount of noise can perturb the result – it is necessary to enforce some regularisation to impose an additional smoothness constraint on the result. By using a plurality of information (from multiple angles), noise that can be present at one angle does not manifest in the particle size distribution if it is

not similarly present at sufficient additional angles.^[23] Because of this effect, the degree of smoothing can be reduced to deliver higher resolution.

6.2.4 Sources of uncertainty and challenges

There are several factors that can either bias or reduce the precision of the measured concentration, and knowledge of these factors is important in uncertainty mitigation. The strongest contributors are in the measurement of the particle size distribution and incorrect optical properties.

The accuracy of the differential scattering cross-section is strongly dependent on the particle size and the optical properties (refractive index and absorption) of the material and dispersant. To ensure that size-related error is minimised precautions can be made to optimize sample cleanliness, minimize the Debye-double layer that surrounds particles in low ionic strength dispersants, and ensure that the sample concentration is suitable for analysis. Since the differential scattering cross-section is nonlinear and is a function of both particle size and optical properties, it is not possible to quantify this effect generally. However, in the simplified case of an isotropic scatterer, 1 % underestimation of the particle size propagates into a 6 % underestimation of the total particle concentration. To compute the differential scattering cross-section, the optical properties (refractive index and absorption) need to be known for the material and dispersant with accuracy of up to two decimal places, which can generally be sourced from literature for non-exotic materials. Furthermore, the refractive index of the dispersed phase can differ from the continuous phase to provide sufficient scattering contrast. In practice, this is a rare occurrence and one that can be mitigated by a prudent dispersant selection.

There is also the issue of the lack of size resolution in DLS and even multi-angle DLS. For example, with a typical DLS setup, it is challenging to resolve 100 nm and 200 nm mixtures of polystyrene since both populations scatter with little angular dependence. However, a mix of 150 nm and 350 nm can be resolved, because 350 nm scattering shows distinct angular dependence, while 150 nm does not.^[24] Nevertheless, the resolution of MDLS is lower compared to other techniques discussed in this document.

6.2.5 Outlook

The MDLS particle concentration technique offers promise for providing fast, screening measurements, suitable for a wide array of material types, particle size ranges and concentrations. Applications have been found in drug delivery vesicles and nanoparticle manufacturing, amongst others. The technique is non-destructive, requires liquid volumes on the order of 1 ml and provides data that is complementary to similar techniques, extending the concentration range of particle tracking analysis or providing sub-population concentrations for aggregate quantification.

6.3 Small-angle X-ray scattering

6.3.1 General

Small-angle X-ray scattering (SAXS) is a well-established technique to obtain structural information on inhomogeneities in materials at the nanoscale and is thus well-suited for nanoparticulate systems. The application of SAXS for the determination of the mean particle size and size distribution has been described in ISO 17867^{[1],[25],[26],[72]}.

When electromagnetic radiation passes through matter, a small fraction of the radiation can be scattered due to electron density differences in the matter. The scattered radiation intensity profile (as a function of the scattering angle or momentum transfer, q), contains information that can be used to deduce morphological characteristics of the material. For sufficiently monodisperse spherical particles, the observed oscillations of the scattered intensity as a function of the momentum transfer, which is directly related to the scattering angle and the wavelength of the incident X-rays, enable the size determination of nanoparticles.

In order to determine the nanoparticle number concentration in a suspending medium, the differential scattering cross-section has to be determined, via the ratio of the scattered intensity to the incident intensity. Assumptions on the particle shape are required, which can be based on microscopy

techniques such as electron microscopy. Furthermore, the electron density difference between the particles and the suspending medium needs to be known. ISO 23484^[73] details the measurement of particle concentration using SAXS.

6.3.2 Sample specifications

For particle number concentration determination with SAXS, the particles need to be stable against sedimentation and flocculation. The particle size range for the concentration determination is identical to the range for sizing as the size determination is a step in the concentration determination. The accessible concentration range depends strongly on the particle size, as the scattered intensity scales with the sixth power of the diameter, and on the particle density, as it also scales with the square of the electron density difference between the particles and the suspending medium.^[21] Typical concentration ranges for gold nanoparticles in water are between 10^{11} kg⁻¹ and 10^{16} kg⁻¹. The concentration determination thus requires the determination of the mean particle size (provided in the same SAXS measurement) as well as prior knowledge of the electron density difference, thus mainly the particle density. A further required parameter is the optical path length in the sample. A straightforward calculation of the differential scattering cross-section is easily possible for sufficiently monodisperse spherical particles based on the form factor, depending only on the momentum transfer q and the particle radius r . Reference materials consisting of nanoparticles with known concentration are not required.

6.3.3 Technical aspects

The SAXS set-up consists of an X-ray source, the optics, a collimation system, a sample holder, a beam stop and a detector. The scattered radiation forms a pattern that contains the information on the size and structure of the sample. This pattern is detected typically by a two-dimensional flat X-ray detector situated behind the sample and perpendicular to the direction of the primary beam. A circular integration of the registered pattern leads to the scattered intensity as a function of the momentum transfer or q -value, which is the magnitude of the scattering vector and is given by $q = (4\pi / \lambda)\sin\theta$, where λ is the wavelength of the incident monochromatic X-rays and θ is half of the scattering angle in free space.

The differential scattering cross-section per volume $\frac{d\Sigma}{d\Omega}(q)$, as a function of the momentum transfer q , can be expressed as the sum of the scattering from an ensemble of particles given in [Formula \(7\)](#):

$$\frac{d\Sigma}{d\Omega}(q) = r_e^2 \cdot C_{nv} \cdot \Delta\rho_e^2 \int_0^\infty g(r) S(q,r) \cdot |P(q,r)|^2 dr \quad (7)$$

where

r is the particle radius;

r_e is the Thomson radius;

C_{nv} is the concentration of scatterers (e.g. nanoparticles) in a volume;

$g(r)$ is the size distribution function;

$S(q,r)$ is the structure factor;

$P(q,r)$ is a form factor;

$\Delta\rho_e$ is the electron density difference or contrast of the SAXS experiment.

$\Delta\rho_e$ is obtained by:

$$\Delta\rho_e = \rho_{eP} - \rho_{eL}$$

where

ρ_{eP} is the electron density of the nanoparticles;

ρ_{eL} is the electron density of the liquid (or matrix or suspending medium).

The electron density of an element can be calculated by [Formula \(8\)](#):

$$\rho_e = (\rho \cdot Z \cdot N_A) / M \quad (8)$$

where

Z is the number of electrons per atom;

N_A is the Avogadro constant;

M is the molar mass.

For water at room temperature, $\rho_{eL} = (333,5 \pm 0,3) \text{ nm}^{-3}$. The accuracy can be increased by using the effective electron density which takes the slight energy dependence of the contrast on the photon energy, E_{ph} , into account by introducing the energy-dependent complex atomic scattering factor. Z is then replaced by $f_1(E_{ph}) + i^* f_2(E_{ph})$.

For nanoparticle suspensions with particle volume fractions below approximately 1 %, the structure factor can be neglected, thus $S(q,r)=1$. If the nanoparticles are sufficiently monodispersed and spherical, the corresponding form factor given in [Formula \(9\)](#) can be used:

$$P(q,r) = \frac{4}{3} \pi r^3 \left(3 \frac{\sin(qr) - qr \cos(qr)}{(qr)^3} \right) \quad (9)$$

A Gaussian size distribution can be assumed for monodispersed samples and is given by [Formula \(10\)](#):

$$g(r) = \exp\left(-\frac{(r-\bar{r})^2}{\sigma^2/2}\right) / \int_0^{\infty} \exp\left(-\frac{(r-\bar{r})^2}{\sigma^2/2}\right) dr \quad (10)$$

where \bar{r} is the mean radius of the particles and σ is the standard deviation of the size distribution.

In a SAXS experiment, the q -axis has to be pre-calibrated for the size determination, either based on the exact knowledge of the wavelength and of the scattering angle, or on a suitable material like silver behenate. For the particle concentration determination, the scattered intensity also needs to be known absolutely. Here, either a reference material like glassy carbon can be employed, or the scattered intensity has to be related to the incident photon flux. If an area detector with very high linearity is used, this can be done directly. Otherwise, or if the incident photon flux Φ_0 is very high (e.g. at synchrotron radiation beamlines), it is also possible to determine the incident photon flux (e.g. with a calibrated ion chamber or photodiode) and to use the previously determined quantum efficiency η_{QE} of the area detector. Using the incident intensity, I_{in} , measured scattered intensity $I(q)$ and other experimentally accessible parameters like the transmission of the sample T , the almost constant solid angle of a detector pixel, Ω , and the sample thickness w , the differential scattering cross-section per volume can be calculated as [Formula \(11\)](#):

$$\frac{d\Sigma}{d\Omega}(q) = \frac{I(q)}{I_{in} \cdot T \cdot \Omega \cdot w} \quad (11)$$

The combination of the formulae leads to [Formula \(12\)](#):

$$\frac{I(q)}{I_{\text{in}} \cdot T \cdot \Omega \cdot w} = r_e^2 \cdot C_{nv} \cdot \Delta\rho_e^2 \int_0^\infty g(r) \cdot |P(q,r)|^2 dr \quad (12)$$

The mean particle radius \bar{r} , the standard deviation σ – both parts of $g(r)$ – and the particle number concentration C_{nv} expressed in metres cubed, are obtained from a fit of this formula to the measured data.

6.3.4 Sources of uncertainty and challenges

As described in 6.3.3, the concentration determination is based on the calculation of the scattered X-ray intensity for monodisperse and spherical particles. Deviations from these requirements lead to increased uncertainties. Furthermore, the presence of smaller particles is easily underestimated in the presence of larger particles as their contribution to the total scattering is much lower, even for identical number concentrations. A main source of uncertainty can arise from the electron density of the particles. While this is less relevant for materials with high electron density such as gold, the electron density of, for example, polystyrene and water is very similar, thus the electron density difference is very low. Even a moderate uncertainty in the particle electron density results therefore in a high uncertainty for the particle concentration.

6.3.5 Outlook

While SAXS is already well established for nanoparticle size determination, the determination of the particle concentration is more challenging and thus less established. Several research projects are currently running to determine the possibilities and limitations for this application. ISO 23484^[73] details the measurement of particle concentration using SAXS.

6.4 Ultraviolet-visible spectroscopy

6.4.1 General

UV-vis spectroscopy is a widely used analytical method capable of measuring the concentration of an analyte species in a transparent fluid.^{[27],[28],[29],[30]} The analyte species need to absorb or scatter light within the measurement range of the spectrophotometer, which is typically in the wavelength-in-vacuum range of 190 nm to 1 000 nm. The most important strengths of UV-vis spectroscopy are that it is inexpensive, readily available and easy to use. Here, the measurement of volume-based concentration is determined and calculated to number concentration assuming an averaged size or a size distribution. For the purpose of quantification, the Beer-Lambert law is used, and both the definition and relationship to quantification is provided in [Formula \(13\)](#).

$$A = \log_{10} \left(\frac{I_0}{I} \right) = \epsilon c l \quad (13)$$

where

- A is the decadic absorbance of light;
- I_0 is the reference intensity of light without analyte;
- I is the measured intensity of light with analyte;
- ϵ is the decadic molar attenuation coefficient, expressed in $\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$;
- c is the concentration of analyte, expressed in mol l^{-1} ;

l is the path length through the sample, expressed in cm.

For nanoparticle analysis, the attenuation of light occurs through scattering and, sometimes, through absorption. For a single nanoparticle, it is possible to calculate the cross-section for scattering and absorption if the refractive index, shape and internal structure of the particle are known. The orientation-averaged extinction cross-section of a particle is related to the decadic molar attenuation coefficient through [Formula \(14\)](#):

$$\sigma = \frac{10^3 \ln(10) \varepsilon}{N_A} \quad (14)$$

where

σ is the orientation-averaged extinction cross-section, expressed in cm^2 ;

N_A is the Avogadro number, $6,022 \times 10^{23} \text{ mol}^{-1}$;

ε is the decadic molar attenuation coefficient, expressed in $\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

Therefore, the number concentration of monodisperse nanoparticles in a sample can be measured using [Formula \(15\)](#):

$$C_{nv} = \frac{10^6 \ln(10) A}{\sigma l} \quad (15)$$

where

C_{nv} is the number concentration of particles, expressed in m^{-3} ;

σ is the orientation-averaged extinction cross-section, expressed in cm^2 ;

l is the path length through the sample, expressed in cm.

6.4.2 Sample specifications

Samples for UV-vis need to be stable suspensions of particles in a transparent fluid. The type of material and size range is unimportant, but the concentration can require adjustment to ensure that the light absorbance is in a reasonable range. Particles with refractive indices close to that of the fluid can be difficult to detect. On the other hand, if a suspension is too concentrated, it will require dilution prior to analysis. In the latter case, it is advisable to ensure that $A < 2$ at the wavelength required to avoid nonlinearity due to multiple scattering, internal light scattering in the spectrometer and statistical noise. Depending upon the specifications of the spectrophotometer the linearity and precision can be compromised if absorbance is too high. A sample of the particle-free dispersant is required as a reference sample. This is particularly important if the fluid contains species that absorb or scatter light in the wavelength range of interest.

6.4.3 Technical aspects

UV-vis spectrophotometers operate by the use of broad-band light sources, typically a quartz tungsten halogen lamp for the visible range and a deuterium arc lamp for the UV region. The light is monochromated, typically using a grating, and then split to pass through a reference cell and a sample cell. The absorbance is directly calculated from the ratio of light intensity passing through the two cells, according to [Formula \(13\)](#). The reference cell needs to have the same dimensions and be made of the same materials as the sample cell. It is also important to ensure that the instrument is calibrated and this can be carried out using specified compounds with known molar attenuation coefficients, such as potassium dichromate.^[29] Certified filters, for example neutral density filters containing rare earth oxides, can also be used to ensure photometric accuracy and linearity. The accuracy of the wavelength scale can be confirmed using reference compounds that have sharp, tabulated absorbance peaks

such as holmium in oxidation state 3, although sharp features in the emission spectra of the lamps can also be used for this purpose. The fraction of stray light, arising from monochromator inefficiency or internal scattering, can be assessed using a cut-off filter and observing the measured absorbance in the wavelength range where no transmitted light is expected. Sodium iodide and sodium nitrate are commonly used with cut-offs below 220 nm and 340 nm wavelength respectively.^[31] The cell, or cuvette, that is used to contain the samples can limit the range of wavelengths that can be analysed. Typically, glass and plastic cuvettes only transmit wavelengths longer than approximately 300 nm; for silica, the cut-off is approximately 200 nm, and quartz can transmit UV light with wavelengths below 190 nm.

The orientation-averaged extinction cross-section of a particle can be calculated using a number of different theories. For transparent particles up to approximately 40 nm in diameter with homogeneous and uniform composition and a different refractive index to the fluid, the Rayleigh equation in [Formula \(16\)](#) can be used typically with less than 5 % error. In this regime, the shape of the particle is relatively unimportant and therefore the expression is given in terms of particle volume because particle size can be expressed in many different ways for non-spherical particles.

$$\sigma = \frac{24 \times 10^{-14} \pi^3 V_p^2}{\lambda^4} \left(\frac{m^2 - 1}{m^2 + 2} \right)^2 \quad (16)$$

where

- σ is the orientation-averaged extinction cross-section in units of cm^2 ;
- V_p is the volume of a particle in units of nm^3 ;
- λ is the wavelength of light in the fluid in units of nm;
- m is the refractive index of the particle divided by the refractive index of the fluid.

For particles with diameters larger than approximately 40 nm, or for particles that have an internal structure such as core-shell particles, calculation of the orientation-averaged extinction cross-section requires more detailed theory. For spherical homogeneous particles, Mie theory^[32] provides accurate results and extensions exist to enable the calculation of ideally concentric, spherical core-shell particles.^[33] For particles with more complicated shapes, such as agglomerated spheres, the T-Matrix superposition technique^{[34],[35]} provides accurate calculations^{[29],[34]}.

For certain materials some mathematical relationships have been developed to enable the measurement of particle concentration without resorting to detailed calculations. For spherical gold nanoparticles in pure water, a simple function of particle diameter is available,^[30] further developments include the influence of particle shape,^[28] agglomeration and dispersant refractive index^[27].

The most practical approach to derive the orientation-averaged extinction cross-section is an indirect method using the measurement of the absorbance of a calibration sample with a known number concentration and of the same material, size and media as the sample under test and use [Formula \(15\)](#). This only provides a physically meaningful result for a monodisperse population of nanoparticles. Due to the nonlinear relationships indicated in [Formula \(16\)](#), the resulting experimental cross-section will only be useful for samples with the same population distributions of size, shape and internal structure suspended in the same fluid. In a mixture of particles, the absorbance of each component is additive and therefore, if there is a mixed population of nanoparticles, each with a distinct absorption spectrum, the concentration of both types of particle can be found if reference spectra for each component are available.

6.4.4 Sources of uncertainty and challenges

The most important sources of uncertainty relate to the particle size distribution and knowledge of the particle refractive index used to calculate the orientation-averaged extinction cross-section. Although particle shape distribution and internal structure are also important, these are difficult to deal with and their effect on the measurement uncertainty is not currently known. Since it is generally not

possible to use UV-vis spectroscopy to determine the size, size distribution and shape of nanoparticles, this information needs to come from different techniques. It is important that those techniques are described and documented fully. Refractive indices are generally not measured but assumed to be the same as those from sources of refractive index data. Even for bulk gold, there is an approximate 20 % relative scatter in refractive index between various literature sources^[28] which represents the limiting uncertainty for concentration measurements of gold nanoparticles using UV-vis spectroscopy. For particles with a refractive index close to that of the fluid, it can be appreciated from [Formula \(16\)](#) that there is a fourth power relationship between the scattering cross-section and the refractive index. Thus, errors in refractive index are critically important if theory is used to calculate scattering cross-sections. In this case, a 10 % error in relative refractive index will result in approximately 40 % uncertainty in a scattering cross-section. Therefore, there is a need to develop methods to measure the refractive index of particles in suspension. This can, for example, be achieved by measuring the polarization of scattered light as a function of scattering angle^[34] and using this technique, it can even be possible to measure the thickness and refractive index of coatings on particles^[36].

Measurement uncertainty can also arise in the case of the forward scattering of light onto the detector and multiple scattering. Forward scattering is pronounced for large particles, as predicted by Mie theory, but the effect can be minimized by ensuring that the acceptance area of the detector collects a small solid angle around the transmitted light passing through the sample. Multiple scattering, in which scattered light is re-scattered into the detector occurs when the sample is too concentrated and this effect can be avoided by ensuring that the absorbance is not large, as noted in [6.4.2](#).

6.4.5 Outlook

UV-vis can be adapted to be used for quality control or in-line monitoring of nanoparticle concentration measurement. There is a need to improve the reference data and ancillary techniques which are required to enable rapid and straightforward interpretation of the data from UV-vis spectra. These include cost-effective methods of measuring the refractive index of particles and particle size distributions.

7 Particle counting techniques

7.1 Particle tracking analysis

7.1.1 General

PTA, also called nanoparticle tracking analysis (NTA), is a commonly used technique for direct measurements of number-based size distribution and the total number concentration of particles on a particle-by-particle basis. Some PTA instruments can measure zeta-potential, light scattering intensity and particle fluorescence. PTA relies on two important properties of nanoparticles in a suspension:

- a) particle ability to scatter light (or to emit fluorescence);
- b) particle ability to move under Brownian motion.

PTA is compatible with a wide range of inorganic and organic particles suspended in various liquid matrices. The technique is comprehensively described in ISO 19430^[74] and ASTM E2834-12^[37], therefore only a short overview is provided in [7.1](#).

7.1.2 Sample specifications

PTA is compatible with a wide variety of particle types, including some complex biological samples. This technique can be used to determine the number concentration of both organic and inorganic materials which scatter light (or emit fluorescence). The light scattering (or fluorescence) properties of the analysed particles can strongly influence the lower size limit of detection. For example, a lower detection limit of approximately 10 nm in diameter is achievable when there is a relatively large difference in the refractive index between the sample and the dispersant media (e.g. gold particles in water), whereas challenges remain in detecting particles below 50 nm in diameter for samples with similar scattering properties to the media (e.g. liposomes in aqueous buffer solution or silica) for measurements based

on the scattered signal. Lower limits of detection can be typically achieved in case of fluorescence measurements. In the case of the scattering signal, the detectable range of particles is also dependent on the wavelength of the laser source applied which needs to be defined along with the detection limit of particle size. The upper size limit of detection is approximately 1 000 nm and is determined by the requirement for particles to move freely under Brownian motion and not to sediment before the measurement is complete, which is particularly important if reliable particle number-concentration is to be measured. It is also important to highlight that care needs to be taken if working with particle sizes close to the limits of detection, as this can have an impact on the accuracy and precision of the number-based concentration determination. For this reason, establishing the limits of quantification is highly recommended^[38].

The particles need to be suspended in a liquid compatible with the instrument's hardware and transparent to the light source. As such, PTA is compatible with simple aqueous and non-aqueous media (depending on the instrument set-up) as well as more complex biological or environmental matrices. However, for complex samples, the accuracy of particle counting is often compromised due to differences in the scattering volume and background scattering noise^[39].

It is important to note that PTA operates in a relatively narrow concentration range (one order of magnitude for most systems) therefore samples need to be diluted or concentrated prior to the analysis to reach the specific concentration range required by the system used (typically, in the range of 10^6 particles·ml⁻¹ to 10^9 particles·ml⁻¹ or 10^9 particles·kg⁻¹ to 10^{13} particles·kg⁻¹ depending on the configuration of the system used^[38]).

PTA requires a specific number of particles per field of view to measure particle number concentration reliably. For smaller particles, the adjustment of camera settings can be undertaken, i.e. the aperture of the detector can be increased or the shutter speed or gain settings adjusted to allow more light and to ensure detection of the same number of particles per field of view as for the larger particles. Alternatively, a laser with shorter wavelength can be used for analysis of smaller particles using the scattering signal or by using fluorescence.

While the technique deals well with multimodal and polydisperse samples, the accuracy of particle concentration determination in individual size fractions in these samples is often compromised as not all the fractions present will be populated at the optimal particle number per field of view^{[1],[9]}.

7.1.3 Technical aspects

PTA is a microscopy-based technique where particles are detected as points of scattered light (or fluorescence emission) and recorded within the field of view using a high-speed digital video camera. These point particles can be directly tracked and counted by an automated software.

To measure the particle number concentration, both the lateral dimensions of the field of view and the depth of field of view are needed to derive the effective sensing volume of the instrument, as explained in detail in ISO 19430^[74]. Accurate calculation of the effective sensing volume also depends on the laser light intensity profile in the analysis field and the particle size-dependent scatter intensity of the sample, hence uncertainty in this measurement can be large. Alternatively, by using nanoparticle reference materials with known particle number concentration, the user can directly determine the effective sensing volume of the instrument in a single step. Once the effective sensing volume is known, it is then possible to derive particle number concentration in the unknown samples by dividing the number of particles counted in the field of view by the sensing volume.

7.1.4 Sources of uncertainty and challenges

The biggest unknown in particle counting with PTA is sample and instrument dependent depth of field of view and therefore the resultant sensing volume. The depth of field of view is usually measured indirectly by the instrument manufacturer as part of the system calibration and/or design qualification, typically conducted using 100 nm diameter polystyrene particles or an optical diffraction grid, with the associated standard uncertainty in the region of 0,4 % possible to achieve.^[39] However, since this parameter is measured by the manufacturers, PTA users can only assess its impact on the uncertainty associated with particle counting. It does not mean that the depth of field of view is the main source of

uncertainty, in fact variability in particle counting is usually the main contributing factor to the overall uncertainty associated with particle number concentration measurements with PTA. Variability in particle counting can be improved by performing the analysis in flow, instead of static mode, since flow mode yields in more particles analysed per video and better statistics.

The main challenges in particle concentration determination with PTA are associated with the narrow concentration range in which technique operates, which often means that the sample requires dilution without compromising the particle colloidal stability. Another point to consider is the lower size limit of detection, which is material dependent, therefore some prior knowledge on the sample is necessary.

7.1.5 Outlook

PTA is already broadly used by the community for particle size, size distribution, fluorescence, light scattering intensity as well as concentration measurements, owing to its simplicity, high throughput and compatibility with a wide range of samples, including real life complex samples, such as viral particles, protein aggregates and exosomes. The technique also allows particle-zeta potential measurements, although it is not the most commonly used feature.^[40] One of the biggest drawbacks of PTA arises from the variable sensing volume which can be overcome by calibration with certified reference materials once they become commercially available. This is likely to further increase the uptake of the technique due to its improved reliability. Other limitations arising from currently accessible particle size range can potentially be overcome by further developments of the instrument's hardware.

7.2 Resistive pulse sensing

7.2.1 General

RPS is an analytical technique that uses electrical signals to measure particle concentration in conductive liquids. This technique measures the excluded volume of a particle which is independent of the particle properties. Therefore, it is suitable for measuring nanoparticles regardless of their refractive index, including biological species which often have a relatively similar refractive index compared to their dispersion media. Other important features of the RPS technique includes its wide accessible particle size range, from 40 nm to 100 µm depending on the apertures used.

RPS is a particle-by-particle technique for determining the size, zeta potential and number-based concentration of particles in electrolyte by measuring a drop in electrical current or voltage as a particle passes through an aperture between two chambers. RPS, also known as the Coulter counter, was first created by Wallace Coulter in 1953^[41] to count and measure the size of microscale particles. Conventional RPS consists of two compartments filled with aqueous electrolyte, equipped with a silver/silver chloride (Ag/AgCl) electrode on each side of a membrane continuously recording ionic current during particle translocation. When an electric potential is applied across the membrane, in the absence of particles, a stable ionic current is measured. If a particle travels through the aperture, it occludes the ionic channel, causing a drop in the current which is termed a 'resistive pulse' due to the replacement of conductive electrolyte solution by the non-conductive solid particle. Most conductive analytes, such as gold particles, can be measured by RPS if there is no conductive path between analytes and electrolyte and, hence, these analytes can be effectively treated like non-conductive particles. The height, width and frequency of these resistive pulses provide the information needed to determine particle size, number concentration and zeta potential of the analytes^[42].

In RPS, the aperture size can be in the nanoscale, allowing the measurement of individual nanoparticles. A common variation of RPS is tunable resistive pulse sensing (TRPS). Other commercial RPS-based instruments for nanoparticle analysis include microfluidic devices using static apertures.^[43] A unique feature of TRPS is tunability. Here, the applied pressure and voltage are tunable to allow for full control of convection and electrokinetic velocity contributions of single nanoparticle translocating the aperture. In one iteration, a flexible elastomer-based pore substrate is used to allow mechanical alteration of the aperture size at the nanoscale level. An important feature of this specific system is that the aperture stretch can be optimized for an analyte. In another iteration of the technique, cylindrical polymeric apertures with fixed aperture sizes are used.

TRPS can be used to measure the nanoparticle number concentration via [Formula \(17\)](#):

$$J = C_n v \quad (17)$$

where

C_n is the particle number concentration, expressed in particles·kg⁻¹;

J is the particle flux, expressed in particles·m⁻²·s⁻¹;

v is the velocity of the particles through the aperture, expressed in m·s⁻¹.

[Formula \(17\)](#) is also known as the Nernst-Planck equation. In most scenarios transport mechanisms, such as gravity, diffusion, dielectrophoresis and drag within the confined space of the aperture can be neglected and will not affect TRPS concentration measurements. When the particle movement is dominated by convection, [Formula \(17\)](#) can be converted to [Formula \(18\)](#):

$$F_{bl} = C_n Q \quad (18)$$

where

F_{bl} is the blockade frequency or the particle count rate;

C_n is the particle number concentration;

Q is the fluid flow rate.

Since F_{bl} is proportional to pressure, the particle count rate F_{bl} becomes proportional to both the concentration and the applied pressure.^[44] As a result, the slope of the particle count rate versus the applied pressure, is proportional to the particle number concentration. In particle concentration measurements by TRPS, knowledge of the aperture characteristics is generally unavailable and therefore the use of a calibration standard with known particle size and number concentration is required to obtain concentration information for the analytes. However, it is possible to calculate particle concentration by predicting the size and geometry of the aperture through the measured background current at a given voltage. Nevertheless, calibration increases measurement reproducibility and traceability and, hence, it is predominantly used.

7.2.2 Sample specifications

TRPS allows nanoparticle number concentration to be determined over a defined size range. This applies to particles ranging from approximately 40 nm to 100 µm. The size range of the analytes depends on the aperture size and the instrument model used. There is no specific sample requirement regarding the shape of analytes. Target concentrations for TRPS measurements depend strongly on the aperture size and can vary between 10⁸ kg⁻¹ to 10¹³ kg⁻¹. [Table 3](#) shows a guide to aperture selection, target calibrant and sample particle concentrations. Note that target concentrations lie well below the respective concentrations at which the probability for coincidence is 5 %. It is also worth mentioning that the TRPS only measures the total number concentration of nanoparticles within a defined size range determined by the aperture selected. For example, a membrane with an aperture diameter of approximately 240 nm can be used to determine the concentration of nanoparticles whose sizes typically range between approximately 40 nm and 240 nm in diameter. Smaller nanoparticles (i.e. <40 nm in diameter) can be difficult to measure depending on the signal to noise ratio, whereas larger nanoparticles will not be able to go through the aperture.

Table 3 — Guide to aperture selection in relation to target particle size range and concentration

Nominal aperture diameter nm	Particle diameter range nm	Target concentration kg ⁻¹
320	40 to 240	1 × 10 ¹³
400	50 to 300	1 × 10 ¹³
800	100 to 600	2 × 10 ¹²
1 600	200 to 1 200	5 × 10 ¹¹
4 000	500 to 3 000	5 × 10 ¹⁰
16 000	2 000 to 12 000	5 × 10 ⁸

The RPS technique has been used to measure the number concentration of inorganic, organic and biological particles in aqueous electrolytes. Note some nanoparticles are likely to aggregate or agglomerate and/or change their physical properties (e.g. nanoparticle size and shape) when placed in different aqueous electrolytes for example at inappropriately chosen high ionic strengths, leading to some measurement challenges in the RPS analysis such as aperture blockages.

In RPS measurements, conductive aqueous electrolyte is required as the dispersant to establish a baseline current. A large selection of aqueous electrolytes can be used, the most common ones being sodium chloride and phosphate buffered saline with physiological buffer range. A salt concentration between approximately 10 mol/l to 400 mol/l is suitable for a typical TRPS measurement. Lower salt concentration (≤ 10 mol/l) can be used for particles with diameters in the micrometre size range. In the case of nanoparticles with an average diameter around 100 nm or less, measurement sensitivity can be improved by increasing the electrolyte concentration (e.g. 150 mol/l to 400 mol/l). High ionic strength, however, means that some nanoparticle suspensions are more likely to agglomerate due to a reduction in length of the electrical double layer and its related reduction of effective surface charge. Users are recommended to choose the electrolyte concentration depending on the analyte of interest and desired experimental conditions and outcomes.

7.2.3 Technical aspects

TRPS concentration measurement over a defined particle size range can be achieved by single and multiple pressure procedures, in which the particle rate for both calibrant and analytes are measured at one, two or more pressures respectively.^[45] Particle number concentration can be calculated from the gradient of the linear particle rate versus applied pressure, whereas the volume-equivalent particle size of the analytes can be determined from the magnitudes of the respective resistive pulse. Particle concentration measurements by TRPS provide information on number-weighted particle size distribution of each size population within an analyte in histogram format, presented as the total number of particles per millilitre and bin size in nanometre. A summary of recommended settings for TRPS particle concentration measurements can be found in [Table 4](#).

Table 4 — Typical settings for particle number concentration measurements

TRPS measurements	Settings
Applied voltage	$\leq 1,6$ V
Applied pressure	$\leq 2,5$ kPa
Particle size range	~ 40 nm to 100 μ m
Concentration range	associated with the size range of nanoparticles

In most TRPS analyses, particles are modelled as spheres and particle sizes refer to the equivalent spherical diameter. However, it is also possible to measure non-spherical particles, including viruses,^[46] bacterial chains^[47] and self-assembled aggregates^[48] using the semi-analytical model. RPS can be combined with predictive logistic regression models, in order to rapidly characterize particle size, aspect ratio, shape and concentration for mixtures of nanorods and nanospheres.^[49] Moreover, high resolution TRPS particle concentration measurements can be achieved using a calibrant-free approach^[50] or an internal calibration technique^[51].

7.2.4 Sources of uncertainty and challenges

A primary concern with any particle counting technique like TRPS is representative sampling. For example, both particle agglomeration and sample settling (e.g. caused by differences in particle size and density) can lead to uncertainties in the concentration measurements. It is therefore recommended to mix samples sufficiently prior to TRPS measurements (see [Annex B](#) for guidance on sample preparation). Poor sampling representativeness can also come from insufficient particles measured which prevent users from achieving statistically meaningful results. In addition to the total number of particles analysed, it is important to consider whether a reasonable particle count rate is present during analysis. A typical particle count rate is between 200 particles per minute and 1 500 particles per minute for reliable and reproducible TRPS measurement. Very slow particle translocation suggests the likelihood of aperture blockage whereas the system has trouble to record viable results with too many particles going through the aperture in a short time scale. It is worth noting that the particle count rate can be optimized by adjusting the applied pressure. If particle concentration is too high, coincidence events can diminish the measured concentration. In this case, the particles need to be further diluted before being assessed with TRPS. Suggested target particle concentrations for TRPS are listed in [Table 3](#).

In the TRPS technique, another source of uncertainty comes from the calibrants used for particle concentration measurements. Since knowledge of the aperture geometry is generally unavailable, accuracy of TRPS particle concentration measurements is strongly dependent on a calibration standard with a known size and concentration. This becomes particularly important when measuring samples which contain multimodal or aggregated populations. The aperture needs to be calibrated with reference materials, ideally particles of known size traceable to the SI.

Measurement uncertainties can also result from poor aperture selection, changes in aperture geometry or particle-aperture interactions. The TRPS-measured total particle concentration depends on the probed size range and lower detection limit of the instrument. As such, it is critical to select an aperture to match the particle population of interest. For measuring multimodal or polydisperse samples, it is common to use multiple apertures of various sizes to match the size range of the analyte. It is also common for users to experience particle-aperture interactions and changes in aperture properties which can be prevented by applying a coating solution to alleviate non-specific binding to the aperture surface prior to the measurements.

While TRPS offers some advantages over other techniques, there are a few challenges, including aperture blockage, requirement of conductive media and limited dynamic range of one aperture setting. In addition, the development of TRPS can be hindered by the availability of reference materials for nanoparticle number concentration measurement.

7.2.5 Outlook

As the TRPS technique continues to evolve, it is anticipated that TRPS will become more capable of measuring complex systems in biological environment. With an increasing number of new reference materials becoming commercially available, the TRPS technique can potentially provide high resolution nanoparticle concentration measurements with improved accuracy.

7.3 Single particle inductively coupled plasma mass spectrometry

7.3.1 General

Since the introduction of Single particle inductively coupled plasma mass spectrometry (spICP-MS) by Degueldre in 2003^[52], 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 that is able to handle the large amount of data produced during spICP-MS experiments, even when using microsecond detection^[53].

In spICP-MS, a very dilute particle suspension is required to minimize the possibility of more than one particle (e.g. double and triple events) reaching the plasma at the same time. The plasma atomises

and ionises the constituents of the nanoparticle, generating a discrete pulse of ions at a corresponding mass-to-charge ratio lasting on the order of a few hundreds of microseconds above the continuous background signal^[25].

spICP-MS can be used to determine nanoparticle concentration using

$$C_n = \frac{N_{NP}}{\eta_{neb} \cdot \frac{1000}{Q_{sam}} \cdot \frac{1}{t_i}} \quad (19)$$

where

C_n is the nanoparticle number concentration, expressed in kg^{-1} ;

N_{NP} is the number of events detected during acquisition time;

η_{neb} is the transport efficiency;

Q_{sam} is the sample uptake mass flow, expressed in $\text{g} \cdot \text{min}^{-1}$;

t_i is the total acquisition time, expressed in min.

spICP-MS is also capable of measuring the dissolved fraction of the element within the same run provided that a fair distinction between the background and/or dissolved signal, and the nanoparticle signal is achieved. This distinction is based on the different behaviour of the analyte inside the plasma in its dissolved or particulate form. This difference comes from the different distribution of the dissolved and nanoparticle species among the aerosol droplets^{[53] [55]}.

7.3.2 Sample specifications

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 single particle mode. Published work includes, but is not limited to the analysis of 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. To date, the sensitivity of ICP-MS instrument is insufficient to detect polystyrene microspheres in the nanoscale.^[56] Alternatively, carbon-based nanoparticles can be measured using the trace catalytic impurities as proxies for the NP^{[57] [58]}.

The accessible lower particle size limit of detection (LOD) will vary depending on the particle composition and the type of the element monitored, but also depends on the instrument set-up, for example working at close to the size LOD can impact both accuracy and repeatability of the measurements. A good overview of size limits of detection achieved for different particles has been provided.^{[55] [59]} This shows the lowest size limits (in the order of a few nanometre) for particle of single elements (e.g. gold) that ionize easily, are stable in dilute aqueous suspension (i.e. do not dissolve) and do not suffer from the contribution of procedural blanks.

Sample dilution is usually required to obtain accurate and reliable particle concentration measurement. However, this technique mostly applies to rather simple matrices. Often, a systematic optimization of sample preparation procedures (e.g. alkaline or enzymatic extractions, filtration) is required to ensure a trade-off between particle extraction efficiency and preservation of their properties. In terms of optimal particle concentration, large sample dilution factors are usually applied but special attention is paid to reduce impact of particle counting on the overall uncertainty with minimal formation of nanoparticle double and triple events.

7.3.3 Technical aspects

In spICP-MS analysis, in order to establish a relationship between the number of particle events detected over a defined analysis window (time scan) and the number of nanoparticles in solution, the transport efficiency needs to be determined, as shown in [Formula \(19\)](#). Some popular techniques

used in the literature for calculation of the transport efficiency include the particle frequency and the particle size methods.^[60] Both approaches are described in detail in ISO/TS 19590^[75] and rely on the use of nanoparticle reference materials which are very scarce and the few existing ones are different from nanoparticles used in real sample matrices.

In the particle frequency method, a monodispersed nanoparticle reference material of known mass concentration is introduced into the ICP-MS, the number of particles is measured over the duration of time scan and the transport efficiency (η_{neb}) is calculated from [Formula \(20\)](#):

$$\eta_{\text{neb}} = \frac{N_{\text{NP}} \cdot d^3 \cdot \rho_{\text{NP}} \cdot \pi \cdot 10^{-9}}{6 C_m \cdot Q_{\text{sam}} \cdot t_i} \cdot 100 \% \quad (20)$$

where

- N_{NP} is the number of events detected during acquisition time;
- d is the mean spherical-volume-equivalent particle core diameter, expressed in nm;
- ρ_{NP} is the particle density, expressed in $\text{g}\cdot\text{cm}^{-3}$;
- C_m is the mass concentration of particle suspension, expressed in $\text{ng}\cdot\text{kg}^{-1}$;
- t_i is the total acquisition time, expressed in min;
- Q_{sam} is the sample uptake mass flow, expressed in $\text{g}\cdot\text{min}^{-1}$.

[Formula \(20\)](#) assumes spherical geometry of single-element particle. Particle density is also often assumed to be similar to the bulk material, which has been shown to be a good assumption for particles composed of gold but not for many other types of particles. For example, silicon dioxide can have densities ranging from below $1,9 \text{ g}\cdot\text{cm}^{-3}$ in the hydrated amorphous form of Stöber silica to above $2,6 \text{ g}\cdot\text{cm}^{-3}$ for quartz. Because all parameters of [Formula \(20\)](#) come with their associated uncertainty, the uncertainty of η_{neb} estimated following this approach is approximately 11 % for the particle frequency method.^{[61],[62]} The uncertainty in η_{neb} represents the main contributing factor to the overall uncertainty associated with the particle number-based concentration measurements by spICP-MS following the frequency method. Reliable number concentration values can be obtained with this method to a relative expanded uncertainty ranging from 12 %^[62] to 16 %^[61].

Using the known or certified particle number concentration value of a nanoparticle reference material, η , can be calculated from [Formula \(21\)](#):

$$\eta_{\text{neb}} = \frac{N_{\text{NP}} \cdot 1000}{C_n \cdot Q_{\text{sam}} \cdot t_i} \cdot 100 \% \quad (21)$$

where

- t_i is total acquisition time, expressed in min;
- C_n is the particle number-based concentration, expressed in kg^{-1} ;
- N_{NP} is the number of events detected during acquisition time;
- Q_{sam} is the average sample uptake rate, expressed in $\text{g}\cdot\text{min}^{-1}$.

In this case, the uncertainty associated with [Formula \(21\)](#) depends mostly on the uncertainty associated with the 'C' parameter given on the certificate of analysis, which in the case of LGCQC5050¹⁾ is 19 % relative expanded uncertainty ($U_{95}, k = 2$). This also applies to other reference materials (e.g. the widely

1) LGCQC5050 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

used NIST RM series^[82]) for which the C can be calculated indirectly from the parameters given in the certificate.

In the size method, a reference nanoparticle suspension of known particle diameter is used for the calculation of transport efficiency. Moreover, a dissolved standard solution with a known mass concentration of the same element of interest is measured. Transport efficiency is then calculated from [Formula \(22\)](#).

$$\eta_{\text{neb}} = \frac{R_{\text{ionic}}}{R_{\text{NP}}} \cdot 100 \% \quad (22)$$

where

R_{ionic} is the instrument's response to ions, expressed in counts per second per microgram, $\text{cps} \cdot \mu\text{g}^{-1}$;

R_{NP} is the instrument's response to the particle suspension, expressed in $\text{cps} \cdot \mu\text{g}^{-1}$.

R_{ionic} and R_{NP} can in turn be calculated as follows from [Formulae \(23\)](#) and [\(24\)](#), respectively:

$$R_{\text{ionic}} = \frac{R_{\text{fion}} \cdot 6 \cdot 10^7}{Q_{\text{sam}} \cdot t_{\text{d}}} \quad (23)$$

where

R_{fion} is the instrument's response factor to ionic standard, derived from regression analysis of the calibration curve, expressed in $\text{cps} \cdot \mu\text{g}^{-1} \cdot \text{kg}$;

Q_{sam} is the average sample uptake rate, expressed in $\text{g} \cdot \text{min}^{-1}$;

t_{d} is the dwell time used, expressed in ms.

$$R_{\text{NP}} = \frac{I_{\text{NP}} - I_{\text{diss}}}{m_{\text{NP}}} \quad (24)$$

where

I_{NP} is the average particle intensity, expressed in cps;

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

m_{NP} is the mass of element in a single particle, expressed in μg .

m_{NP} can be calculated from [Formula \(25\)](#):

$$m_{\text{NP}} = \frac{d^3 \cdot \rho_{\text{NP}} \cdot \pi}{6 \cdot 10^{15}} \quad (25)$$

where

d is the mean spherical-volume-equivalent particle core diameter, expressed in nm;

ρ_{NP} is the particle density, expressed in $\text{g} \cdot \text{cm}^{-3}$.

Similarly to the particle frequency method, the particle size method is also based on several assumptions such as: particle sphericity, equal transport efficiencies between particulate and dissolved solutions and 100 % ionization efficiencies of the nanoparticles. Particle spherical volume equivalent core size and particle density also feed into the formula, meaning that the uncertainty associated with η_{neb} estimated following both approaches are similar. However, there are several literature reports highlighting differences in the η_{neb} values obtained with the two approaches, which have been attributed to particle

losses in the containers and tubing and other effects such as possible off axis trajectories of particles in the plasma and differences in the transmission of atoms coming from dissolved analyte or from nanoparticles.

More recently, methodology based on the use of the dynamic mass flow (DMF) approach which does not require a reference material for determination of the transport efficiency has been reported.^[61] Instead, this method operates by dynamically and continuously weighing a vial from which a sample or NP solution is introduced through the nebulizer and to which the solution exiting the spray chamber is returned while the ICP-MS system is in equilibrium. Note that as some solution can be retained within the introduction system, it is essential to nebulize each solution for sufficient time to allow a state of equilibrium to be achieved. Thereafter, the rate of reduction of the vial weight can be considered to represent a true measure of the mass flow of sample reaching the plasma, under the conditions reported elsewhere^[63].

The transport efficiency value is then calculated using [Formula \(26\)](#):

$$\eta_{\text{neb}} = \frac{M_{\text{fpl}}}{M_{\text{fup}}} \quad (26)$$

where

M_{fpl} is the slope from the regression analysis representing mass flow reaching plasma, expressed in $\text{g}\cdot\text{min}^{-1}$;

M_{fup} is the slope from the regression analysis representing mass flow of sample uptake, expressed in $\text{g}\cdot\text{min}^{-1}$.

In this case, the η_{neb} determination relies on weighing of the suspension over time, so when working with interface type and low temperature conditions as specified in the literature,^[61] its associated uncertainty has been demonstrated to be mostly based on mass measurements which can be accomplished with high accuracy and precision.

Such methodology although somewhat laborious (due to the consecutive measurements of mass flows), shows promise for the validation of other laboratory techniques for particle number concentration. It is important to note that demonstration of the accuracy of this method has been reported to date^[61] by using a conventional Micromist nebuliser with a cooled Scott-type spray chamber at 2 °C. The method has not been reported to work outside these conditions, although some practitioners have had success up to 10 °C. Working with a cooled spray chamber helps to reduce the amount of water vapour (produced from evaporation of water from aerosol in the spray chamber) entering the plasma, thus minimising the contribution of this source of error to the uncertainty of the mass-based η_{neb} . Moreover, the DMF method can only be applied successfully if there is no significant fractionation of the nanoparticles between the aerosol sent to the plasma and the solution returned to the vial.

Under the conditions reported to date,^[61] accurate number concentration data has been achieved for different types of particles using an ICP interface or sample introduction system that can be conventionally used by ICP practitioners and highlights the real benefits of the DMF approach to assign reliable number concentration values (with a relative expanded uncertainty of approximately 10 %) to nanoparticles available from manufacturers, some of which are similar to those in real samples. This expands current opportunities for analysis of number concentration by spICP-MS considering that adequate reference or test materials are scarce and that production of a range of new materials to cover the range of nanoparticles in real samples would represent a massive long-term effort.

In [Formula \(19\)](#), some of the parameters such as the acquisition time and the sample uptake mass flow can be measured with high accuracy. Therefore, one other critical parameter that requires careful optimization is the number of nanoparticles detected. In order to optimize particle counting, several factors have to be taken into account, including ICP conditions, dwell time, threshold set up between the background signal and the particle events and the sample dilution factor. With regards to ICP optimization, the aim is to obtain maximum signal of the element of interest with a minimum background contribution.

The particle detection threshold is defined by the cut-off between background counts and counts arising from particle events. For nanoparticles whose size is close to the background, the position of the detection threshold can have a higher impact on the overall measurement uncertainty, therefore this parameter needs to be carefully considered for accurate measurement of the number concentration of particles. A basic approach for the discrimination of the particle events over the baseline produced by the presence of the background/dissolved analyte is the application of $(n - \sigma)$ criteria, where σ is the standard deviation of the baseline. Coefficients of three and five are commonly applied, although 7σ and 8σ criteria have also been used by some authors. Depending on the criterion adopted, the number of false positives can be significant.

The uncertainty for particle counting is related to systematic (bias) and random errors (due to the counting) which can be minimized by working at an optimal particle flux in which random errors and systematic errors are equal, while minimizing the formation of double and triple events.

Recent developments in the ICP instrumentation, such as micro-second dwell times and/or quasi-simultaneous multi-isotopic TOF (time of flight) detectors allowing multi-element and/or multi-isotope detection in spICP-MS mode, are of particular interest in characterization of complex materials, e.g. core/shell nanocomposites. In particular, as size limits of detection are less affected by the presence of dissolved species/background by using dwell times in the microsecond range, compared to milliseconds, the microsecond dwell detection capability has enabled the selective detection of nanoparticles from high levels of dissolved fractions; this is especially attractive for particles prone to dissolution (e.g. silver particles).

7.3.4 Sources of uncertainty and challenges

The two main factors contributing to the overall uncertainty in the measurement of number concentration by spICP-MS are uncertainty in the measurement of transport efficiency and variability in number of particles detected in a time scan of the sample.^[62] Measurement of transport efficiency using nanoparticle reference materials such as in the particle frequency method provides a direct measure of aerosol transport into the plasma but uncertainty in the particle diameter, variability in the number of particles detected in a time scan of the reference material, uncertainty in the mass concentration and uncertainty in the particle density contribute to the overall uncertainty of the measured transport efficiency. For the DMF method, under the working conditions reported in the literature,^[61] the uncertainty in the determination of transport efficiency has been demonstrated to be mostly based on mass measurements which can be accomplished with high accuracy and precision.

The main challenges in particle concentration determination with spICP-MS are associated with the very high dilution factors required by the technique. Dilutions have to be performed in a way that does not compromise the particle characteristics and stability and does not induce instrumental artefacts, such as multiple events. Another point to consider is the elemental composition of the material and the presence of the dissolved element in the sample, which all impact the lower size limits of detection. Care needs to be taken when working with millisecond dwell times to appropriately account for split events.

7.3.5 Outlook

spICP-MS as a tool for the determination of particle number concentration has matured significantly over the last decade. The measurement capability of spICP-MS, initially proven for simple suspensions of very stable gold and silver particles, has now been extended to more complex particles (e.g. silica and titania) in complex matrices (e.g. food, cosmetics, biological tissues) and has been applied to help the industry to comply with existing regulations.

Although many advances have been achieved so far, further developments are still anticipated because of some existing challenges. Better sample introduction interfaces need to be developed to achieve higher transport efficiencies with good long-term repeatability, enabling high sample throughput. More effort is also expected on the sample preparation arena, in particular when working with solid samples, to put in place validated techniques that are able to handle the large volumes of samples needed for toxicological and risk assessment studies.

The lack of reference materials for instrument calibration remains a key challenge. However, the recent development of a calibrant-free approach could open a new door to the characterization of reference and/or quality control materials that can be used with higher throughput techniques which require a reference material (e.g. the frequency technique) for a range of applications.

Finally, further software development is required in particular for techniques such as ICP-time of flight (ToF)MS which enables users to generate multi-isotopic information at the microsecond level in single particle mode. This will help obtain better detection selectivity for number concentration determination of particles of mixed elemental composition and core/shell materials.

7.4 Condensation particle counter and differential mobility analysing system

7.4.1 General

Several techniques are available to measure the airborne number concentration of nanoparticles.^[76] One such technique is a condensation particle counter and, when combined with size classification, the technique also provides number-based particle size distributions. Aerosol particle size is typically measured by differential mobility analysing system and the technique is described in ISO 15900.^[77] Size-specific nanoparticle number concentration can be provided when a CPC is used with a differential mobility analyser (DMA) and electrostatic classifier in a DMAS configuration. The technique can also be extended to liquid samples containing nanoparticles; here, the suspensions are typically introduced into the CPC or DMAS by electrospray or nebulizer.

The CPC technique is used to determine the number concentration of small aerosol particles usually less than a few micrometres in diameter. Condensation of supersaturated vapours is used to grow nanoscale particles to droplets of sizes that can be optically detected. Most CPCs use alcohol (e.g. butanol, isopropanol) or water as the working fluid. The most common CPCs use laminar flow and diffusional heat transfer. Continuous flow laminar CPCs have more precise temperature control than other types of CPCs and they have fewer particle losses than instruments which use turbulent flow.

The counting of the droplets is performed by optical light scattering. The droplet passes through a detection area where it is illuminated by a focused light beam and a portion of the scattered light is detected with a photodetector. The frequency of this event leads, with the known volume of sampled air, to the particle number concentration. At low concentrations, the CPC counts individual particles and allows an absolute determination of particle number concentration (single particle count mode). At higher concentrations some instruments include an evaluation of the total scattered light intensity without single particle counting and thus estimate the number concentration, based on assumptions of final particle size and optical properties (photometric mode). The photometric mode has much higher uncertainty associated with the number concentration of aerosol particles than the single count mode, as discussed in [7.4.2](#).

With DMAS, aerosol particles in the nanoscale size range can be measured to determine the parameters of the size distribution and the number concentration. The technique scans or steps through the size range over a period of time, of the order of a minute, and thus is most suitable for measuring the parameters of a steady-state concentration and size distribution. Additional uncertainty in the number concentration will arise if the concentration is varying during the measurement. Fast mobility sizing instruments, with response times on the order of one second, are available. These fast instruments use multiple aerosol electrometers instead of a CPC to count the particles and require higher concentrations than a DMAS to perform a measurement. The consequence of using fast aerosol spectrometers for sizing is that their use for number concentration has a much higher uncertainty.

7.4.2 Sample specifications

CPCs measure particle number concentrations of airborne particles in real-time. The measurement range of typical commercial instruments is between approximately 5 nm to 3 000 nm and concentration range between 10^6 m^{-3} and $5 \times 10^{10} \text{ m}^{-3}$ (single particle count mode) or between 10^6 m^{-3} and 10^{13} m^{-3} (photometric mode) depending on the instrument manufacturer and model. Most CPCs have lower size detection limits between 5 nm and 20 nm, whereas some speciality CPCs have detection lower limits

below 5 nm. Most recent CPCs are even able to detect particles down to 1 nm, by using diethylene glycol as either a working fluid or a size magnifier technique, or both.

In general, the detection efficiency of a CPC depends on the particle size and composition, and, to a lesser extent, the number concentration of the aerosol. The lower size detection limit of a CPC is characterized by d_{50} , a diameter at which the counting efficiency is 50 %. Besides d_{50} , the steepness of the detection efficiency curve is another quality criterion for a CPC. EN 16897^[78] and ISO 27891^[79] provide typical examples of the dependency of the counting efficiency on particle size. Particles larger than about 1 μm are frequently lost due to inertial impaction.

The maximum detectable number concentration of a CPC in single particle count mode is limited by coincidence counts. Coincidence will lead to an underestimation of the concentration and needs to be avoided. Devices with a photometric mode and detection system can measure the number concentration above this coincidence threshold. In some cases, there can be counting discrepancies (non-steady increase or decrease in the concentration value) when the CPC switches from single particle count mode to its photometric mode. The accuracy of the single count mode is usually $\pm 10\%$, while the photometric mode is known to be less accurate ($\pm 20\%$) and, hence, is less preferred depending on the measurement situation and the accuracy required. When the particle number concentration exceeds the maximum single particle count mode concentration, it is recommended that a dilution system, if possible, is used to maintain single particle count mode. Recent CPCs include software-based coincidence correction algorithms to extend the range of measurable number concentration and improve accuracy for high concentrations. Water-based CPCs can underreport particle number concentrations when measuring hydrophobic aerosols.

With DMAS, aerosol particles in a size range typically from 5 nm to 1 000 nm (dependent upon the specific instrument configuration) can be measured. One limitation is that only approximately one third of the particles entering a DMAS are sized and counted due to the aerosol neutralizer imparting a Boltzmann charge distribution, with algorithms based on aerosol physics used to estimate the concentration of particles not measured. Issues such as multiple charging of individual particles and diffusion losses can be corrected for in the software, however they do introduce additional uncertainties.

7.4.3 Technical aspects

7.4.3.1 Sampling line

There are two types of sampling losses that bias the measured concentration: the losses inside the sampling line (if used) and the losses inside the CPC or DMAS. Particle losses inside the CPC or DMAS are usually not explicitly considered but are often incorporated during calibration in the manufacturer's software. When the aerosol is sampled using a sampling line, particle losses (such as diffusion, inertial, impaction, interception, electrostatic and thermophoretic) need to be minimized. Diffusion losses are highly size dependent below 100 nm, increasing as particle size decreases. To minimize losses, the sampling line preferably is made of a material which is electrically conductive and of length as short as possible without bends, kinks or elbows. The flow in the sampling line is normally laminar^[76].

7.4.3.2 Background particle concentration

Depending upon the measurement scenario, the influences of indoor and outdoor aerosol particles on the number concentration measured with a CPC or DMAS can be significant. In general, if a measurement is to be made to isolate the impact of an activity or task in an open area such as a workplace, laboratory or office, the influence of background particle concentration is determined by taking measurements prior to the start of an activity or task and after or in the far-field background (a suitable distance to avoid interference from the activity or task) using a second measurement. The specific type of background sampling will depend on the unique sampling goal. For environmental test chambers (e.g. emissions testing of products), air entering the chamber normally passes through a high-efficiency particulate air (HEPA) filter to reduce background number concentration to an acceptable limit. High numbers of small background particles can strongly influence the result and, if possible, can be subtracted from the desired measurement.

7.4.3.3 Analysis of liquid-borne particles

The CPC technique can be extended to liquid samples containing nanoparticles. These suspensions are typically introduced into the CPC or DMAS by electrospray or nebulizers. Electrospray forces the conductive fluid through a capillary where an electric field is applied at the tip. As the fluid is pulled from the tip, it forms a Taylor cone that generates a stream of droplets. The droplets are mixed with flowing air or a mixture of air and CO₂. As the droplets evaporate, the remaining charged nanoparticles are neutralized in an ionization chamber. The way the liquid sample is delivered to the electrospray unit can vary depending on the instrument set up.^[63] In a typical setup, the electrospray delivers mostly singly charged particles to the DMA column where they are classified by mobility size and then counted by the CPC.

The use of a Collison nebulizer followed by an aerosol dryer is also effective in producing a nanoparticle aerosol, typically multiply charged, which needs to be neutralized if it is to be classified by mobility size using a DMA. There are many challenges in producing an aerosol with a number concentration that can be related to the concentration in suspension, as there is limited understanding of the transfer efficiency from suspension to aerosol, as well as transport losses and coagulation. Measurement of aerosolized nanoparticle suspensions is more effective at determining the concentration of the particles in aerosol than the concentration of the nanoparticles in suspension.

7.4.3.4 Approaches to the calibration for concentration measurements

The CPC instrument requires calibration, which can be performed through two distinct approaches: by comparison with a Faraday-cup aerosol electrometer (FCAE) and by comparison with a reference CPC. Both these approaches are described in ISO 27891^[79], together with critical considerations to evaluate the associated uncertainty. The result of a calibration is the particle detection efficiency for an individual CPC instrument with specified operating parameters, and for specific cases of particle size, particle type and particle number concentration.

ISO 27891^[79] recommends that before and after a series of measurements are performed, the air flow and zero count of the instrument are checked. The air flow is measured using a calibrated gas flow meter and the zero-count check is carried out with a HEPA filter fitted at the inlet of the CPC. When multiple instruments of the same model are available, their performance can be compared via measurements of laboratory-generated calibration/reference aerosols such as particle size standards.

DMAS calibration for number concentration is typically performed by calibrating the CPC component of the DMAS as described earlier in 7.4.

When an aerosol is sampled using a sampling line, the multiple loss mechanisms can be calculated and corrected for with the knowledge of the flow rate, the sample line diameter, bends, temperature gradients and the particle size distribution. When some or none of these parameters are not known, then the corrections for losses can be approximated in some cases, with greater uncertainties. The use of a dilution system requires measurement of the dilution factor, which introduces another source of uncertainty to the measurement of number concentration.

For nanoparticles in suspension, internal calibrants have shown promise to increase the accuracy of particle concentration measurements^{[64],[65]}.

7.4.3.5 Measurement

Particles are aspirated into the CPC probe inlet at a known flow rate and grow in size by passing through a saturated vapour from a working fluid as they travel through the instrument. Particles exposed to supersaturation that are larger than the Kelvin diameter for the noted supersaturation level are activated, thereby undergoing nucleation and subsequent condensational growth. The grown particles (droplets composed of the working fluid) travel through a measurement chamber where they are illuminated by a laser and counted by a photodetector.

Particle number concentration (in m^{-3}) is calculated according to [Formula \(27\)](#):

$$C_n = \frac{n_{\text{CPC}}}{1\,000 \cdot tQ} \quad (27)$$

where

C_n is the particle number concentration, expressed in m^{-3} ;

n_{CPC} is the number of particles counted by the CPC;

t is the sampling time, expressed in s (usually 1 s);

Q is the sample flow rate, expressed in $\text{l}\cdot\text{s}^{-1}$;

1 000 is the conversion factor from l to m^3 .

For DMAS measurements of number concentration, particles are neutralized, classified by their mobility diameter and counted with a CPC. The instrument software performs corrections to account for the fraction of the particles within the Boltzmann charge distribution that are not counted, multiple charges and internal diffusion losses before producing the mobility diameter distribution information. The number concentration measurement is calculated by integrating the mobility diameter distribution over all measured size bins.

7.4.4 Sources of uncertainty and challenges

7.4.4.1 Introduction

The accuracy of a CPC depends on several parameters, such as the accuracy and variation of the air flow rate, measurement bias due to loss of working fluid, count losses at very high concentrations due to coincidence and vapour depletion effects, inefficient condensation on the surface of particles for very small particles, and losses from the aerosol inlet to the detection zone. Other influences such as the time to adapt to rapidly changing concentrations or effects due to a highly charged aerosol can play an additional role. More information, especially on uncertainty components, can be found in ISO 27891^[79] and EN 16966.^[76] The accuracy of DMAS depends on the same parameters for its CPC component, uncertainties associated with the many corrections and calibration of the high voltage in the DMA.

7.4.4.2 Flow rates

For particle number concentration measurements, the volumetric flow rates of the carrier gas mixture can be measured with calibrated mass flow meters, provided the temperature and pressure are also measured. If attempting to assess the concentration in suspension, it is essential to also measure the air displacement injection/spraying flow rate. Uncertainties in these flow rates directly impact the accuracy of measurement of the number concentration of the nanoparticles in solutions. When performing DMA size selection upstream of the CPC, the size resolution is inversely proportional to the aerosol-to-sheath flow rate ratio and, therefore, can be controlled by modulating these flows.

7.4.4.3 Depletion of working fluid

Working fluid is consumed during particle concentration measurements and needs to remain at a sufficient level to ensure accurate counting statistics. Most commercial CPCs have a built-in indicator (light or audible sound) to indicate that the working fluid volume is low. Anytime the working fluid is replenished, data collected prior to and immediately after the event needs to be evaluated and a determination made as to whether the depleted fluid level introduced a bias into the measurements.

7.4.4.4 Aerosolization of liquid-borne particles

Dissolved solutes in a nanoparticle liquid dispersion, such as salts and unbound molecules, form small residual particles during the aerosolization process that can give rise to a particle population that can overlap with that of the nanoparticles. Even ppm levels of impurities can produce significant numbers of residual particles. These non-volatile species can also deposit onto the nanoparticle surface, resulting in a bias of the size measurement towards a larger mobility diameter. These effects are particularly significant for smaller particles. One method for removing the small residual particles is the use of diffusion screens. Sample pre-treatment, for example, through repeated centrifuge-based cleaning to remove non-volatile additives (e.g. surfactants), can be effective in removing unbound solutes, but can also cause particle aggregation and loss. Volatile ammonium acetate salt is commonly used to control the solution conductivity for effective electro spraying. If the “salt” particles generated by the electro spray are very small compared with the particles of interest, then it is possible to simply exclude them from the particle count by using a DMAS. It is not possible to tune a CPC to exclude these residual particles; however, if there is prior knowledge of the size distribution of residual particles, then a CPC instrument with lower size cut-off that exceeds the size of residual particles can be used to exclude them. With a DMAS, the portion of the size distribution with residual particle can be corrected for or removed when measuring the number concentration of the aerosolized nanoparticles from suspension.

Another challenge to measure the number concentration in suspension from the aerosol number concentration is producing a steady aerosol concentration during aerosolisation. This does not apply when measuring the aerosol number concentration only. For electro spray systems, particle adsorption in the capillary can significantly reduce the particle number concentration and reduce the sample flow rate. Any restriction within the capillary reduces an already limited aperture size, generally on the order of tens of micrometres. Commonly used silica capillaries are generally pre-treated with a surface coating; however, the effectiveness of the coating depends on the particles of interest. A potential reduction of sample flow can be monitored by the transport current and needs to be corrected. Capillaries need to be cleaned after each use to maintain good performance. For Collision nebulizers, the challenge is in knowing the transfer efficiency from suspension to aerosol. For DMAS measurements of number concentration, it is essential to make corrections to account for the fraction of the particles within the Boltzmann charge distribution that are not counted, the multiple charges on particles, and the internal diffusion losses.

An alternative method exists to measure the number concentration in a solution based on an electro spray artefact: droplet-induced aggregation. A suspension consisting of only monomers will still yield some dimers, trimers, etc. when measured by a DMAS due to the aerosolisation process where two or more particles are captured within a single droplet. This process depends on the monomer diameter, suspension number concentration and aerosolisation droplet diameter. The measurement requires a monodisperse particle and monodisperse aerosolisation droplet distribution. The dimer-to-monomer ratio (and similar for trimers, etc.) is measured over a range of dilutions. The rate at which the dimer-to-monomer ratio changes with dilution can be used to calculate the solution number concentration^[66] with significant uncertainties, including the polydispersity of both the nominally monodisperse particle and droplet size distributions.

The connection between the aerosolisation device and the DMAS needs to include a pressure relaxing bypass. The flow through this bypass will bias the measurement and needs to be monitored by a calibrated flowmeter and recalculated. The connection between the aerosolisation device and the DMAS needs to be conductive to avoid electrostatic deposition and as short as possible to minimize diffusion losses. All mechanisms for sampling losses need to be calculated and corrections applied.

The feeding system to an electro spray device can further influence the steady aerosol concentration. It is essential to ensure that the feeding system always delivers the same amount of liquid to the electro spray system, with the same pressure. Similarly, for a Collision nebulizer, it is necessary for the air flow and pressure to remain consistent to aid in maintaining a steady aerosol concentration.

7.4.5 Outlook

CPC and DMAS instruments provide well-established and powerful techniques for the measurement of particle number concentration in aerosols. While robust methods, some emphasis needs to be placed

on the further optimization of the measurement efficiency and improving reproducibility of results. The two techniques can provide different results for the number concentration, which to some degree can be addressed by careful calibration but can also require intra- or interlaboratory comparison to quantify.

Substantial issues also remain for the quantitative measurement of nanoparticle number concentration in liquid suspensions. More efficient and robust electrospray or nebulizer systems are needed to address the point of greatest uncertainty and variability for the analysis of liquid dispersions. While less robust, a broader range of commercially available calibration standards and methods would be highly desirable and can significantly improve the applicability of assessing nanoparticle concentrations in suspension.

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Annex A (informative)

Summary of VAMAS international interlaboratory studies

A.1 Introduction

A large international interlaboratory comparison of five measurement methods for nanoparticle number concentration was organized under the umbrella of the Versailles Project on Advanced Materials and Standards (VAMAS). VAMAS is an international organization that supports world trade in products dependent on advanced materials technologies, through international collaborative projects aimed at providing the technical basis for harmonized measurements, testing, specifications and standards. It organizes pre-normative interlaboratory studies to test methods that can be a precursor to standardization in ISO.

This annex summarizes the rationale and outcome of the Project 10 of the VAMAS Technical Working Area (TWA) 34 (nanoparticle populations)^[68]. The lead organization, the National Physical Laboratory, provided 53 laboratories across the world with a nanoparticle test sample together with a measurement protocol and a reporting form in October 2017. The results were collected largely in 2018. To gather comparative data across different methods, the different laboratories measured the same sample, i.e. 30 nm gold nanoparticles, with multiple techniques, namely spICP-MS, PTA, SAXS, UV-visible spectroscopy and DCS. The aim of the study was twofold: on one hand, the study wanted to provide comparative data on the accuracy and precision of the methods; on the other hand, the study wanted also to identify the best practice in the use of the methods and the related sample preparation procedures.

In order to also evaluate the accuracy of the methods, the VAMAS study was coordinated with the pilot study P194 of the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) of the International Bureau of Weights and Measurements (BIPM)²⁾ that was led by LGC and ran in parallel. This consisted of an interlaboratory comparison amongst the international community of national measurement institutes (NMIs) of the measurement of colloidal number concentration of the same batch of particles utilized for the VAMAS study.

A.2 Sample

The samples for both studies were produced by LGC (Teddington UK) as 5 ml vials with part number LGCQC5050 and consisted of gold colloids in water with a nominal size of 30 nm. The vials were sterilised by gamma-irradiation and homogeneity and stability tests were performed by LGC to ensure the product was consistent and stable for the entire duration of the studies. The BIPM pilot interlaboratory study resulted in a consensus value for the number concentration of the colloidal gold nanoparticle sample of $(1,42 \pm 0,12) \times 10^{14} \text{ kg}^{-1}$ where the uncertainty is expressed with a confidence level of 68 % ($k = 1$). Each participant to the VAMAS study was provided with five vials of the LGCQC5050 product.

A.3 VAMAS measurement protocol

A.3.1 Preparatory steps

Both electronic and paper versions of the measurement protocol were provided to all the VAMAS participants together with the samples. The protocol was authored by a team of scientists at NPL and LGC and published as the NPL Report AS 98.^[67] This contains recommendations on how to handle and prepare the samples and how to undertake the various measurements and determine the concentration

2) This study has not been published yet.