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The NanoDefine Methods Manual

Part 2: Evaluation of methods



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Legal Note

This document contains general recommendations supporting the user in the decision whether a material is a nanomaterial according to the EC Recommendation on the Definition of Nanomaterial (Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). OJ L 275, pp. 38-40). However, users are reminded that the texts of the appropriate EC legal acts are the only authentic legal reference and that the information in this document does not constitute legal advice. Usage of the information remains under the sole responsibility of the user. The NanoDefine Consortium Partners do not accept any liability with regard to the contents of this document.

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NanoDefine

Development of an integrated approach based on validated and standardised methods to support the implementation of the EC recommendation for a definition of nanomaterial

The NanoDefine Methods Manual

Part 1: The NanoDefiner Framework and Tools

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement n° 604347

Website: <http://www.nanodefine.eu/>
Project co-ordinator: Wageningen Food Safety Research (WFSR), NL

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About the NanoDefine Methods Manual

The present series of reports, **the NanoDefine Methods Manual**, has been developed within the NanoDefine project 'Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation for a definition of nanomaterial', funded by the European Union's 7th Framework Programme, under grant agreement 604347.

In 2011 the European Commission (EC) published a recommendation for a definition of the term 'nanomaterial', the EC NM Definition, as a reference to determine whether an unknown material can be considered as a 'nanomaterial' for regulatory purposes¹. One challenge is the development of methods that reliably identify, characterize and quantify nanomaterials (NM) both as substances and in various products and matrices.

The overall goal of NanoDefine was to support the implementation of the EC NM Definition. It can also support the implementation of any NM definition based on particle size. The project has developed an integrated approach, which allows identifying any material as a nano- or not a nanomaterial according to the EC NM Definition. NanoDefine explicitly supported the governance challenges associated with the implementation of legislation concerning nanomaterials by:

- addressing the issues on availability of suitable measuring techniques, reference materials, validated methods, acceptable to all stakeholders (authorities, policy makers, commercial firms),
- developing an integrated and interdisciplinary approach and a close international co-operation and networking with academia, commercial firms and standardization bodies.

Thus, the NanoDefine Methods Manual provides guidance on practical implementation of the EC NM Definition throughout the nanomaterial characterization process, and on the characterization techniques employed as well as their application range and limits. It assists the user in choosing the most appropriate measurement method(s) to identify any substance or mixture for a specific purpose, according to the EC NM Definition of a nanomaterial. The NanoDefine project also explored how to assess a material against the criteria of the definition through proxy solutions, i.e. by applying measurement techniques that indirectly determine the x_{50} . Those findings were developed through empirically based scientific work and are included in Part 1 of this Manual. As they go beyond the text of the EC NM Definition, they may be used as practical approach to indicate whether a material is a nanomaterial or not, but keeping in mind that they should not be taken as recommendation for the implementation of the EC NM Definition in a regulatory context.

The NanoDefine Methods Manual consists of the following three parts:

- Part 1: The NanoDefiner Framework and Tools
- Part 2: Evaluation of Methods
- Part 3: Standard Operating Procedures (SOPs)

Part 1 covers the NanoDefiner framework, general information on measurement methods and performance criteria and tools developed by NanoDefine such as a materials categorisation system, a decision support flow scheme and an e-tool.

Part 2 discusses the outcome of the evaluation of the nanomaterials characterisation methods for measuring size.

Part 3 presents the 23 Standard Operating Procedures developed within the NanoDefine project.

The current document is part 2.

Abbreviations and acronyms used in the Manual

| | |
|------------------|---|
| AC | Analytical Centrifugation |
| AF4 | Asymmetrical Flow Field-Flow-Fractionation |
| AFM | Atomic Force Microscopy |
| ALS | Angular Light Scattering |
| Aq. | Aqueous |
| AR | Aspect Ratio |
| AUC | Analytical Ultra Centrifugation |
| BET | Brunauer-Emmett-Teller |
| BSA | Bovine Serum Albumin |
| CM | Characterisation Method |
| CEN | European Committee for Standardization |
| CFFF | Centrifugal Field-Flow-Fractionation |
| CLS | Centrifugal Liquid Sedimentation |
| CPC | Condensation Particle Counter |
| DEMA | Differential Electrical Mobility Analysis (also spray-DEMA) |
| DMA | Differential Mobility Analyser |
| DLS | Dynamic Light Scattering |
| DSFS | Decision Support Flow Scheme |
| DUM | Dynamic Ultramicroscopy |
| EC | European Commission |
| EC NM Definition | EC Recommendation on the Definition of a Nanomaterial |
| EDX / EDS | Energy Dispersive X-ray spectrometry |
| EELS | Electron Energy Loss Spectroscopy |
| EFTEM | Energy-Filtered Transmission Electron Microscopy |
| EHS | Environment, Health and Safety |
| EM | Electron Microscopy |
| ESD | Equivalent Spherical Diameter |
| ESI-SMPS | Engineering System International SMPS |
| ESZ | Electrical Sensing Zone |
| FFF | Field-Flow-Fractionation |
| FTIR | Fourier-transform Infrared Spectroscopy |
| HSE | Health, Safety and Environment |
| ICP-MS | Inductively Coupled Plasma - Mass Spectrometry |
| ICP-OES | Inductively Coupled Plasma - Optical Emission Spectrometry |
| KB | Knowledge Base |
| LD | Laser Diffraction |
| LoD | Limit of Detection |
| LS | Light Scattering |

| | |
|----------|--|
| MALS | Multi-Angle Light Scattering |
| MALLS | Multi angle laser light scattering |
| MCS | Material Categorisation Scheme |
| MT | Measurement Technique |
| MWCNT | Multi-walled Carbon Nanotube |
| m/z | Mass-to-Charge Ratio |
| NaDS | Sodium Dodecyl Sulphate |
| NM | Nanomaterial |
| NTA | Nanoparticle Tracking Analysis |
| NP | Nanoparticle |
| PSD | Particle Size Distribution |
| PTA | Particle Tracking Analysis |
| QELS | Quasi Elastic Light Scattering |
| RI | Refractive Index |
| SAXS | Small-Angle X-ray Scattering |
| SDS | Safety Data Sheet |
| SEM | Scanning Electron Microscopy |
| SEM-EDX | SEM-Energy Dispersive X-ray Analysis |
| SedFFF | Sedimentation Field-Flow-Fractionation |
| SFM | Scanning Force Microscopy |
| SLS | Static Light Scattering |
| SMPS | Scanning Mobility Particle Sizer |
| SOP | Standard Operating Procedure |
| spICP-MS | Single Particle ICP-MS |
| TEM | Transmission Electron Microscopy |
| TRPS | Tuneable Resistive Pulse Sensing |
| UF | Ultrafine |
| USB | Ultrasonic Bath Sonicator |
| USP | Ultrasonic Probe Sonicator |
| USSp | Ultrasonic Spectroscopy |
| UV | Ultra Violet |
| UV-vis | Ultra Violet - Visible |
| VS | Vial Sonicator |
| VSSA | Volume-Specific Specific Surface Area |

Executive summary

This document is Part 2 ("Evaluation of methods") of the NanoDefine Methods Manual. It is based on the results of a comprehensive study performed within the NanoDefine project ('NanoDefine') of the available measurement techniques, which are candidates for performing a reliable analysis of the number-based size distribution of a particulate material, with the goal to identify nanomaterials according to the European Commission recommendation on the definition of nanomaterial¹. NanoDefine was executed under the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement n° 604347.

Based on the performance criteria established in NanoDefine the potential measurement techniques were evaluated (i) according to studies available in the literature, (ii) in a comparative interlaboratory study with selected real world materials ("NanoDefine priority materials"), as well as (iii) through the expertise of the NanoDefine consortium partners. The detailed information on the evaluation process is presented in NanoDefine Technical Reports which can be found on the project website at <http://www.nanodefine.eu/index.php/nanodefine-publications/nanodefine-technical-reports>.

This document discusses most of the available size measurement techniques for nanomaterials. The different types of the methods that allow the determination of size and size distributions are explained and an overview of techniques and their capabilities is presented in user-friendly overview tables. These tables provide the reader with a possibility for quick selection of the method(s) which may be appropriate for the characterisation of given material. For the final selection of the method(s) to be employed it is highly recommended to use the detailed performance tables. These are described in the four main sections of the report. Each section introduces one of the four types of methods: counting, fractioning, spectroscopic ensemble and integral methods and explains the basic principles of that type of method as well as its performance, advantages and disadvantages.


This report presents the outcomes of the evaluation in two types of tables per method: one table that presents general performance information highlighting also advantages and disadvantages of the technique. The second table provides additional and very detailed information on the capabilities of the method.

1 Introduction

This report is Part 2 of the NanoDefine Methods Manual and provides the 'Evaluation of methods'. It reflects the outcomes of a comprehensive study performed within the NanoDefine project on the available measurement techniques, which are candidates for performing a reliable analysis of the number-based size distribution of a particulate material, with the goal to identify nanomaterials according to the European Commission recommendation on the definition of nanomaterial¹ the EC NM Definition. Based on the performance criteria already established in NanoDefine and the NanoDefine Technical Reports, which all can be found on the project website², these measurement techniques were evaluated (i) according to studies available in the literature, (ii) in a comparative interlaboratory study with selected real world materials ("NanoDefine priority materials", see Annex 1 for a list of these materials), and (iii) through the expertise of the NanoDefine consortium partners. The detailed information about the evaluation process is presented in the NanoDefine Technical Reports.

This document discusses most of the available size measurement techniques for nanomaterials, see Figure 1. The methods described are divided into four main groups, which are counting, spectroscopic ensemble, fractioning and integral methods. For each evaluated measurement technique, the basic principles are briefly explained and described in a dedicated section.


An overview of the techniques and their capabilities is presented in the form of three types of user-friendly tables. The first table type of table is presented in section 0, which presents overview tables of the evaluation of all the methods (see Table 3 to Table 6), providing the reader with the possibility for a quick selection of the method(s) which may be appropriate for the characterisation of given material. As the purpose of this report is to present the outcomes of evaluation the size measurement methods these overview tables are also the conclusions of this report.

The second and third types of tables, general performance tables and technical characteristics tables, give additional and much more detailed information on the capabilities of each method. The performance tables give an overview of main features, main advantages and main disadvantages for each measurement technique. The technical characteristics tables first present the material-related technical characteristics of the criteria, and then the metrological aspects of the technique, and the content of the technical characteristics tables is explained in section 2.2.1. The chapters 3 to 6 of the report have 11 sections that present one general performance table and one technical characteristics table for each measurement technique. In particular, these tables highlight any further information that should be considered for the choice of the most appropriate method with a yellow warning sign  to draw attention of the reader. Furthermore, the column "notes" lists additional relevant information that may be of particular importance for specific cases.

It is highly recommended to use the technical characteristics tables, which are described in this report, for final selection of the method(s) to be employed.

2 Measurement techniques

This chapter presents the summary of the results of a comprehensive study performed within the NanoDefine project. NanoDefine studied, among others, the available measurement techniques, which were candidates for the reliable analysis of the number-based size distribution of a particulate material with the goal to identify nanomaterials according to the EC NM Definition.

The following chapters explain the basic principles of each evaluated method and general performance information is presented in a dedicated table per technique, which also highlight main advantages and disadvantages of the technique. Furthermore, additional detailed information on the performance and suitability of each method is presented as a table which allows the user a quick and simple identification of the question (criterion) and clear answer. Additional information to take into consideration when choosing the most appropriate method is highlighted with the sign . It is also strongly suggested to pay attention to the information included in the column “notes” as it may be important for specific cases.

As already stated in chapter 1 different sets of tables help the reader to identify and select the (most) appropriate technique for given material. A non-exhaustive list of relevant international standards (ISO) on particle sizing is included in Annex 2 of this document.

2.1 General introduction to the methods

There are several means and physical phenomena that allow for the determination of size and size distributions (e.g. imaging, sedimentation, light extinction). A more general distinction of particle sizing techniques is based on how the weights of the individual size fractions are determined.

- counting techniques (measuring particle properties at the level of individual particles)
- ensemble techniques (measuring the spectral or parametric response of a representative particle ensemble of the total particle system)
- fractionating techniques (measuring the amount or concentration of size/property classes after fractionating the particle system)
- integral methods (measuring effective properties at the level of a whole particle system, without being able to provide a size distribution)

Figure 1 gives an overview of measurement techniques that can be used for each of the four types of methods, and a description of each type of method is given below.

Screening methods give an initial indication of whether a given material is a nanomaterial according to the EC NM Definition or not and may under favourable conditions allow identifying nanomaterials. These methods are called tier 1 methods. The tier 2 methods (currently only EM methods are recommended by NanoDefine) are confirmatory methods that allow to unambiguously determine if a given material is a nanomaterial according to the EC NM Definition or not.

2.1.1 Counting methods

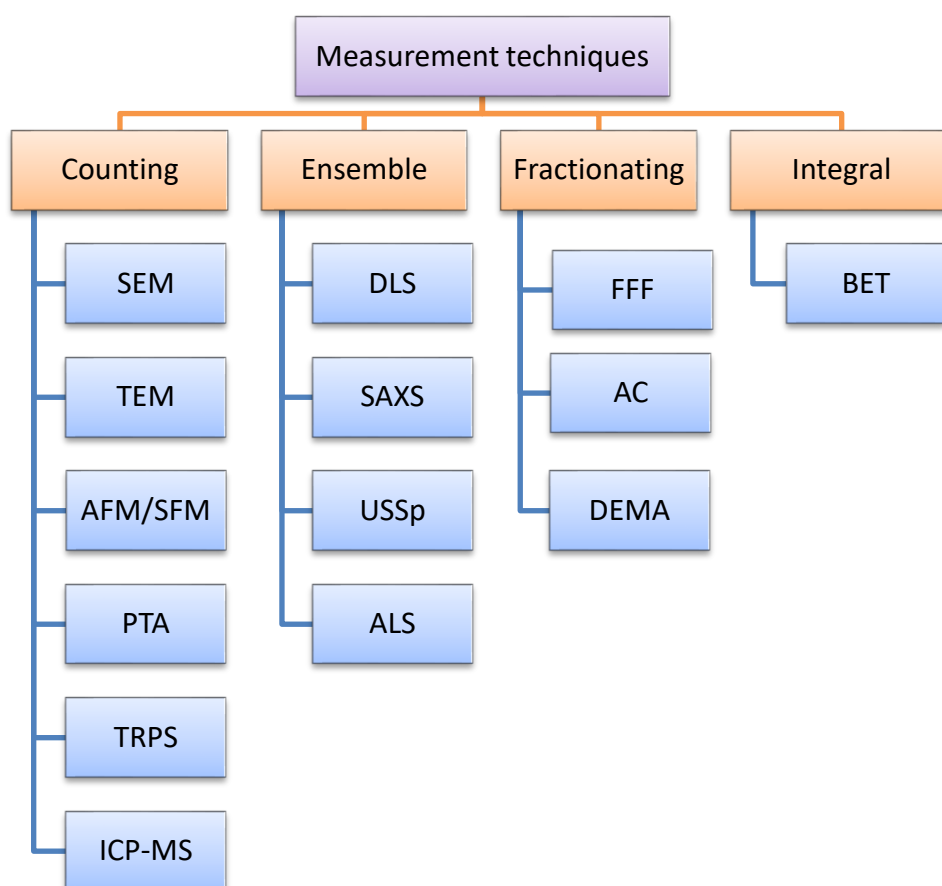
In the counting methods individual particles are measured and counts of similar-sized particles are placed into a "size bin" or size class to construct a size distribution.

Counting methods inherently yield particle number weighted distributions (Q_0) of a certain particle property or of a physical quantity that is related to a certain particle property (e.g. particle size, or the average displacement as a measure of the diffusion coefficient). They rely on the individualisation of the

particle sample, which can be either achieved by analysing microscopy images (e.g. from electron microscopes), by sufficient sample dilution or by reduction of the sample or measurement volume. The probed particle property may be either geometric (in particular for image analysis), optical (e.g. scattering cross section), or related to mobility (diffusion coefficient).

2.1.2 Ensemble methods

In the ensemble methods (Spectroscopic) all particles in the sample are measured at the same time and the size distribution is extracted from a combined signal from all particles. The immediate result of this type of method is the variation of the measured signal g over the spectral parameter s (time, space or frequency). Each size fraction x has a characteristic spectrum $k_r(s,x)$, which in general covers the whole range. Assuming that each size fraction contributes independently and linearly to the measured signal, the determination of the size distribution requires the inversion of a linear integral equation (Fredholm type). The intrinsic type of quantity is not necessarily obvious; it refers to the impact of a single particle to the integrated signal. The probed particle property of an ensemble method frequently relates to the particle mobility (diffusion) or to its interaction with external fields (scattering, extinction).



ABBREVIATIONS: COUNTING: EM: Electron microscopy. SFM: Scanning force microscopy (SFM) / AFM: atomic force microscopy. PTA: Particle tracking analysis / DUM: Dynamic ultramicroscopy. TRPS: Tunable Resistive Pulse Sensing / ESZ: Electrical sensing zone / nano Coulter counter. spICP-MS: Single particle ICP-MS. ENSEMBLE: DLS: Dynamic light scattering. SAXS: Small-angle X-ray scattering. USSp: Ultrasonic spectroscopy. ALS: Angular light scattering, including LD: Laser diffraction (LD). FRACTIONING: DEMA: Differential electrical mobility analysis. FFF: Field-Flow-Fractionation. AC: Analytical centrifugation. INTEGRAL METHODS: BET for determination of volume specific surface area (VSSA).

Figure 1: Measurement techniques for particle size distribution described in this report

2.1.3 Fractionating methods

In the fractionating methods an external force/process is used to separate particles according to their size; subsequently the quantities of the separated different sizes or size fractions are determined to construct a size distribution.

Fractionating methods include the two steps of fractionation and detection. The former can either result in a physical separation of the different size classes or in the depletion of coarse or fine particles in the measurement zone. In the case of colloidal suspensions, the fractionating effect is usually related to the mobility of the particles (e.g. settling velocity). The detection system monitors the fractionation process and thus serves for evaluating the class frequencies. Detection frequently employs phase shift, extinction or scattering of some radiation (e.g. X-rays). The applied detection system determines the type of quantity in which the size fractions are intrinsically weighted (e.g. extinction of X-rays is mass proportional – Q_3).

2.1.4 Integral methods

In addition to the methods that resolve the distribution of particle sizes, there are a few methods which solely measure an integral (effective/mean) property of a particle system such as the specific surface area (S_v or S_m) or the turbidity of a suspension. These properties can be directly converted into mean values of a PSD (e.g. $S_v \rightarrow$ harmonic mean of the volume weighted PSD). Note that ensemble methods, in principle, also yield such integral properties (e.g. the mean decay of signal fluctuation in DLS gives χ_{cum} , i.e. the harmonic mean of the intensity weighted size distribution).

2.2 Performance criteria

For the purpose of the NanoDefiner framework performance criteria of each measurement method were elaborated in detail; they include: applicability to different groups of substances (chemical scope of the method), applicability to polydisperse samples, capability to measure aggregates, agglomerates, constituent particles (in agglomerates/aggregates) and/or non-spherical particles, accuracy of the results determined with the measurement technique, standardisation status (traceability of the measured values / availability of CRMs).

Each measurement technique was evaluated against these criteria depending on the substance to analyse (if the measurement technique is suitable for this type of substance) and on the technical factors. The applied criteria, which are discussed below, are not listed according to their priority. The presented tables include more information than eventually was included into the final Material Categorisation Scheme and NanoDefiner e-tool, but as these detailed data are available they are included in the report for information and possible use.

2.2.1 Applicability to different groups of substances

This section discusses criteria which affect the suitability and performance of particles sizing methods. Particle sizing methods were assessed against these criteria, and the detailed results can be found in the NanoDefine methods performance tables in chapters 3 to 6 of this report.

2.2.1.1 Nanoparticles in powder, or liquid suspensions or embedded in a matrix

Certain measurement techniques are only applicable to nanoparticles dispersed in a liquid phase; others are for aerosols or for powders and granulates. For the characterisation of nanomaterials e.g. in articles or formulations it is necessary to know if a method can characterise nanoparticles embedded in a matrix. One should also take into consideration that some nanoparticles may change significantly during sample

preparation, which restricts the possible dispersion medium and thus excludes certain measurement techniques. As the physical state of the sample has a major impact on the choice of the measurement technique the primary grouping was done based on the type of the physical state of the sample:

- Dispersed in liquids
- Solid particulate form (powder...)
- Dispersed or embedded in different types of matrices (paste, resin, elastomer...)

2.2.1.2 Dispersibility according to dispersion protocols

Some measurement techniques may be applied only to dispersed materials (in liquid and gas). Therefore it is essential to know if the substance can be dispersed by standardised protocols that specify both a dispersion medium and a dispersion protocol.

Measured size distributions can be severely affected by sample preparation protocols, for instance by the choice of the dispersion media and the particle concentration but also by the selected dispersant, which controls the state of aggregation and agglomeration. It should be underlined that the surface chemistry of particles strongly affects their dispersibility behaviour hence they are divided in five major groups:

- Dispersible in aqueous media (by generalised protocols)
- Dispersible in non-polar liquids (by generalised protocols)
- Dispersible in polar liquids other than water (by generalised protocols)
- Dispersible in material-specific media (by specific protocols)
- Can be aerosolized

2.2.1.3 Nature of the substance

One of the most important criteria is the nature of the substance considering that the measurement technique has to be chosen and/or adapted accordingly. For instance, the lower size limit of spICP-MS is directly related to the elemental composition of the material, and organic particles cannot be detected (in the nanorange).




Consequently materials can be grouped as follows:

- Inorganic materials such as metals, ceramics, salts, oxides (significant content of inorganic elements homogeneously incorporated in all constituent particles)
- Particles which exhibit size-dependent absorption of photons / fluorescence (metals, quantum dots³...)
- Carbon-based (CNT, nanodiamond, carbon black...)
- Organic, particulate (polymers, dyes, etc., nanonized, precipitated)
- Organic, non-particulate (dendrimers, liposomes, supramolecular assemblies...)
- Biological (nucleic acid, peptide, protein)
- Composite particle
- Other

A **composite** is a solid substance where each particle consists of two or more phase-separated constituents⁴. Depending on the internal structure, **composite particles** can be divided into three types, see Table 1. As the structure of the composite particle may influence the result of the analysis it is

necessary to acquire detailed knowledge on the composite particles structure in order to apply a suitable measurement technique including the correct data evaluation.

Table 1: Types of composite particles (Reproduced from Ref. [5] with permission from the Royal Society of Chemistry).

| Type of composite particle | Visualisation |
|---|---|
| Core-shell particles consist of at least two components, one of which (the core) lies within the other that forms the outer layer (the shell). |  |
| Multishell particles are core-shell particles with more than one outer layer (shell) |  |
| Particles with inclusions are particles in which the components are phase-separated from each other and one phase is dispersed in the other and forms the inclusions. The number and size of the domains can vary, and their spatial distribution within the particles is often not uniform. |  |

2.2.1.4 Particle shape and number of small dimensions

Many of the currently employed measurement techniques implicitly assume that the particles are spherical or yield an equivalent spherical size, which severely limits their applicability to particles with non-spherical shape. Furthermore, plate- or fibre-like particles requires specific methods, which allow to measure the smallest dimensions of the particles as often this is the dimension which should be assessed to determine if the material meets the EC NM Definition requirements. An additional difficulty occurs if the analysed sample consists of a mixture of particles of different shapes. In such case, only EM and possibly scanning probe microscopy could yield reliable results. Even with EM, the analysis of plate-like particles is problematic as the smallest dimension (thickness) could be difficult to access.

Considering the characteristics of available analytical methods, the criteria of shape and number of nanoscale dimensions of nanoparticles can assume the following values:

- 1, 2 or 3 (number of nanoscale dimensions)
- Mixture of nanoparticles with different shapes

In principle, indication of the number of small dimensions is sufficient, but often it is helpful to characterise particle shape with more descriptive terms. They are also included here regardless of some redundancy with the criterion of number of small dimensions. Descriptive criteria for particle shape are:

- One small dimension: plates (flat shapes incl. irregular flakes) (ratio thickness:lateral extension ≤ 0.25)
- Two small dimensions: fibres (elongated shapes such as tubes, fibres, rods) (length:diameter ≥ 3)
- Three small dimensions: Spherical, equiaxial or similar (e.g. prismatic, cubic, tetrahedral)
- Mixture of nanoparticles with different shapes
- Other (incl. unknown)

2.2.1.5 Thermal degradation sensitivity

Some measurement techniques may lead to a thermal load on the sample and consequently alter their chemical or physical properties. Therefore it is important to know if a given measurement technique can cause damages to heat sensitive samples, which would hamper the validity of such measurements. Subsequently substances may be categorised as below:

- Sensitive above 0 °C
- Sensitive above 25 °C (room temperature)
- Sensitive above 37 °C (body temperature)
- Sensitive above 50 °C
- Sensitive above 100 °C
- Sensitive above 150 °C
- Sensitive above 500 °C
- Sensitive above 1000 °C

The entry 'yes' in the performance table means that the method can be used to measure a material with the stated sensitivity.

2.2.1.6 Cooling degradation sensitivity

It is important to know if the measurement technique may cause damage to temperature sensitive samples during a cooling process, hence jeopardising the validity of such measurements. Consequently substances may be divided in six general groups:

- Sensitive below 25 °C
- Sensitive below 0 °C
- Sensitive below - 18 °C (freezer)
- Sensitive below - 35 °C (deep freezer)
- Sensitive below - 78 °C (dry ice)
- Sensitive below – 195 °C (liquid nitrogen)

The entry 'yes' in the performance table means that the method can be used to measure a material with the stated sensitivity.

2.2.1.7 Electron beam sensitivity

This criterion takes into account that some substances are sensitive to electron irradiation and therefore cannot be characterised reliably with EM, or require more sophisticated EM techniques, such as cryo-EM or low-dose techniques:

- Sensitive to electron beam
- Not sensitive to electron beam

The entry 'yes' in the performance table means that the method can be used to measure a material with the stated sensitivity.

2.2.1.8 Particle size dispersity and modality

All samples have, to a certain degree, a polydisperse particle size distribution. Therefore it is important to recognize if a specific measurement technique can be used to analyse polydisperse samples without obtaining false results. Hence dispersity and modality of the sample has to be considered as an important factor in the performance of the method. The following general groups of particle size dispersity have been identified:

- (Quasi-) Monodisperse
- Polydisperse
- Multimodal
- Monomodal

It is also important to define at which degree of polydispersity a sample may not be considered monodisperse anymore.

2.2.1.9 Optional criteria

Electrical conductivity

Another important criterion to be considered is electrical conductivity of the sample as certain measurement techniques are more appropriate for electrically conductive substances.

- Conductive
- Semiconductive
- Insulator

Magnetic properties

Magnetic particles may be characterised with some specific measurement techniques such as magnetic force microscopy (MFM)⁶ or magnetic particle spectrometer^{7, 8}. Thermomagnetic⁹, direct current (dc) magnetisation¹⁰ and alternating current (ac) susceptibility¹¹ measurements enable also to determine the size distribution of magnetic particles. In this regard, nanoparticles can be classified as:

- Magnetic
- Non magnetic

Functionalisation / no functionalisation of the surface

One important criterion is to know if the nanoparticles are surface functionalised or not. Measurement techniques have to be adapted according to this characteristic.

- Functionalised
- Not functionalised

It is also important to determine the difference between functionalisation of the surface and the presence of a shell layer. For the purpose of the NanoDefiner e-tool it was established that nanoparticles completely covered with a uniform layer belong to the category composite materials.

Most of the measurement techniques give can measure the size of the functionalised nanoparticles, but do not give any information on the type of functionalisation.

Agglomeration/ aggregation state

Nanoparticles can be aggregated or have a tendency to agglomerate. This specific feature of a material needs to be accounted for in the performed data analysis; nanoparticles can be categorised as:

- Nanoparticles are aggregated
- Nanoparticles are not aggregated
- Nanoparticles are agglomerated
- Nanoparticles are not agglomerated

2.2.2 Capabilities of the measurement techniques

2.2.2.1 What type of measurement technique is it? (Counting, ensemble technique or fractionating)

- Single particle counting
- Calculate number or concentration from ensemble methods
- Method combination (hyphenated methods)

2.2.2.2 Working range

The working range is the range in which the method provides reliable results. The working range may be dependent also on the material and its preparation and on the instrument type.

- Size range
- Concentration range
- Minimum sample intake (How much material is needed?)
- Linearity/proportionality
- Limits of detection/quantification
- Sensitivity (Counting or detection efficiency as a function of size)

2.2.2.3 Trueness

Trueness is defined as a difference between the averages of several measurements on the same sample or material and the true value of the measured property (associated quantitative term "bias").

Trueness may be expressed either in terms of size or in terms of amount of particles.

2.2.2.4 Robustness

Robustness is defined as an influence of slight variations in the test protocol on the outcome of the test.

2.2.2.5 Precision

Precision is a test result variation within one test series (repeatability) or several test series (intermediate precision), either in terms of size or in terms of amount of particles.

2.2.2.6 Resolution

Resolution means to which degree a certain size fraction can be distinguished from another (e.g. minimum distance or size ratio needed between different modes so that they can be identified in a mixture of monodisperse samples).

2.2.2.7 Size distribution

The following criterion was considered: Does the method provide a size distribution¹² or a certain average value?

2.2.2.8 Selectivity

Selectivity is how well the measurement technique can distinguish between:

- nanoparticles and non-nanoparticles of the same composition
- nanoparticles and non-nanoparticles of another composition (e.g. mixtures of powders)
- nanoparticles of another composition (mixtures of powders)

Moreover, if the substance to analyse is not pure, it is essential to know if and how the results are affected by impurities, including dissolved ionic species from the same substance. "Yes" in the performance table means that the measurement technique can distinguish between the nanomaterial and the impurity, whereas a "No" mean that the impurities will be detected but the measurement technique cannot distinguish between the nanoparticles and impurity particles.

2.2.2.9 Capability to measure aggregation

Can the method identify and/or measure agglomerates or aggregates of particles?

2.2.2.10 Capability to measure single particles

Can the method measure the size and number of individual particles?

2.2.2.11 Counting constituent particles in aggregates

Is the method able to count constituent particles in aggregates?

2.2.2.12 Chemical composition

Does the method provide information on the chemical composition?

2.2.2.13 Specification of the measurand (diameter)

Size measurement is method dependent.¹³ Different methods address different measurands (equivalent diameters) which need to be specified (hydrodynamic diameter, Stokes diameter, projected area diameter, etc.).

2.2.2.14 Non-destructive / destructive

This criterion indicates whether the method is destructive or not.

2.2.3 Template for technical characteristics

An overview of the outcomes of the evaluation of each measurement technique is presented in a self-explanatory performance table with standardised information content, giving an over view of the main features of each method, and main advantages and disadvantages.

The outcomes of the evaluation of each measurement technique are presented in a dedicated technical characteristics table with standardised information content; an empty table is shown as Table 2. For clarity it is divided into two sections. The first (blue rows) is related to the type of substance being analysed, and indicating whether the method is suitable for characterising materials with specific properties. The second section (orange rows) is related to the technique, indicating the characteristics of

each measurement technique according to the specified criteria. This report presents detailed performance tables filled in for each of the measurement techniques investigated in NanoDefine.

Table 2: Technical characteristics for assessment of measurement techniques

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|--|--|------------------------------|-------|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | | |
| | Solid particulate form | | |
| | Dispersed or embedded in matrices | | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | | |
| | Dispersible in non-polar liquids | | |
| | Dispersible in polar liquids other than water | | |
| | Dispersible in material-specific media | | |
| | Can be aerosolised | | |
| Substance Nature | Inorganic | | |
| | Size-dependent absorption / fluorescence | | |
| | Carbon based | | |
| | Organic, particulate | | |
| | Organic, non-particulate | | |
| | Biological | | |
| | Composite | | |
| Composite particles (see section 2.3.3.1) | Core/shell | | |
| | Multishell particles | | |
| | Particles with inclusions | | |
| Number of small dimensions | 1 (e.g. thickness of nanoplates) | | |
| | 2 (e.g. diameter of nanofibres) | | |
| | 3 | | |
| Shape of nanoparticles | Sphere or similar | | |
| | Equiaxial | | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | | |
| | Flakes and discs (thickness: lateral extension ≤ 0.25) | | |
| | Other | | |
| Thermal degradation sensitivity (Must be compatible with Measurement Technique working range: x-y °C) | Above 0 °C | | |
| | Sensitive above 25 °C | | |
| | Sensitive above 37 °C | | |
| | Sensitive above 50 °C | | |

| | | | |
|--|---|--|--|
| | Sensitive above 100 °C | | |
| | Sensitive above 150 °C | | |
| | Sensitive above 500 °C | | |
| | Sensitive above 1000 °C | | |
| Cooling degradation sensitivity (Must be compatible with Measurement Technique working range: x-y °C) | Sensitive below 25 °C | | |
| | Sensitive below 0 °C | | |
| | Sensitive below -18 °C | | |
| | Sensitive below -35 °C | | |
| | Sensitive below -78 °C | | |
| | Sensitive below -195 °C | | |
| Electron beam sensitivity | Electron beam sensitive | | |
| | Not electron beam sensitive | | |
| Particle size dispersity and modality | Monodisperse | | |
| | Polydisperse | | |
| | Monomodal | | |
| | Multimodal | | |
| Conductivity properties (electrical) | Conductive | | |
| | Semiconductive | | |
| | Insulator | | |
| Magnetic properties | Magnetic | | |
| | Non magnetic | | |
| Functionalization / no functionalisation | Functionalised | | |
| | Not functionalised | | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | | |
| | Nanoparticles are not aggregated | | |
| | Nanoparticles are agglomerated | | |
| | Nanoparticles are not agglomerated | | |
| Counting, fractionating or ensemble technique | Single particle counting | | |
| | Measures or calculates number or number concentration from fractionating techniques | | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | | |
| | Integral technique | | |
| | Used in hyphenated methods | | |
| Working range | Size range | | |
| | Concentration range | | |
| | Minimum needed sample amount | | |
| | Linearity/proportionality | | |
| | Limits of detection/quantification | | |
| | Sensitivity (counting efficiency) as a function of size | | |

| | | | |
|--|--|--|--|
| Trueness | Indicate the trueness of this measurement technique in measuring the particle size | | |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | | |
| Robustness | Specify the robustness of this measurement technique | | |
| Precision | Specify the precision of the measurement technique | | |
| Resolution | Specify the resolution of this measurement technique | | |
| Size distribution | Is it possible to measure size distribution? | | |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | | |
| | Discrimination between NPs and non-NPs of another chemical composition | | |
| | Discrimination from NPs of another chemical composition | | |
| | Impurities | | |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | | |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | | |
| Chemical composition | Does this measurement technique analyse chemical composition? | | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | | |
| Destructive measurement technique or not | Is it a destructive measurement technique? | | |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | | |
| Sample support | Does this measurement technique need preparation on suitable supports? | | |

2.3 Evaluation and performance of the techniques: overview

A general overview of the recommended particle size measurement techniques described in this chapter is shown in Table 3 to Table 6. The criteria selected are of two representative natures: direct relation to the EC NM Definition and analytical one. For the sake of clarity, only the scores 'fair', 'good' and 'very good' were highlighted in the tables. Please note that these tables only aim to give a general overview of the recommended methods and for selecting an appropriate method the detailed performance tables given in chapters 3, 5, 4 and 6 in this report should be used.

The overview tables include the measurement technique scanning electron microscopy working in transmission mode (TSEM), which does not have a detailed performance table associated. TSEM is mentioned in the EM section.

Table 3 provides an overview of the performance of the measurement techniques with regard to measuring size. Table 4 gives an overview and an evaluation of the measurement techniques' capacities when measuring different types of materials. The overview in Table 5 is for the evaluation of the measurement techniques with regard to their capacities for measuring single particles, agglomerates and aggregates in the samples. Table 6 collects an overview of how the measurement techniques perform with regard to "Additional information", i.e. if it is a direct counting technique, how easily the result of the measurement is convertible to the number-weighted PSD (including quantitative and size accuracy), how well the technique can measure the smallest dimension of each particle, and if it gives access to measuring constituent particles, the chemical selectivity of the method, if it can measure the material as it is and availability of ISO standards.

Table 3: Evaluation of the methods: Size range

| Type of method | Method | | Size range | | | | | | | |
|----------------|--------|----------|------------|-----------|-----------|----------------|-----------|-----------|-----------|-----------|
| | | | nm | | | µm | | | | |
| | | | 1-10 | 10-30 | 30-100 | 0.1-1 | 1-10 | >10 | | |
| Counting | EM | SEM | | Good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | TSEM | Good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | TEM | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | SFM/AFM | Good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | PTA | | Fair | Good | Very good | Very good | Very good | Very good | Very good |
| | | TRPS | | Fair | Good | Very good | Very good | Very good | Very good | Very good |
| | | spICP-MS | | Fair | Good | Very good | Very good | Very good | Very good | Very good |
| Ensemble | | DLS | Good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | SAXS | Good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | USSp | | Fair | Good | Very good | Very good | Very good | Very good | Very good |
| | | ALS | | Fair | Good | Very good | Very good | Very good | Very good | Very good |
| Fractionating | | FFF | Good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | AC | Fair | Good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | DEMA | Good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| Integral | | BET | Good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| Legend | | | Fair | Good | Very good | Not applicable | | | | |

Table 4: Evaluation of the methods: Material

| Type of method | Method | | Sample | | | Type of material | | | | | | Shape | | | |
|----------------|----------|-----------|----------------------|------------------------|--------------------|------------------|--------------|----------------------|----------------|------------|----------------------|-----------|-----------|-----------|---------------------|
| | | | Dispersed in liquids | Solid particulate form | Embedded in matrix | Inorganic | Carbon based | Organic, particulate | Biological | Core/Shell | Multishell particles | Inclusion | Sphere | Equiaxial | Tubes, fibres, rods |
| Counting | EM | SEM | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | TSEM | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | | TEM | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | SFM | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | PTA | Very good | | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | TRPS | Very good | | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | spICP-MS | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| Ensemble | DLS | Very good | | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | SAXS | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | USSp | Very good | | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | ALS | Very good | | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| Fractionating | FFF | Very good | | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | AC | Very good | | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| | DEMA | | | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| Integral | BET | | Very good | | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good | Very good |
| Legend | | | | | | Fair | Good | Very good | Not applicable | | | | | | |

Table 5: Evaluation of the methods: single particles, agglomerates and aggregates

| Type of method | Method | | Size distribution | Measures aggregates/ agglomerates | Measures individual particles | Counting constituent particles in aggregates | Measures constituent particles in aggregated / agglomerated samples | Measures constituent particles in not aggregated / agglomerated samples |
|----------------|--------|------|-------------------|-----------------------------------|-------------------------------|--|---|---|
| Counting | EM | SEM | Very good | Very good | Very good | Very good | Very good | Very good |
| | | TSEM | Very good | Very good | Very good | Very good | Very good | Very good |
| | | TEM | Very good | Very good | Very good | Very good | Very good | Very good |
| | SFM | | Fair | Fair | Fair | Fair | | |
| | PTA | | Very good | Very good | Very good | | | |
| | TRPS | | Very good | | Very good | | | Very good |
| | ICP-MS | | Very good | | Very good | | | Very good |
| Ensemble | DLS | | Very good | Fair | | | | Very good |
| | SAXS | | Very good | Fair | | Fair | | Very good |
| | USSp | | Very good | | | Very good | Very good | Very good |
| | ALS | | | | | | | |
| Fractionating | FFF | | Very good | | | | | Very good |
| | AC | | Very good | | | | | Very good |
| | DEMA | | Very good | | Very good | | | Very good |
| Integral | BET | | | | Fair | Very good | Very good | |
| Legend | | | Fair | Good | Very good | Not applicable | | |

Table 6: Evaluation of the methods: additional information

| Type of method | Method | | Direct counting technique | Access to the smallest dimension of each particle | Measurement of the material as it is | ISO standards available | Size Accuracy | Chemical selectivity | Access to constituent particles? |
|----------------|----------|------|---------------------------|---|--------------------------------------|-------------------------|---------------|----------------------|----------------------------------|
| Counting | EM | SEM | Very good | Very good | Very good | Very good | Very good | (+EDX) | Very good |
| | | TSEM | Very good | Very good | Very good | Very good | Very good | (+EDX) | Very good |
| | | TEM | Very good | Very good | Very good | Very good | Very good | (+EDX) | Very good |
| | SFM | | Very good | Very good | Very good | Very good | Very good | | Fair |
| | PTA | | Very good | Fair | Very good | Very good | Very good | | Fair |
| | TRPS | | Very good | Fair | Very good | Very good | Very good | | Fair |
| | spICP-MS | | Very good | Fair | Very good | Very good | Very good | Very good | Fair |
| Ensemble | DLS | | Fair | Fair | Very good | Very good | Very good | | Fair |
| | SAXS | | Fair | Fair | Very good | Very good | Very good | Fair | Very good |
| | USSp | | Fair | Fair | Very good | Very good | Very good | | Fair |
| | ALS | | Fair | Fair | Very good | Very good | Very good | | Fair |
| Fractionating | FFF | | Fair | Fair | Very good | Very good | Very good | (+Detector) | Fair |
| | AC | | Fair | Fair | Very good | Very good | Very good | | Fair |
| | DEMA | | Very good | Fair | Very good | Very good | Very good | | Fair |
| Integral | BET | | Fair | Fair | Very good | Very good | | Fair | |
| Legend | | | Fair | Good | Very good | Not applicable | | | |

3 Counting methods

3.1 Electron microscopy

3.1.1 Measurement principle

One analytical method widely used for sample visualization down to the nm scale is electron microscopy (EM), which uses an electron beam for visualization.

The preparation of the nanoparticle sample to be investigated by EM is key to successful analysis of the particle size distribution. Ideally, particles, which are well separated, free of preparation artefacts, and are distributed on the proper support not too far away from each other, shall be accurately imaged. Care must be taken of possible beam or vacuum influence on the size of NPs. Once such an accurate image is taken, the post-measurement of the particle size can be performed with appropriate software packages. Decisive steps in the evaluation of the accurate particle size determined with an electron microscope are the calibration of the image magnification, i.e. of the pixel size including its re-calibration in the processing software, and the setting of the threshold in the image histogram corresponding to the real position of the particle boundaries.

Size analysis with EM relies on counting individual particles. Depending on the number of particles acquired in an image, most often several images are necessary to achieve good counting statistics. The automation tools such as motorized stage and sequential image acquisition should be available in order to speed up the whole measurement process. Also automatic image processing of batch images speeds up the determination of the size distribution.

In scanning electron microscopy (SEM), images are constructed based on electrons coming from the sample surface¹⁴. In case of transmission electron microscopy (TEM), images are constructed based on electrons passing through the sample. Both SEM and TEM give 2-dimensional projections of 3-dimensional particles. EM images facilitate the determination of number-weighted size distributions by analysing each identifiable particle individually¹⁴. EM also allows an assessment of the morphology of particles. The analysis of flattened particles (e.g. disks, flakes) could be problematic because the smallest axis of the particle could be hidden. This could lead to a measured size that is overestimated compared to the size relevant for the EC NM Definition. Most EM measurements are performed in high vacuum chambers. Therefore, the use of EM is limited to the analysis of particles which are not sensitive to the combined action of high vacuum and electron beams.

In SEM, the sample to be examined is bombarded with a finely (i.e. in nm range) focused electron beam which is scanned over a defined field. Low-energy secondary electrons are released after inelastic collisions with the atoms in the specimen, and high-energy backscattered electrons after elastic collisions with the atoms in the specimen. Depending on their kinetic energy, the information range carried by the released electrons varies from micrometre (typical for backscattered electrons) down to nanometre (typical for secondary electrons). Hence, the secondary electrons are suited for high-resolution morphological characterization of the specimen surface at nanometre scale and even individual nanoparticles may be visualized and lateral dimensions measured with SEM. Depending on the instrument used, but also strongly on the challenging sample preparation onto typical sample substrates/holders, accurate size characterization of nanoparticles is possible with sizes down to about 7 nm¹⁵.

Whereas in the case of an SEM typical beam voltages up to 30 kV may be applied and secondary electrons/ backscattered electrons are collected by various detectors, in the case of TEM the beam voltage is up to 300 kV. The samples to be analysed must be in the range of the electron

transparency so that the electrons transmitted through the thin sample can be collected. The highly energetic electron beam is even finer than in the SEM case (more sophisticated aberration correctors being often also available), so that spatial resolution well below 1 nm can be attained. TEM has similar requirements as SEM for NPs regarding sample preparation, suitable substrate (typically the so-called TEM grids), vacuum suitability¹⁵, calibration of the pixel size and automation of both acquisition and image processing. By combining TEM imaging and semi-automatic image analysis accurate characterization of the size, shape, and surface morphology of colloidal nanomaterials,¹⁶ aggregated nanomaterials^{17,18}, and primary particles in aggregates (or, in terms of the EC NM Definition, constituent particles)¹⁹ is enabled. A characterization methodology which includes a systematic selection procedure for unbiased random image collection, semi-automatic image analysis and data processing has been validated for size, shape and surface morphology measurements of silica nanoparticles. The expanded uncertainty of size measurements of two colloidal silica certified reference materials was estimated to be about 3 %.

TEM can be operated in the scanning mode, i.e. STEM, when the electron beam is focused into a narrow spot which is scanned over the sample. According to the range of angles by which they are scattered in the sample, the transmitted electrons can be differentiated as: bright-field electrons are those electrons slightly or not at all scattered and dark-field electrons are those collected concentrically to the optical microscope axis. The so-called HAADF-STEM (High-Angle Annular Dark-Field STEM) imaging mode results when only the strongly scattered electrons are collected with an annular dark-field detector. The contrast of this type of imaging with atomic resolution is directly related to the atomic number of the elements constituting the sample (Z-contrast image).

One hybrid type of electron microscopy is constituted by the SEM able to work in the transmission mode, i.e. TSEM, T-SEM or STEM in SEM etc. This means that by using TEM grids as support for NPs, the transmitted electrons (of lower energies than in the TEM case) are more or less absorbed by the NPs and a so-called STEM detector placed under the sample holder detects them. The alternative is to use a dedicated transmission sample holder, which enables performing TSEM with the available secondary electrons/backscattered electrons detector without the need to use an additional STEM detector. It was recently demonstrated that both types of transmission modes with SEM are well suited for metrological measurement of NP size and size distribution down to about 10 nm^{20, 21, 22}.

3.1.2 Performance

Current limitations in application include:

- Strong dependency on sample preparation (incl. suitable substrate)
- Possibility to use vacuum for certain types of material (organic, coated etc.)
- SEM is not able to measure accurately the size of NPs below about 7 nm (depending on instrument)
- Automation (batch image acquisition and batch image processing)
- Time-consumption that depends on the complexity of sample preparation and degree of automation
- High cost, but still in the same order of magnitude as e.g. ICP-MS
- Accuracy in identification of the NPs in the image processing software
- The size resolution of EM is good with the following analytical lower limits:
- SEM minimal NP size to be measured accurately: about 7 nm depending on the instrument employed.

- TEM minimal NP size to be measured accurately: below 1 nm depending on the applied contrast and on the instrument employed.
- TSEM minimal NP size to be measured accurately: about 10 nm depending on the instrument employed.

Systematic results of metrological measurement of NP size and size distribution by SEM, TEM and TSEM were carried out recently in the frame of various, specifically dedicated round robin tests¹¹. According to these reported results, EM provides traceable results, which are also consistent, i.e. comparable, with those obtained by AFM, SAXS and SMPS, but not with DLS.

Table 7 and Table 8 below give the general performance of electron microscopy and the detailed performance table for this method, respectively.

Table 7: General performance of electron microscopy (EM)

| Main features | |
|--|---|
| Type of samples | particles properly deposited onto substrates or particles embedded in an electron-transparent medium |
| Type of sizing | counting technique (by identifying individual objects in images) |
| Particle property measured | selected properties of the 2D particle image, e.g. Feret diameter, area equivalent circle diameter (ECD), wide range of 1D and 2D size, shape and surface measurands |
| Type of quantity | particle number |
| Size range | <ul style="list-style-type: none"> • SEM: 7 nm - 1000 μm • TEM (incl. HAADF-STEM): <1 nm - 1000 μm • TSEM: 10 nm - 100 μm |
| Concentration range | "0" (individual particles) ... monolayer (immobilised particles) |
| Information content | <ul style="list-style-type: none"> • very high, i.e. can well resolve details of the size distribution • good in x-y direction (i.e. parallel to the substrate) |
| Main advantages | |
| <ul style="list-style-type: none"> • intrinsically yields number-weighted size distributions • facilitates determination of particle size and shape as well as aggregate structure and surface measurands • in principle can distinguish aggregates from their constituent particles and allows for size measurement of the constituents • size, shape and surface measurands can be measured on 2D images • sub-nm resolution for TEM, nm resolution for SEM • access to smallest dimension of particles in X-Y plane • high resolution of particle size distribution • capable of chemical specificity of single particles by the attached EDX • crystallographic information is available by electron diffraction • significant instrumental developments (spatial resolution, automation, EDX detector sensitivity, table-top instruments, etc.) • automated image processing: available and in further development | |

Main disadvantages

- strongly dependent on sample preparation (immobilised particles on substrate need to be representative for the material)
- needs vacuum and expensive instrumentation
- limited dynamic range (highest size/lowest size < 40) based on one image only

Table 8: Technical characteristics for electron microscopy (EM)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|---|---------------------------|---|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | No | Possible if a cryo-stage is available ²³ Yes ⚠ successful deposition on substrates required |
| | Solid particulate form | Yes | |
| | Dispersed or embedded in matrices | Yes ⚠ | Only at the surface, in thin films or in ultramicrotomed sections |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | No | Yes ⚠ successful deposition on substrates required |
| | Dispersible in non-polar liquids | No | |
| | Dispersible in polar liquids other than water | No | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite particles | Yes | |
| | Other | Yes | |

| | | | |
|---|--|---------------|---|
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes | Can detect the internal structure in some cases |
| | Multishell particles | Yes | Difficult |
| | Particles with inclusions | Yes | Only outer size. Can detect the internal structure in some cases |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No (⚠ Yes) | Typically ⚠ For specialised solutions (EELS, EFTEM) |
| | 2 (e.g. diameter of nanofibres) | Yes | |
| | 3 | Yes | Measurement of Z (height) is difficult in SEM'. In TEM it is possible (not routine) |
| Shape of particles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods (length: diameter ≥ 3) | Yes | |
| | Flakes and discs (thickness: lateral extension ≤ 0.25) | No (⚠ Yes) | Typically for specialised solutions (EELS, EFTEM) |
| | Other | No (⚠ Yes) | Typically for specialised solutions (EELS, EFTEM) |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 15-25 °C) | Above 0 °C | No | ⚠ Yes, with cryo stage |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| | Sensitive above 1000 °C | Yes | |
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 15-25 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |

| | | | |
|---|--|-------|---|
| Electron beam sensitivity | Electron beam sensitive | No | Low-dose measurement may work for particles with weak electron beam sensitivity |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | |
| | Monomodal | Yes | |
| | Multimodal | Yes | |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | With altered image quality |
| | Insulator | Yes | SEM: low-voltage option or conductive high resolution sputter-coating needed |
| Magnetic properties | Magnetic | Yes | Depending on the strength of the magnetic field the performance of the technique may worsen |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Yes only for sophisticated sample preparation or fairly small agglomerates |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | Yes | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble measurement techniques | No | |
| | Integral technique | No | |
| | Used in hyphenated methods | Yes | E.g. with EDS or EELS |

| | | | |
|--|--|--|---|
| Working range | Size range | 7 nm to 1000 μm (SEM) 1 nm to 1000 μm (TEM) | Lower range depends on instrument type, sample type and preparation, ⚠ full size range requires more than one image (at different magnifications) |
| | Concentration range | N/A | Accurate measurements only for single particles deposited on a substrate → optimum concentration depends on size and deposition procedure. For example, a droplet of 0.1-1 μL at 0.1 %-vol. conc. is typically sufficient |
| | Minimum needed sample amount | 0.1 μL | Minimum 500 NPs for a monodisperse/ monomodal sample ¹³ |
| | Linearity/proportionality | Yes | When differentiating between linearity in size and linearity in quantity then: Size: yes Quantity: N/A or (Yes), as the correct quantification is mainly determined by sample preparation (representative and homogeneous deposition on substrate) and measurement procedures (image acquisition) |
| | Limits of detection/quantification | 1 nm to 10 nm | Depending on instrument, sample type and preparation, etc. |
| | Sensitivity (counting efficiency) as a function of size | good | |
| | Trueness | Indicate the trueness of this measurement technique in measuring the particle size | good |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | good | To be evaluated for specific cases |
| Robustness | Specify the robustness of this measurement technique | average | Strong dependency on sample preparation |
| Precision | Specify the precision of the measurement technique | 1 nm to 10 nm | Depending on many parameters, mainly preparation; better for TEM and poorer for SEM |
| Resolution | Specify the resolution of this | 1 nm to | |

| | | | |
|--|--|---------------------------------|---|
| | measurement technique | 10 nm | |
| Size distribution | Is it possible to measure particle size distribution? | Yes | |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | Yes | Depending on the size of the big particles and the width of the size distribution |
| | Discrimination between NPs and non-NPs of another chemical composition | Yes | |
| | Discrimination from NPs of another chemical composition | Yes ⚠ | In cases when image contrast between the particulate species is high enough |
| | Impurities | Yes ⚠ | Depends on the nature of the impurity |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | Yes ⚠ | Difficult and possible only after sampling on substrates and measurements performed in vacuum |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | Yes ⚠ | Depending on contrast and size of aggregate |
| Chemical composition | Does this measurement technique analyse chemical composition? | No Yes | Typically Modern instruments with EDS and EELS (hyphenation) |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | “Diameter” “Ferret diameter” | All size and shape parameters available that can be deduced by 2D image analysis |
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes | Sample must be prepared on substrates or as thin films, etc. |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | Yes | |
| Sample support | Does this measurement technique need preparation on suitable supports? | Yes | |

3.2 Scanning force microscopy (SFM), or atomic force microscopy (AFM)

3.2.1 Measurement principle

Atomic force microscopy (AFM) or scanning force microscopy (SFM) is a technique in which a sharp tip, a needle, is fixed on a cantilever and moved along the surface being analysed. Different ways to measure exist: the cantilever can tap the surface, touch the object constantly, and not be in contact with it. The shape of the tip as well as the substrate can influence the AFM images.

Particles need to be fixed to the surface in order to be characterised and not to be moved by the tip. AFM is an imaging method and can be used to measure the size of polydisperse and polyshaped particles. Organic particles can also be analysed with this technique.

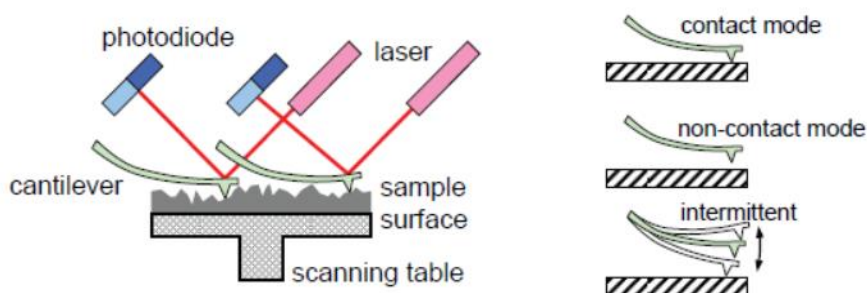


Figure 2: Measurement principle of SFM

This technique, able to image colloidal particles or powders, was developed by Binnig and his co-workers in the 1980s.²⁴ AFM (or SFM) is the most used type of scanning probe microscopy (SPM). The AFM and its related techniques are based on the interaction between a very fine probe tip with the atoms or molecules at the surface of the sample (see Figure 2)^{25, 26}. This can be used to resolve surface morphologies or particles on a substrate with vertical/out-of-plane resolution of 0.1 nm to 10 nm (lateral/in-plane resolution: 10 nm). The AFM is usually employed for the characterisation of films and surfaces (e.g. roughness), whereas the morphological characterisation of particles is a less frequent application. Its real strength is the sensitivity to the forces between probe and sample, which allows an evaluation of surface chemistry (e.g. functional groups, hydrophobicity) and the quantification of particle interactions, or interactions between particles and surfaces (e.g. adhesion, friction^{27, 28}). Depending on the situation, forces that are measured in AFM include mechanical contact force, van der Waals forces, capillary forces, chemical bonding, electrostatic forces, magnetic forces, etc. As well as force, additional quantities may simultaneously be measured through the use of specialised types of probe. Samples in air or in liquid can be analysed, but the sample must adhere to a substrate and be rigid and well dispersed on it. The roughness of the substrate must be significantly smaller than the size of the nanoparticles being measured. The AFM can be operated in several modes. In general, imaging modes are divided into static (also called contact) modes and a variety of dynamic (or non-contact) modes where the cantilever is vibrated (Figure 2). The use of the AFM in biology, biochemistry and bionanotechnology, also for the characterization of nanomaterials (size, shape), are reviewed in an article by Kada et al.²⁹

The results of imaging methods are (mainly) number-weighted particle size distributions (PSDs). It means that the sample size (number of probed particles) should be sufficiently high for ensuring low uncertainty in class frequencies. Moreover, the sample size required to achieve a certain

confidence level increases with polydispersity. The accuracy of the measured particle properties depends on a variety of factors (e.g. magnification or spatial resolution of the scanning mode, or image processing). Most crucial, however, is the representativeness of the imaged particles for the whole particle system. That requires that the particle deposition on the substrate is neither size-selective nor inhomogeneous.³⁰ In general, sample preparation is a key issue for imaging methods.

3.2.2 Performance

The AFM technique is applicable to both powders and suspensions, and the particles need to be immobilised and be well dispersed on a support surface. Almost any material can be measured. The technique can only qualitatively distinguish between individual particles and agglomerates/aggregates and thus the size of constituent particles inside aggregates/agglomerates cannot be measured reliably. AFM measures external dimensions and the best resolution is achieved in the direction perpendicular to the surface on which the particles are immobilised. This technique provides an access to the minimum dimension of a particle, which is especially important for platelets-like shaped particles (thickness). The roughness of the substrate may introduce errors thus it must be significantly smaller than the size of the nanoparticles being measured. AFM can directly provide number-based particle size distributions.

Table 9 and Table 10 below give the general performance of scanning force microscopy (SFM) / atomic force microscopy (AFM) and the detailed performance table for this method, respectively.

Table 9: General performance of scanning force microscopy (SFM)/ atomic force microscopy (AFM)

| Main features | |
|--|---|
| Type of samples | particles of (almost) any material, in vacuum, air or liquid immobilised on a substrate |
| Type of sizing | counting technique (by identifying individual objects in images) |
| Particle property measured | dimensions of the 2D particle image and particle height above the level of a substrate |
| Type of quantity | particle number |
| Size range | <ul style="list-style-type: none"> • 1 nm to <10 µm (z-size, "height", most reliable), • 10 nm to 100 µm (lateral size, depends on tip geometry) |
| Concentration range | "0" (individual particles) ... monolayer (immobilised particles) |
| Information content | <ul style="list-style-type: none"> • relatively high, i.e. can well resolve details of the size distribution • good in z-direction (i.e. in "height") • not reliable in x-y direction (laterally, parallel to the substrate) |
| Main advantages | |
| <ul style="list-style-type: none"> • intrinsically yields number-weighted size distributions, Q0 • facilitates determination of particle size and shape as well as surface properties • access to the minimum dimension of a particle • measures a wide range of materials • instruments are widely available and not expensive | |

| Main disadvantages |
|--|
| <ul style="list-style-type: none"> strongly dependent on sample preparation (immobilised particles on substrate need to be representative for the material) lateral size measurements are less reliable than altitude measurements (dependent on tip conditions) low sample throughput, slow limited dynamic range |

Table 10: Technical characteristics for scanning force microscopy (SFM) / atomic force microscopy (AFM)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | No | ⚠ Yes after successful immobilization on substrates |
| | Solid particulate form | Yes ⚠ | After successful immobilization on substrates, generally from a liquid dispersion |
| | Dispersed or embedded in different kinds of matrices | Yes ⚠ | Only at the surface or in thin films or in ultramicrotomed sections, as long as the surface is smooth enough with respect to the particle size and there is enough contrast with the matrix; possible bias due to random sectioning at non-controlled distance from the diameter plane (for spheres) |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | No | ⚠ Yes after successful immobilization on substrates |
| | Dispersible in non-polar liquids | No | |
| | Dispersible in polar liquids other than water | No | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |

| | | | |
|--|---|-------|--------------------|
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite particles | Yes | |
| | Other | Yes | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | Only external size |
| | Multishell particles | Yes ⚠ | |
| | Particles with inclusions | Yes ⚠ | |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | Yes | |
| | 2 (e.g. diameter of nanofibres) | Yes | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | Yes | |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | Yes | |
| | Other | Yes | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 15-40°C) | Above 0 °C | No | |
| | Sensitivity above 25 °C | Yes | |
| | Sensitivity above 37 °C | Yes | |
| | Sensitivity above 50 °C | Yes | |
| | Sensitivity above 100 °C | Yes | |
| | Sensitivity above 150 °C | Yes | |
| | Sensitivity above 500 °C | Yes | |
| | Sensitivity above 1000 °C | Yes | |
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |

| | | | |
|---|---|-------|---|
| Technique working range: 15-40 °C) | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | |
| | Monomodal | Yes | |
| | Multimodal | Yes | |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes | Only aggregate size, with limitations (size and roughness) |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Only for small agglomerates or sophisticated sample preparation |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | Yes | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | No | |
| | Integral technique | No | |
| | Used in hyphenated methods | No | |

| | | | |
|--|--|--|---|
| Working range | Size range | > about 1 nm for height; >10 nm for lateral size; up to 10 μm | Range varies in dependence on instrument type, sample type and preparation |
| | Concentration range | N/A | Accurate measurements only for single particles deposited on a substrate \rightarrow optimum concentration depends on size and deposition procedure, e.g. a droplet of 0.1-1 μL at 0.1%-vol. conc. is typically sufficient |
| | Minimum needed sample amount | 0.1 μL for liquid suspension 1 mg for powder | Minimum sample size is about 500 particles, i.e. 0.1 – 1 μL at 0.1%-vol. conc. |
| | Linearity/proportionality | Yes | |
| | Limits of detection/quantification | 1 nm / several tens of nm | About 1 nm for height and several tens of nm for lateral dimensions (depending on tip convolution) |
| | Sensitivity (counting efficiency) as a function of size | good | Low throughput |
| Trueness | Indicate the trueness of this measurement technique in measuring the particle size | very good | Only for height, as convolution with the tip geometry leads to a bias in lateral dimensions |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | good | Depending on polydispersity and sample preparation; to be evaluated for specific cases |
| Robustness | Specify the robustness of this measurement technique | average | Strong dependency on sample preparation; to be evaluated for specific cases |
| Precision | Specify the precision of the measurement technique | < 1 nm for height | Depending on many parameters; precision on lateral size depends, among others, on tip convolution. Depends also on the type of material. |

| | | | |
|--|--|-----------------------|---|
| Resolution | Specify the resolution of this measurement technique | 1 nm to 5 nm (height) | Depending on dimension (height versus lateral), instrument type and imaging conditions and material type |
| Size distribution | Is it possible to measure size distribution? | Yes ⚠ | If the size difference is not too big |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | Yes ⚠ | If the size difference is not too big |
| | Discrimination between NPs and non-NPs of another chemical composition | Yes ⚠ | If the size difference is not too big. In cases when image contrast is high enough |
| | Discrimination from NPs of another chemical composition | average | In cases when hardness properties are significantly different (in intermittent contact mode); modes based on other properties (electrical, magnetic, thermal...) could be helpful, but need to be evaluated |
| | Impurities | No | Particulate impurities (in the appropriate size range and with sufficient contrast) can be detected and counted. Dissolved impurities are ignored |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | Although coupling with Raman or FTIR does exist, but remains limited (in resolution, among others) and not widespread |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | height / lateral size | Bias on lateral size due to tip convolution |

| | | | |
|--|--|-----|---|
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes | Sample must be immobilised on substrates or as thin films, etc. |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | Also possible, but not required. |
| Sample support | Does this measurement technique need preparation on suitable supports? | Yes | |

3.3 Particle tracking analysis (PTA), dynamic ultramicroscopy (DUM)

3.3.1 Measurement principle

Fine colloidal particles are usually smaller than the spatial resolution of an ordinary light microscope, which means that they are invisible with regard to an affine projection. However, when they are laterally illuminated by very intense light against a dark background (dark field microscopy), it is possible to see the scattering patterns with an optical microscope. Such an instrument is named ultramicroscope. When ultramicroscopy is used for particle sizing, one evaluates the Brownian motion of the scattering centres (i.e. particles), because of which this type of sizing is called particle tracking analysis (PTA) or dynamic ultramicroscopy (DUM).

In contrast to the majority of sizing techniques for NP suspensions PTA is in principle capable of measuring the particle number concentration. However, the reliability of such a measurement depends very much on material properties and particle size distribution width. The current state-of-the-art concentration measurement is not very reliable in the general case and needs further investigation³¹.

3.3.2 Performance

The application limits of ultramicroscopy result from the requirements that the inter-particle distances should be much larger than the optical resolution limit of the microscope, which can be achieved by appropriate dilution, and that the scattered light of all individual particles is sufficiently strong for detection. This requirement is not fulfilled for particles below a material-specific size limit. Additionally, there are principal difficulties in detecting weak scatters in the presence of strong scatters. That means, though providing number weighted PSD (similar to ordinary microscopy), PTA has a bias to strongly scattering particles (similar to dynamic light scattering³²). This concerns very broad size distributions as well as multi-component particle systems.

Table 11 and Table 12 give the general performance of the method particle tracking analysis (PTA) / dynamic ultramicroscopy (DUM) and the detailed performance criteria for this method, respectively.

Table 11: General performance of particle tracking analysis (PTA) / dynamic ultramicroscopy (DUM)

| Main features | |
|---|--|
| Type of samples | particles (solid, liquid, gaseous) in liquid dispersion medium |
| Type of sizing | counting technique (by identifying individual objects in video images) |
| Particle property measured | diffusion coefficient equivalent diameter; translational hydrodynamic diameter |
| Type of quantity | particle number |
| Size range | 10 nm to 1 µm (depending on the scattering properties of the material) e.g. Au, Ag 10 nm to 1 µm, polystyrene latex (PSL) 50 nm to 1 µm, SiO ₂ 70 nm to 1 µm |
| Concentration range | << 1 vol.-% |
| Information content | relatively high, i.e. can well resolve details of the size distribution |
| Main advantages | |
| <ul style="list-style-type: none"> • intrinsically yields number-weighted size distributions, Q0 • fairly good resolution of particle size distribution • provides particle number concentration of the analysed sample | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • does not resolve particle shape, but measures a hydrodynamic equivalent diameter • measures aggregate size rather than size of the constituent particles • lower size limit is >10 nm and depends on material properties • poor sensitivity in the fine particle range • limited dynamic range | |

Table 12: Technical characteristics for particle tracking analysis (PTA) / dynamic ultramicroscopy (DUM)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|---|-------------------------------------|--------------|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes | |
| | Dispersible in polar liquids other than water | Yes | |

| | | | |
|---|---|-------|--|
| | Dispersible in material-specific media | Yes ⚠ | Dispersion medium must be compatible with the instrument |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes ⚠ | Requires that scattering is sufficiently strong |
| | Organic, non-particulate | Yes ⚠ | Requires that scattering is sufficiently strong (typically if size >> 100 nm) |
| | Biological | Yes ⚠ | Requires that the particle is stationary (i.e. shall not move itself, as e.g. bacteria may do) |
| | Composite particles | No | |
| | Other | | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | Only outer size |
| | Multishell particles | Yes ⚠ | |
| | Particles with inclusions | Yes ⚠ | |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No | Does not resolve shape |
| | 2 (e.g. diameter of nanofibres) | No | |
| | 3 | Yes | |
| Shape of particles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | No | |
| | Other | | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 10-40 °C) | Above 0 °C | No | |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |

| | | | |
|---|---|-------|--|
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| | Sensitive above 1000 °C | Yes | |
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 10-40 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes ⚠ | Insensitive to fine NPs for very broad PSDs |
| | Monomodal | Yes | |
| | Multimodal | Yes ⚠ | Insensitive to fine NPs for very broad PSDs |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes ⚠ | Measurement technique measures only aggregate size |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Measurement technique measures only agglomerate size |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | Yes | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number | No | |

| | | | |
|--|--|---|---|
| | concentration from spectroscopic ensemble techniques | | |
| | Integral technique | No | |
| | Used in hyphenated methods ¹ | Yes | |
| Working range | Size range | 10 nm – 1 µm | Lower limit is material dependent |
| | Concentration range | 10 ⁷ to 10 ⁹ particles/mL | i.e. << 0.1 vol-% |
| | Minimum needed sample amount | 10 mL | |
| | Linearity/proportionality | Yes ⚠ No | Yes with regard to diffusion velocity, i.e. size Not really, with regard to concentration |
| | Limits of detection/quantification | | Depend on optical contrast and size, e.g. 10 nm for Au, 70 nm for SiO ₂ |
| | Sensitivity (counting efficiency) as a function of particle size | good ⚠ | Poor for very fine NPs in the presence of large particles |
| Trueness of particle size measurement | Indicate the trueness of this measurement technique in measuring the particle size | good | Size: “falseness” if wrong calibration of microscope and wrong model parameters (e.g. viscosity) |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions by this measurement technique | good | Number/frequency: falseness if inappropriate illumination and image analysis or if too a high particle concentration; measurement technique has a bias to strongly scattering particles |

¹ There are 2 independent signals: average track length and spot size, i.e. diffusion coefficient and scattering intensity; a 2-dimensional plot of a monoconstituent sample would therefore show the functional relationship between size and scattering cross section (i.e. a strong, non-linear correlation); for multiconstituent samples we would expect different groups of such functional dependencies, which offers a possibility to couple size analysis with material or shape analysis

| | | | |
|--|--|-------------------------------------|--|
| Robustness | Specify the robustness of this measurement technique | poor | |
| Precision | Specify the precision of the measurement technique | 5-10% | PSD parameters to be defined (PSD mean, width, etc.) |
| Resolution | Specify the size resolution of this measurement technique | 5-7% | |
| Size distribution | Is it possible to measure particle size distribution? | Yes | |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | Yes | |
| | Discrimination between NPs and non-NPs of another chemical composition | No | |
| | Discrimination from NPs of another chemical composition | No | |
| | Impurities | No | Particulate impurities (in the appropriate size range and with sufficient contrast) will be detected and counted. Dissolved impurities are ignored |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | Yes | |
| Counting constituent particles in aggregates | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | Translational hydrodynamic diameter | (long time self-) diffusion coefficient of particles |
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes | Usually, because of dilution |

| | | | |
|--------------------------|--|----|--|
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suitable supports? | No | |

3.4 Tunable resistive pulse sensing (TRPS)² / electrical sensing zone (ESZ) / nano Coulter counter

3.4.1 Measurement principle

A Coulter counter is an apparatus for counting and sizing particles suspended in electrolytes³³; the measuring technique is also called electrical sensing zone. It is used for cells, bacteria, prokaryotic cells and virus particles and more recently for fine particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. As fluid containing particles or cells is drawn through each micro channel, each particle causes a brief change to the electrical resistance of the liquid. The counter detects these changes in electrical resistance. The Coulter principle relies on the fact that particles moving in an electric field cause measurable disturbances in that field. The magnitudes of these disturbances are proportional to the volume of the particles in the field^{34, 35, 36}. For accurate measurement a few prerequisites should be fulfilled. Firstly, the particles should be suspended in a conducting liquid. Secondly, the electrical field should be physically constricted so that the movement of particles in the field causes detectable changes in the current. Finally, the sample should be sufficiently dilute so that only one particle at a time passes through the physical constriction, preventing an artefact known as coincidence.

3.4.2 Performance

The Coulter counter needs calibration (usually with spherical polymer latex). This calibration holds true for any other non-conducting material with particles that do not deviate considerably from spherical shape. The calibration constants should be changed for non-spherical particles (even though the signal is still proportional to the particle volume). Conducting particles require a defined adjustment of the applied voltage. Porous particles or aggregates need appropriate models or calibration, which means that the morphology of such particles has to be known.

The electrical sensing zone technique principally allows the measurement of number-weighted size distributions with high resolution. In addition, it can be used to measure the particle number concentration and their volume concentration (because the signal is volume proportional).

² TRPS (tunable resistive pulse sensing) is the preferred designation of the manufacturer (ison) for their newly introduced instrument (qnano): particles dispersed in water with dissolved salt move through the single pore of an elastic separator (hence the 'tunable' detection interval) which separates two electrodes that detect the ion current. Whenever a single particle blocks the pore, the current reduces, and the duration and depth of this 'pulse' provide information on size. The sequential detection of blockade events constitutes a size distribution in number metrics without further conversion. This detection principle is related, but not identical to the conventional ESZ (electrical sensing zone), and hence the designation 'Nano Coulter counter' for TRPS is not preferred.

Table 13 and Table 14 below give the general performance of the method Tuneable Resistive Pulse Sensing and the performance table for this method, respectively.

Table 13: General performance of Tunable Resistive Pulse Sensing (TRPS) / Electrical sensing zone (ESZ) / nano Coulter counter

| Main features | |
|--|---|
| Type of samples | particles (solid, liquid, gaseous) in liquid dispersion medium |
| Type of sizing | counting technique (by identifying individual objects in continuous signals) |
| Particle property measured | particle volume equivalent sphere diameter: volume equivalent diameter |
| Type of quantity | particle number |
| size range | <ul style="list-style-type: none"> • minimum size ≥ 50 nm • maximum is in the range 1-10 μm |
| concentration range | $10^5 - 10^{12}$ particles / mL |
| information content | very high, i.e. can well resolve details of the size distribution |
| Main advantages | |
| <ul style="list-style-type: none"> • intrinsically yields number-weighted size distributions, Q0 • good resolution of particle size distribution • provides number concentration of analysed sample | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • does not resolve particle shape, but measures a volume equivalent diameter, • measures aggregate size rather than size of the constituent particles • lower size limit is clearly above 10 nm • limited dynamic range because of clogging | |

Table 14: Technical characteristics for tunable resistive pulse sensing (TRPS)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characteri- sation (Yes/No) | Notes |
|---|--|---|--------------|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar | No | |

| | | | |
|---|---|-------|---|
| | liquids | | |
| | Dispersible in polar liquids other than water | Yes | |
| | Dispersible in material-specific media | Yes | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | No | |
| | Biological | Yes | |
| | Composite particles | Yes ⚠ | Only outer size |
| | Other | | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | Only outer size |
| | Multishell particles | Yes ⚠ | |
| | Particles with inclusions | Yes | Possible in certain cases |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No | Measurement technique does not resolve shape |
| | 2 (e.g. diameter of nanofibres) | No | Volume correct, if aspect ratio length/diameter < 3 |
| | 3 | Yes | Measures particle volume replacing liquid volume |
| Shape of nanoparticles | Sphere or similar | Yes ⚠ | Measurement technique can measure objects of different shapes, but only provides an equivalent diameter |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | No | |
| | Other | No | |

| | | | |
|---|-----------------------------|-----|--|
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 15-25 °C) | Above 0 °C | No | |
| | Sensitivity above 25 °C | Yes | |
| | Sensitivity above 37 °C | Yes | |
| | Sensitivity above 50 °C | Yes | |
| | Sensitivity above 100 °C | Yes | |
| | Sensitivity above 150 °C | Yes | |
| | Sensitivity above 500 °C | Yes | |
| | Sensitivity above 1000 °C | Yes | |
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 15-25 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | |
| | Monomodal | Yes | |
| | Multimodal | Yes | |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |

| | | | |
|---|---|--|--|
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes ⚠ | Measurement technique measures only aggregate size |
| | Nanoparticles are not aggregated | Yes | Provided that they fit to the measurement range of the instrument |
| | Nanoparticles are agglomerated | Yes ⚠ | Measurement technique measures only agglomerate size, and only if they are small enough to avoid capillary clogging |
| | Nanoparticles are not agglomerated | Yes | Provided that they fit to the measurement range of the instrument |
| Counting, fractionating or ensemble technique | Single particle counting | Yes | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | No | |
| | Integral technique | No | |
| | Used in hyphenated methods | No | |
| Working range | Size range | 50 nm - 10 µm ⚠ | Lower size limit depends on sensing pore size, which may be blocked. 50 nm - 10 µm for flow through membrane; 0.4µm - 1200 µm classical for capillary flow |
| | Concentration range | 10 ⁵ - 10 ¹² particles/mL for porous membrane; 10 ¹ -10 ⁹ mL ⁻¹ ; capillary flow | |
| | Minimum needed sample amount | 500 µL | |
| | Linearity/proportionality | Yes | Pulse amplitude linear to displaced electrolyte volume |
| | Limits of detection/quantification | 50 nm | For membrane flow technique |

| | | | |
|--|--|------------|--|
| | Sensitivity (counting efficiency) as a function of size | good | |
| Trueness | Indicate the trueness of this measurement technique in measuring the particle size | good | |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | good | |
| Robustness | Specify the robustness of this measurement technique | average | |
| Precision | Specify the precision of the measurement technique | | Not examined in NanoDefine; in general it depends on the number of particle counts |
| Resolution | Specify the resolution of this measurement technique | $\geq 2\%$ | |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | Yes | |
| | Discrimination between NPs and non-NPs of another chemical composition | No | |
| | Discrimination from NPs of another chemical composition | No | Limited discrimination reported to be possible |
| | Impurities | No | Particulate impurities (in the right size range and with sufficient contrast) are detected. Dissolved impurities are ignored |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |

| | | | |
|--|---|----------------------------|--|
| Measurement of individual particles | Does this measurement technique characterise individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | Volume equivalent diameter | |
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes | |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suitable supports? | No | |

3.5 Single particle ICP-MS (spICP-MS)

3.5.1 Measurement principle

Single particle inductively coupled plasma - mass spectrometry (spICP-MS) is based on the measurement of highly diluted nanoparticle dispersions by ICP-MS operated in time-resolved mode for a pre-selected mass-to-charge ratio (m/z) value^{37, 38, 39, 40, 41}. Ideally, individual particles enter the ion source and are atomised and ionised in the plasma torch to produce a plume of element ions that is transferred to the mass spectrometric detector. The discrete measurement intervals (dwell times) of the MS are set to a value (≤ 10 ms) that allows the registration of the signal of the ion plume from only one particle. A prerequisite to operate in the single particle modus is (besides the short dwell times) that the concentration of particles is small enough to avoid simultaneous ionisation of more than one particle or the generation of overlapping ion plumes per dwell time. When these requirements are met, the signal intensity is proportional to the mass of the respective elements in the particle. The diameter of spherical particles can then be calculated from the measured mass based on the known or assumed stoichiometry and density of the target analyte. The number concentration of the particles in the measured dispersion can be inferred from the number of signals, the infusion rate, nebulisation efficiency and the acquisition time.

3.5.2 Performance

This measurement technique has a number of unique features. It is a relatively robust technique and can be run on conventional ICP-MS instruments that are widely available in both commercial and official control laboratories. Sample preparation is simple (often only dilution) and the measurement time per sample very short (1 min) which allows high throughput analysis. Furthermore, it is chemically specific and provides actual number-based size distributions.

Current application limits include:

- The detection limits in terms of size are constrained by (i) the sensitivity of the detector for the target element, and (ii) isobaric interferences/background for the target isotope. In general, sensitivity is higher for heavier elements.
- The correct size determination is limited to spherical particles of known density.
- Current instruments only allow mono-isotopic detection, i.e. different particles that carry the same target element cannot be distinguished (e.g. Ag NP from Ag/Au NP). New instruments are on the edge to allow bi-isotopic detection (at the cost of compromising correct quantification and thus size determination).
- Constituent particles in aggregates are not resolved, in agglomerates only by appropriate dispersion in the sample preparation step (not in the instrument).

Table 15 and Table 16 below give the general performance of the method single particle Inductively Coupled Plasma – Mass Spectrometry and the performance table for this method, respectively.

Table 15: General performance of single particle inductively coupled plasma – mass spectrometry (spICP-MS)








| Main features | |
|----------------------------|--|
| Type of samples | particles (typically solid) in liquid dispersion medium |
| Type of sizing | counting technique (by identifying individual objects in continuous signals) |
| Particle property measured | mass of specified elements equivalent diameter: mass or volume equivalent diameter |
| Type of quantity | particle number |
| Size range | depending on target element, e.g.: Au 15 – 1000 nm, Ag 20 - 1000 nm, TiO ₂ 50 – 1000 nm, SiO ₂ 200 – 1000 nm |
| Concentration range | depending on element, particle size, instrument (e.g. Ag 60 nm: 5 – 500 ng/L) |
| Information content | <ul style="list-style-type: none"> • very high with respect to size distribution, i.e. can well resolve details of the size distribution • good (particle size, particle number concentration, mass concentration) |

| Main advantages |
|--|
| <ul style="list-style-type: none"> • intrinsically yields number-weighted size distributions, Q0 • good resolution of particle size distribution • provides number concentration of the analysed sample • chemically specific • rapid and cost-efficient |
| Main disadvantages |
| <ul style="list-style-type: none"> • does not resolve particle shape, but measures a mass or volume equivalent diameter and needs the particle density • measures aggregate size rather than size of the constituent particles • lower size limit is >10 nm and depends on material properties • cannot be applied to all kinds of material • accurate size determination limited to spherical particles |

Table 16: Technical characteristics for single particle inductively coupled plasma – mass spectrometry (spICP-MS)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characte risation (Yes/No) | Notes |
|---|---|--|---|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes ⚠ | Only aqueous liquids |
| | Solid particulate form | No | |
| | Dispersed or embedded in matrices | No | |
| Dispersibility by dispersion protocols (instrument requires that test material is in liquid dispersion) | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | No | |
| | Dispersible in polar liquids other than water | No | |
| | Dispersible in material-specific media | ⚠ No | Only aqueous media (which can be modified with e.g. dispersants, buffers, low percentage of organic solvents) |
| | Can be aerosolized | No | |

| | | | |
|---|---|---|---|
| Substance Nature | Inorganic | Yes  | Sensitivity and interferences depending on element |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | No | |
| | Organic, particulate | No | |
| | Organic, non-particulate | No | |
| | Biological | No | |
| | Composite particles | Yes  | Requires that particle contains detectable elements (of inorganic nature) |
| | Other | | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes  | If particle contains detectable elements and the composition of core and shell is known |
| | Multishell particles | Yes  | |
| | Particles with inclusions | Yes  | |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No | |
| | 2 (e.g. diameter of nanofibres) | No | |
| | 3 | Yes | Measurement technique does not resolve shape |
| Shape of particles | Sphere or similar | Yes  | Measurement technique can measure objects of different shapes, but only provides an equivalent diameter |
| | Equiaxial | Yes  | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | No | |
| | Other | No | |
| Thermal degradation sensitivity (Must be compatible with measurement technique working range: 15-40 °C) | Above 0 °C | No | |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| | Sensitive above 1000 °C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |

| | | | |
|---|---|---------------|--|
| (Must be compatible with measurement technique working range: 15-40 °C) | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron-beam sensitivity | Electron-beam sensitive | Yes | |
| | Not electron-beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | |
| | Monomodal | Yes | |
| | Multimodal | Yes | |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes ⚠ | Measurement technique measures only aggregate size |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Measurement technique measures only agglomerate size |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | Yes | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | No | |
| | Integral technique | No | |
| | Used in hyphenated methods | Yes | E.g. with FFF |
| Working range | Size range | 15 to 1000 nm | Depending on element (e.g. Ag: 15 – 1000 nm, TiO ₂ 50 to 1000 nm) |

| | | | |
|--|--|------------------------------|---|
| | Concentration range | N/A | Depending on element, particle size, instrument (e.g. Ag 60 nm: 5 – 500 ng/L). Result is mass |
| | Minimum needed sample amount | 5 mL of injection dispersion | Usually not an issue due to the high dilution factors (1000 – 100 000) of the original sample |
| | Linearity/proportionality | size: no quantity: yes | Linear range depending on element, particle size, instrument |
| | Limits of detection (LoD)/quantification | N/A | Depending on element it is between 10 and 30 nm |
| | Sensitivity (counting efficiency) as a function of size | N/A | |
| Trueness | Indicate the trueness of the results of this measurement technique in measuring the particle size | good | Depending on analyte, matrix, laboratory |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | good | Starting from 2 x LoD |
| Robustness | Specify the robustness of this measurement technique | good | |
| Precision | Specify the precision of the measurement technique | 0.1% to 2% | for $x_{50,0}$ ⁵³ |
| Resolution | Specify the resolution of this measurement technique | 1% | |
| Size distribution | Is it possible to measure size distribution? | Yes | Size distribution will be measured as a mass distribution |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | Yes | |
| | Discrimination between NPs and non-NPs of another chemical composition | No | The technique is tuned to a single m/z value during measurement |
| | Discrimination from NPs of another chemical composition | No | The technique is tuned to a single m/z value during measurement |
| | Impurities | Yes ⚠ | Dissolved impurities are ignored |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |

| | | | |
|--|---|--------------------------|--|
| Measurement of individual particles | Does this measurement technique characterise individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | Yes | At the moment only one m/z |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | mass equivalent diameter | Can be used to calculate diameter for spherical, non-porous particles by using a density |
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes | |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suitable supports? | No | |

4 Ensemble methods (spectroscopic)

4.1 Dynamic light scattering (DLS)

4.1.1 Measurement principle

Dynamic Light Scattering (DLS), also called Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering (QELS), is a technique for characterisation of colloidal systems based on the scattering of visible light resulting from the difference in refractive index between the dispersed colloids and the dispersion medium. Fluctuations of scattered light may be caused by any changes in the microstructure of the suspension, e.g. by particle motion or vibrations of particle networks. For this reason, there are manifold applications for DLS, e.g. particle sizing, molecular weight determination, studying particle aggregation, monitoring phase transition in colloidal suspensions, or measuring the strength of colloidal gels.

The principle in DLS is measurement of fluctuations in laser light scattered by vibrating particles suspended in a liquid as function of time (Figure 3). The vibration is due to Brownian motion caused by collision with solvent molecules of the liquid. The Brownian motion varies as a function of particle size and causes variation in the intensity of scattered light as function of time. A correlator compares the signal measured at a time t_0 with different very short time delays Δt (autocorrelation). As the particles move, the correlation between t_0 and subsequent Δt signals decreases with time, from a perfect correlation (1) at t_0 , to a complete decorrelation (0) at infinite time (order of milliseconds). In the case of large particles, the signal changes slowly and the correlation persists for a long time, whereas small particles have fast Brownian movement causing rapid decorrelation.

Thus, a DLS instrument measures the velocity of Brownian motion, defined by the translational diffusion coefficient D of the particles. The particle size, or more precisely its hydrodynamic diameter d_h , is then estimated using the Stokes-Einstein equation assuming spherical shape:

$$d_h = \frac{kT}{3\pi\eta D}$$

k : Boltzmann's constant

D : translational diffusion coefficient

T : absolute temperature

η : viscosity

It should be noted that even if a particle is truly spherical, the spherical DLS size is fundamentally different from the physical spherical size. The hydrodynamic size includes the double-layer of highly polarized water molecules around the physical particle. When the particle morphology is highly non-spherical, the hydrodynamic size should be understood as the equivalent hydrodynamic spherical size. Establishment of mean hydrodynamic size and size distributions (intensity, number, volume) is reached software algorithms, by fitting the correlation function in the data treatment.

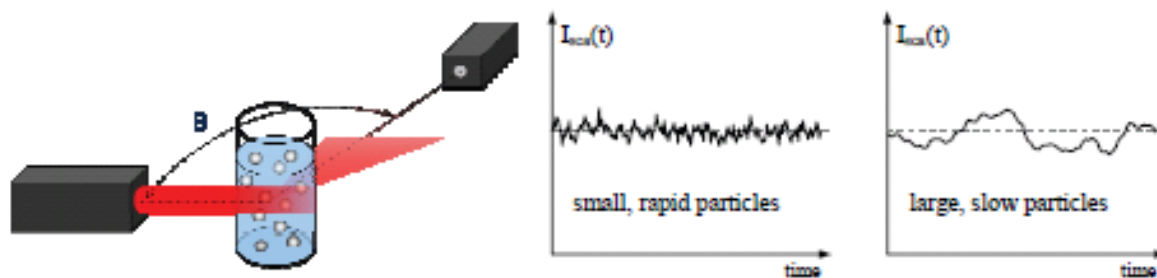


Figure 3: Measurement principle of DLS

The intensity fluctuations of DLS experiments can be analysed in terms of (ISO 22412 and ⁴³):

- a frequency spectrum (frequency analysis, FA), or
- a time correlation function (photon correlation spectroscopy, PCS).

PCS requires a different hardware than FA, but it can be shown that the results of both techniques are equivalent^{42, 43}. Today, a large variety of commercial or “self-made” DLS instruments are used. Apart from data processing (FA or PCS), they can be distinguished with regard to laser optics and signal modulation.

In quiescent, dilute suspensions, the light fluctuations result essentially from the Brownian displacement of the single particles and thus reflect the particles’ translational diffusion coefficient D_t . For spherical particles, this parameter (D_t) is inversely proportional to the sphere diameter (Stokes-Einstein equation).

4.1.2 Performance

Limits for the applicability of DLS are mainly set by size and concentration of particles. First of all, the concentration should be low enough to avoid strong multiple scattering. For particle sizing the concentration should be low enough to ensure measurement of short time self-diffusion of particles. As a rule of thumb, this may be achieved for concentrations below 0.01 to 0.1 vol.-%, but lower concentration values may be necessary for very fine nanoparticles.

Additionally to the upper concentration limits, there is also one at the lower range, where the intensity fluctuations start to become affected by the statistic variation of particle number in the measurement zone⁴⁴, a minimum of 100 particles should stay in the measurement zone. This is fulfilled for most colloidal suspensions; problems may arise for micrometre particles (i.e. $\geq 1 \mu\text{m}$).

Sedimentation sets a limit to the detection of large particles because the particle displacement of micrometre particles is governed by sedimentation rather than by diffusion. This is of particular importance for polydisperse particle systems, where diffusion and sedimentation are coupled⁴⁵. Moreover, sedimentation may affect the size distribution in the measurement zone. However, in the colloidal size range ($x \leq 1 \mu\text{m}$) there is virtually no impact of sedimentation on DLS results⁴⁶. A lower size limit exists only as much as the scattering intensity of the particles should considerably exceed that of the fluid molecules. Xu⁴³ proposes a minimum factor of 2.5; however, for highly reliable DLS this value should be multiplied by 10.

During the last two decades, dynamic light scattering has evolved into a major characterisation technique for colloidal suspensions. A recent interlaboratory study of the characterisation of a monomodal colloidal silica⁴⁷ showed that state-of-the-art DLS instrumentation facilitates a highly

reproducible and very reliable acquisition of correlation function and corresponding mean particle size x_{cum} . The study involved 17 participants from EU and USA, which provided 19 independent data sets from 6 different commercial instruments covering sideward scattering (90°) and backscattering. An earlier study with a different test material already indicated the high interlaboratory comparability⁴⁸.

Table 17 and Table 18 below give the general performance of the method dynamic light scattering and the detailed performance table for this method, respectively.

Table 17: General performance for dynamic light scattering (DLS)

| Main features | |
|--|---|
| Type of samples | particles (solid, liquid, gaseous) in liquid dispersion medium |
| Type of sizing | spectroscopic ensemble technique |
| Particle property measured | diffusion coefficient, equivalent diameter: (apparent) hydrodynamic diameter (apparent because signal is affected by Brownian rotation) |
| Type of quantity | intrinsically: intensity of scattered light; for NPs: $I_{sca} \propto x^6$ |
| Size range | 1 nm to 1 μ m |
| Concentration range | ≤ 1 vol.-% (depends on the material) |
| Information content | relatively low, only few details of the PSD can be resolved |
| Main advantages | |
| <ul style="list-style-type: none"> • lower size limit <10 nm • relatively wide dynamic range • when DLS detects NPs then there are certainly NPs, • fast and robust • a minimum amount of information about the sample is needed to run the analysis • testing is non-destructive (sample may be recovered) • small amount of sample is needed to run a test | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • number-weighted size distributions Q0 cannot directly be measured • does not resolve particle shape, but measures a hydrodynamic/mobility equivalent diameter, non-spherical particles will introduce errors • measures aggregate size rather than size of the constituent particles • size distribution obtained by inverting a signal spectrum \rightarrow principally limits resolution • low resolution | |

Table 18: Technical characteristics for dynamic light scattering (DLS)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes | |
| | Dispersible in polar liquids other than water | Yes | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes ⚠ | See size criteria |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite particles | Yes ⚠ | Outer particle size |
| | Other | No | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | Outer particle size |
| | Multishell particles | Yes ⚠ | |
| | Particles with inclusions | Yes ⚠ | |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No | |
| | 2 (e.g. diameter of nanofibres) | No | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes ⚠ | Conventional DLS measurement and analysis do not resolve shape |
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | |
| | Flakes and discs (thickness:lateral | No | |

| | | | |
|--|---|-----------------------|---|
| | extension ≤ 0.25) | | |
| | Other | | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 5-60 °C) | Above 0 °C | No | |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| | Sensitive above 1000 °C | Yes | |
| | Cooling degradation sensitivity (Must be compatible with Measurement Technique working range: 5-60 °C) | Sensitive below 25 °C | Yes |
| Sensitive below 0 °C | | Yes | |
| Sensitive below -18 °C | | Yes | |
| Sensitive below -35 °C | | Yes | |
| Sensitive below -78 °C | | Yes | |
| Sensitive below -195 °C | | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | Insensitive to fine NPs for (very) broad PSDs |
| | Monomodal | Yes | |
| | Multimodal | Yes | Insensitive to fine NPs for (very) broad PSDs |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes ⚠ | Only aggregate size |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Only agglomerate size |
| | Nanoparticles are not agglomerated | Yes | |

| | | | |
|---|---|-----------------------|---|
| Counting, fractionating or ensemble technique | Single particle counting | No | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | Yes | Intrinsically: intensity-weighted size distribution |
| | Integral technique | No | |
| | Used in hyphenated methods | Yes | E.g. in FFF for measuring hydrodynamic diameter at narrow size fractions |
| Working range | Size range | 3 nm – 5 μ m | |
| | Concentration range | 0.00001 to 0.3 vol.-% | Depending on optical contrast, particle size |
| | Minimum needed sample amount | 0.1 mL to 2 mL | Depending on instrumentation |
| | Linearity/proportionality | No Yes | No, with regard to size (decay of ACF [autocorrelation function] or spectral shift) Yes, with regard to concentration (total intensity) for diluted systems (where linear dependence on concentration) |
| | Limits of detection/quantification | 1 nm to 20 nm | Depending on optical contrast and size |
| | Sensitivity (counting efficiency) as a function of size | good | Insensitive to very fine particles (weak scattering signal) and very coarse particles (do not contribute to signal fluctuation and disappear from measurement zone) |
| Trueness of particle size measurement | Indicate the trueness of this measurement technique | good | Size: “falseness” if wrong model parameters (e.g. viscosity, wavelength) or if too a high concentration (multiple scattering, hydrodyn. interaction) |

| | | | |
|--|--|---------------------------|---|
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | moderate | Intensity weights: “falseness” if multiple scattering or if too high laser intensities |
| Robustness | Specify the robustness of this measurement technique | good | |
| Precision | Specify the precision of the measurement technique | 0.2% to 20% 0.4% to 5% | For $x_{50,0}$ (NanoDefine) For $x_{50,int}^3$ (NanoDefine) |
| Resolution | Specify the resolution of this measurement technique | 20% to 50% | |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | Yes ⚠ | If NP dominate |
| | Discrimination between NPs and non-NPs of another chemical composition | No | |
| | Discrimination from NPs of another chemical composition | No | |
| | Impurities | No | Particulate impurities (in the right size range and with sufficient contrast) are detected. Dissolved impurities are ignored |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | No | |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size | Specify: for example hydrodynamic... | apparent hydrodyn | Short time self-diffusion: translation affected by |

³ $x_{50,int}$ is a median of (scattering) intensity-weighted size distribution Q_{int} , which is intrinsically measured by DLS; the term Q_{int} is given in ISO 22412:2017

| (diameter) | | amic diameter | rotation for non-spherical objects |
|--|--|---------------|--|
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes | If dilution is required; otherwise not |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suited supports? | No | |

4.2 Small-angle X-ray scattering (SAXS)

4.2.1 Measurement principle

Small-angle X-ray scattering (SAXS) is a technique based on the interaction between X-rays and matter to probe the structure of materials. X-rays passing through a medium that is not completely homogenous is forced to deviate from the straight direction. The scattering angle, i.e. the deviation from the straight line (see

Figure 4), depends on the wavelength of X-rays and the size of the particles on which they are scattered. The processed data are the intensity, I , of X-ray scattered by a sample as a function of angular position of a detector.

The intensity can be expressed in relative units or in absolute scale. The absolute scale is independent from test parameters such as X-ray wavelength, experimental background, time of acquisition and sample thickness. In both cases, 2D raw data images are converted into diffractograms displaying the scattered intensity I as a function of scattering vector \mathbf{q} defined by:

$$|\mathbf{q}| = \frac{4\pi \sin \theta}{\lambda}$$

with λ : X-ray wavelength.

The intensity pattern of the scattered radiation can give information about the particle size, size distribution, as well as the shape of the particles and their nanostructure. Size and shape of the particles are obtained by fitting of the measured scattering curves (intensity vs angle).

Two main applications of SAXS are characterization of nanoparticles and determination of large surface areas. The SAXS theory is based on fundamental physical processes⁴⁹, and it is very mature. A study of six European metrology institutes proved recently that SAXS allows traceable size determination of monomodal, spherical nanoparticles¹¹.

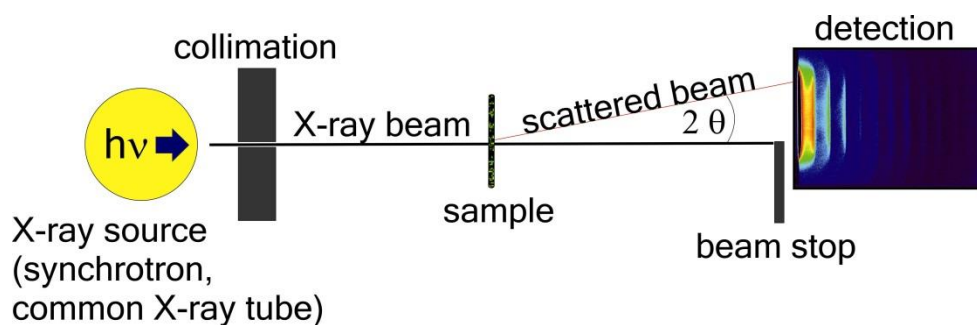


Figure 4: Measurement principle of SAXS

4.2.2 Performance

The method is accurate, non-destructive and requires only a minimum of sample preparation. SAXS covers only the lower range of interest from 1 to 100 nm. The experimental devices at synchrotrons and common laboratories are well developed. Numerous technical improvements have been made during the last five years. High throughput instruments were realized by using sample changing robots⁵⁰ and new X-ray detectors for low noise data recording are available⁵¹ for esynchrotron and laboratory SAXS. Currently, numerous SAXS manufacturers in Europe have improved SAXS instruments. These instruments are suitable for routine and standardized measurements in accordance with ISO standards.

Table 19 and Table 20 below give the general performance of the method small-angle X-ray scattering and the detailed performance table for this method, respectively.







Table 19: General performance for small-angle X-ray scattering (SAXS)

| Main features | |
|----------------------------|--|
| Type of samples | particles (solid, liquid) dispersed in liquid media or in solid matrices; or solid particles in powder form (any two-phase systems where the phases have different density) |
| Type of sizing | spectroscopic ensemble technique |
| Particle property measured | scattering pattern (which reflects particle size, shape and orientation as well as state of aggregation) a) size analysis for particle with defined shape: external dimensions of the particles (e.g. diameter and length of rod-like particle) b) size analysis for particles of arbitrary (irregular) shape → scattering equivalent diameter |
| Type of quantity | intrinsically: particle surface (for typical size analysis) |
| Size range | 1 to 100 nm (upper limit depends on specific instrument) |
| Concentration range | 0.01 vol.-% to 100 vol.-% (lower limit depends strongly on size and density of particles, as well as on the quality of the instrument) |

| | |
|---|--|
| Information content | relatively low with respect to size distribution, i.e. only few details of the PSD can be resolved |
| Main advantages | |
| <ul style="list-style-type: none"> • can, in principle, resolve particle shape and aggregate structure • facilitates distinction between aggregates and their constituent particles • particles can be detected with minimal sample preparation. No or little danger exists for sample preparation artefacts • can be employed to determine the volume specific surface area (VSSA) • advanced instrumentation allows for determination of particle number concentration | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • number-weighted size distribution cannot directly be measured • typically limited to particles ≤ 100 nm (even with advanced instrumentation ≤ 400 nm) • size distribution obtained by inverting a signal spectrum \rightarrow principally limits resolution • low resolution • shape cannot be resolved for highly polydisperse materials | |

Table 20: Technical characteristics for small-angle X-ray scattering (SAXS)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characteri- sation (Yes/No) | Notes |
|---|---|---|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | Determination of VSSA may be possible |
| | Dispersed or embedded in matrices | Yes  | As long as the X-rays transmit through the material |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes  | In-situ measurement |
| | Dispersible in non-polar liquids | Yes  | |
| | Dispersible in polar liquids other than water | Yes  | |
| | Dispersible in material-specific media | Yes  | |
| | Can be aerosolized | No | Aerosols can be measured with special setups ⁵² |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes  | Size dependent absorption or fluorescence in the UV-Vis range does not affect the SAXS results |
| | Carbon based | Yes | |

| | | | |
|---|---|-------------|--|
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite particles | Yes | |
| | Other | | |
| Composite particles (see Section 2.2.1.3) | Core/shell | No Yes ⚠ | For unknown materials with increased polydispersity it is rather difficult to predict the measurement result of SAXS. Thickness of individual coatings can be determined if core and shell have different electron densities |
| | Multishell particles | Yes ⚠ | |
| | Particles with inclusions | Yes | |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | Yes ⚠ | Size and size distribution can be measured after knowledge of shape (from EM) |
| | 2 (e.g. diameter of nanofibres) | Yes ⚠ | |
| | 3 | Yes | |
| Shape of particles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | Yes | Yes for low polydispersity |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | Yes | Yes for low polydispersity |
| | Other | No ⚠ | If different shapes are mixed in the same sample SAXS analysis is possible only with additional information such as shape information from imaging methods |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: -10-60 °C) | Above 0 °C | No | |
| | Sensitivity above 25 °C | Yes | |
| | Sensitivity above 37 °C | Yes | |
| | Sensitivity above 50 °C | Yes | |
| | Sensitivity above 100 °C | Yes | |
| | Sensitivity above 150 °C | Yes | |
| | Sensitivity above 500 °C | Yes | |
| | Sensitivity above 1000 °C | Yes | |

| | | | |
|--|------------------------------------|-------|---|
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: -10-60 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes ⚠ | If polydispersity is above ~20%, data evaluation may become ambiguous without further information |
| | Monomodal | Yes | |
| | Multimodal | Yes ⚠ | If modes are too close to each other, distinction of the modes is difficult |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes | |
| | Nanoparticles are not agglomerated | Yes | |

| | | | |
|---|---|---------------|---|
| Counting, fractionating or ensemble technique | Single particle counting | No | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | Yes | Intrinsically: surface-weighted size distribution |
| | Integral technique | No | |
| | Used in hyphenated methods ⁴ | No | |
| Working range | Size range | 1 nm – 100 nm | Typically 1 to 100 nm, but size range depends on specific instrument. Specialized instruments work up to 10 micrometres. |
| | Concentration range | <= 1 vol% | The concentration range depends strongly on the electron density of the particles. The higher the electron density is, the more sensitive SAXS is |
| | Minimum needed sample amount | 1 to 100 µL | Depending on the instrument and sample holder used |
| | Linearity/proportionality | Yes | |
| | Limits of detection/quantification | 1 nm | |
| | Sensitivity (counting efficiency) as a function of particle size | good | Sensitivity increases with size |
| Trueness of particle size measurement | Indicate the trueness of this measurement technique in measuring the particle size | good | SAXS is a metrologically traceable method ¹¹ |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions by this measurement technique | average | Can be quantified if uncertainties are considered properly ⁵¹ |
| Robustness | Specify the robustness of this measurement technique | good | Results are robust for defined SOPs of data processing |
| Precision | Specify the precision of the measurement technique | 1% to 5% | For $x_{50,0}$ ⁵³ |

⁴ There are 2 independent signals: average track length and spot size, i.e. diffusion coefficient and scattering intensity; a 2-dimensional plot of a monoconstituent sample would therefore show the functional relationship between size and scattering cross section (i.e. a strong, non-linear correlation); for multiconstituent samples we would expect different groups of such functional dependencies, which offers a possibility to couple size analysis with material or shape analysis

| | | | |
|--|--|-----------------|--|
| Resolution | Specify the size resolution of this measurement technique | 5% to 20% | |
| Size distribution | Is it possible to measure particle size distribution? | Yes | |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | No | Size range is typically limited to ≤ 100 nm |
| | Discrimination between NPs and non-NPs of another chemical composition | No | |
| | Discrimination from NPs of another chemical composition | Yes | With contrast variation techniques |
| | Impurities | Yes | |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | Yes | Restricted to small agglomerates/aggregates (typically < 100 nm) |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | No | |
| Counting constituent particles in aggregates | Is the measurement technique able to characterise single constituent particles in aggregates? | No | SAXS is able to measure the (average size) of particles within aggregates, but not by counting as it is a spectroscopic method |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | physical length | Based on density differences |
| Destructive measurement technique or not | Is it a destructive measurement technique? | No | (high-flux) synchrotron radiation may induce damage |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | Yes, if required ⚠ |
| Sample support | Does this measurement technique need preparation on suitable supports? | No | |

4.3 Ultrasonic spectroscopy (USSp)

4.3.1 Measurement principle

Ultrasonic spectroscopy is the generic term for all particle sizing techniques that are based on the frequency-dependent measurement of sound velocity or attenuation in the ultrasonic domain (mostly within 100 kHz to 200 MHz). While velocity spectroscopy is mainly used for the study of inter- and intramolecular processes, attenuation spectroscopy has found its major application in particle sizing^{54,55}. The most promising feature of acoustic characterisation techniques is their applicability to highly concentrated particle systems (up to 70 vol.-%) under non-equilibrium conditions⁵⁶. That means it offers the opportunity to monitor the state of dispersion of dense product streams, to control the de-agglomeration of suspensions or the homogenisation of emulsions, and to study polymerisation or crystallisation processes.

As mentioned before, the attenuation spectroscopy has found application in particle sizing⁵⁵. The principle of this technique is that plane sound waves moving through a particle suspension are attenuated in a predictable manner according to size and concentration of the particles in suspension.

Attenuation of an ultrasonic wave passing through a suspension may be modelled given a set of mechanical, thermodynamic and transport properties describing both the continuous and particulate media. The relationship between spectral data and particle size is illustrated by the attenuation curves. Each curve shows the attenuation of sound waves of a particular frequency as a function of the size of a monosize population of fixed volume concentration. Due to the measurement noise along with modelling errors, for the reliable analysis, measurements should be performed for a greater number of frequencies.

4.3.2 Performance

In principle, ultrasonic spectroscopy can be used for the characterisation of particles in the colloidal and micrometre size range, provided that the particle concentration is sufficiently high (at least 1 vol.-%) and that the signal contribution by the particles is significant compared to those by the continuous phase (may be a problem for attenuation measurements in highly viscous solvents).

A major difficulty for the particle sizing by USSp is that the acoustic behaviour depends on a variety of material parameters. This is of particular relevance for emulsions (14 properties), whereas for aqueous suspensions only the viscosity and sound speed of liquid and the density contrast have to be known⁵⁷.

In colloidal suspensions, the sound propagation is typically governed by the acoustophoretic motion of particles. For monodisperse spheroids that are not extremely flat or stretched ($1/10 < \text{aspect ratio} < 10/1$) the attenuation spectrum essentially reflects the volume specific surface area of the particles⁵⁸. Similar results would probably be obtained for any convex particle shape. For particle aggregates, the inner structure is decisive. Regarding the type of quantity, acoustically measured size distributions are ideally volume-weighted distributions.

It could be shown that the results of ultrasonic spectroscopy agree fairly well with those of other characterization methods and are hardly affected by the extent of sample dilution⁵⁹. Interlaboratory comparisons of ultrasonic spectroscopy measurements on suspensions of inorganic particles also showed good agreement⁶⁰. That is why ultrasonic spectroscopy is considered as a powerful tool for

monitoring colloidal processes. However, the method does not allow for a very sharp resolution of size distributions in the colloidal size range⁶¹.

Table 21 and Table 22 below give the general performance of Ultrasonic spectroscopy and the detailed performance table for this method, respectively.

Table 21: General performance of ultrasonic spectroscopy (USSp)

| Main features | |
|--|--|
| Type of samples | particles (solid, liquid) in liquid dispersion medium |
| Type of sizing | spectroscopic ensemble technique |
| Particle property measured | depends on size range and kind of material <ul style="list-style-type: none"> • e.g. coarse particles $\geq 10 \mu\text{m}$: acoustic scattering • e.g. fine particles $\leq 1 \mu\text{m}$ with high density contrast: acoustophoretic motion |
| Type of quantity | depends on way of signal processing; typically: particle volume |
| Size range | 10 nm - 100 μm |
| Concentration range | > 1 vol % |
| Information content | <ul style="list-style-type: none"> • relatively low for $x \leq 1 \mu\text{m}$, i.e. only few details of the PSD can be resolved • relatively high for $x \geq 10 \mu\text{m}$, i.e. can well resolve details of the size distribution |
| Main advantages | |
| <ul style="list-style-type: none"> • for aggregates of NPs USSp detects the internal aggregate structure rather than the outer proportions, • lower size limit: 10 nm • wide dynamic range • does not require dilution for dense, turbid suspensions (which may affect the state of dispersion) • fast and robust, technique relatively easy to implement | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • number-weighted size distributions cannot directly be measured • does not resolve particle shape, but measures an acoustical equivalent diameter • size distribution obtained by inverting a signal spectrum \rightarrow principally limits resolution • low resolution of size distribution in the range $\leq 10 \mu\text{m}$ • does not allow the characterisation of dilute suspensions, i.e. requires a lot of substance • needs intense data evaluation based on mathematical modelling | |

Table 22: Technical characteristics for ultrasonic spectroscopy (USSp)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|---|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes ⚠ | If not too viscous |
| | Dispersible in polar liquids other than water | Yes ⚠ | If not too viscous |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes ⚠ | But not always, e.g. not feasible when density contrast NP/medium is not large enough |
| | Organic, non-particulate | No | |
| | Biological | No | |
| | Composite particles | Yes ⚠ | Particle outer size, provided that effective material properties are known |
| | Other | No | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | Particle outer size |
| | Multishell particles | Yes ⚠ | Particle outer size |
| | Particles with inclusions | No | Yes, particle outer size, if mixture composition is known ⚠ |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No | |
| | 2 (e.g. diameter of nanofibres) | No | |
| | 3 | Yes | |

| | | | |
|--|---|-----|--|
| Shape of nanoparticles | Sphere or similar | Yes | Measurement technique can measure objects of different shapes, but provides an equivalent diameter |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | No | |
| | Other | | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: -10-60 °C) | Above 0 °C | No | |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| | Sensitive above 1000 °C | Yes | |
| Cooling degradation sensitivity(of test material) (Must be compatible with Measurement Technique working range: -10-60 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | Difficult for NPs with broad PSD |
| | Monomodal | Yes | |
| | Multimodal | Yes | Difficult for NPs with broad PSD |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / | Functionalised | Yes | |

| | | | |
|---|---|----------------|--|
| no functionalisation | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes ⚠ | Reflects internal lengths (e.g. pore size, inter-particle distance) |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Reflects internal lengths (e.g. pore size, inter-particle distance) |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | No | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | Yes | Intrinsically: volume-weighted size distribution |
| | Integral technique | No | |
| | Used in hyphenated methods | No | |
| Working range | Size range | 1 nm to 100 µm | |
| | Concentration range | 2 to 70 vol.-% | |
| | Minimum needed sample amount | 1 mL to 100 mL | Depends on instrumentation |
| | Linearity/proportionality | No Yes | No, with regard to size Yes, with regard to quantity (volume) for dilute suspensions |
| | Limits of detection/quantification | 1 nm to 50 nm | Depends on density/ thermoacoustic contrast and size |
| | Sensitivity (counting efficiency) as a function of size | N/A | |
| Trueness of particle size measurement | Indicate the trueness of this measurement technique | average | Size: “falseness” if wrong model parameters (e.g. viscosity, sound speed) or if too a high particle concentration (>> 10 vol.-%) |

| | | | |
|--|--|----------------------------|---|
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | average | Volume weights: “falseness” if frequency range too narrow or PSD too broad (low information content below 5 µm) |
| Robustness | Specify the robustness of this measurement technique | good | |
| Precision | Specify the precision of the measurement technique | 1% to 5% | Estimate based on Dukhin et al. 2012 ⁶⁰ |
| Resolution | Specify the resolution of this measurement technique | 10% to 50% 5% to 20% | For x < 10 µm For x > 10 µm |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | Discrimination between NPs and non-NPs of the same composition | Yes | |
| | Discrimination between NPs and non-NPs of another composition | Yes ⚠ | No, if similar acoustic properties |
| | Discrimination from NPs of another chemical composition | No | |
| | Impurities | No | Dissolved impurities are ignored |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | ⚠ However for known suspension/emulsion it is possible to recognise agglomeration from signals |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | No | |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | Yes ⚠ | Reflects internal lengths (e.g. pore size, inter-particle distance) |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | “acoustophoretic diameter” | For inorganic particles in water (i.e. high-frequency hydrodynamic diameter) which is approx. the specific surface area |
| Destructive measurement technique or not | Is it a destructive measurement technique? | No | |

| | | | |
|--------------------------|--|----|--|
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suited supports? | No | |

4.4 Angular light scattering (ALS), including laser diffraction (LD)

4.4.1 Measurement principle

Angular light scattering (ALS) techniques measure the spatial distribution of scattered light. Historically, this has been realised by two different concepts of instrumentation which cover distinct size ranges. These are the *static light scattering* (SLS), which is conventionally employed for fine colloids, and the *laser diffraction*, which was originally used for micrometre particles only. Even though the measurement ranges of both techniques have actually converged in the recent past, there still remain qualitative differences in the sensor set-up and in data analysis, which justify their separate treatment.

SLS is operated for a wide range of scattering angles (typically of 10° to 150°). The time averaged angular distribution of scattered light is then commonly employed for the characterisation of macromolecules (molecular weight, radius of gyration, the second virial coefficients), but can be used to study suspensions of inorganic colloids as well (e.g. ⁶²). However, the angular distribution of scattered light is insensitive to particle size for nanoparticles, in which case only an average particle size can be determined. For very fine nanoparticles (<10 nm) the size information may be even completely lost. It could be shown that above this critical size, SLS measures the average particle size with fairly good reproducibility⁶³.

The term *laser diffraction* (LD) spectroscopy comprises angular light scattering techniques, which are primarily designed to resolve the scattering pattern at small scattering angles. Historically, LD instruments and software were restricted to the characterisation of micrometre objects for which the scattering pattern is mainly caused by diffraction and can be explained by Fraunhofer's theory from 1821. In the micrometre range, which is diffraction dominated, size distribution can be determined with high accuracy and good resolution^{64, 65}. In order to extend the instrument applicability to colloidal particle systems, several modifications have been realised, e.g.: variation of wavelength and polarisation or inclusion of wide angle scattering (⁴³(p. 111-181); ISO 13320). These modifications have evidently enhanced the sensitivity to colloidal particles far below 1 µm, but not in a uniform, reproducible way as interlaboratory comparisons prove (e.g. ⁶⁶). Even so, laser diffraction may serve as a useful tool for the characterisation of colloidal suspensions, in particular for monitoring dispersion procedures or for evaluating the coarse particle content ($\geq 1 \mu\text{m}$).

4.4.2 Performance

'Optical' measurement techniques, including ALS and LS, are prone to underestimate the amount of NPs and, thus, to overestimate the number-weighted median $x_{50,0}$. ALS also has a lower detection limit well above 1 nm, which restricts its applicability and reduces its general reliability regarding the quantification of nanoparticle fractions^{53, 66}. ALS was shown⁵³ to have deficiencies in measuring

the number-weighted median size ($x_{50,0}$) over the particularly interesting size range of 10–1000 nm, which is an important restriction for the applicability to identifying NMs.



Table 23 and Table 24 below give the general performance of angular light scattering and the detailed performance table for this method, respectively.

Table 23: General performance of angular light scattering (ALS)

| Main features | |
|--|---|
| Type of samples | particles (solid, liquid, gaseous) in liquid media or particles (solid, liquid) in gas phase |
| Type of sizing | spectroscopic ensemble technique |
| Particle property measured | in general: scattering equivalent diameter for $x \gg 1 \mu\text{m}$ (diffraction regime): dimensions of the projected image |
| Type of quantity | particle volume |
| Size range | 100 nm - 1000 μm |
| Concentration range | <0.1 vol.-% |
| Information content | <ul style="list-style-type: none"> relatively low for $x \leq 1 \mu\text{m}$ relatively high for $x \geq 1 \mu\text{m}$ |
| Main advantages | |
| <ul style="list-style-type: none"> fast and reliable particle sizing technique high resolution of PSDs in the range $x > 1 \mu\text{m}$ small amount of sample is needed to run a test | |
| Main disadvantages | |
| <ul style="list-style-type: none"> does not yield number-weighted size distributions weak performance in the colloidal size range, in particular <100 nm typically requires dilution of samples needs data evaluation based on mathematical modelling does not resolve particle shape, but measures an equivalent diameter | |

Table 24: Technical characteristics for angular light scattering (ALS)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characteri sation (Yes/No) | Notes |
|---|---|--|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes | |
| | Dispersible in polar liquids other than water | Yes | |
| | Dispersible in material-specific media | Yes | |
| | Can be aerosolized | Yes | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | Optical properties should be known |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | No | |
| | Biological | Yes | No for nanosized viruses Yes for cells ≥ 100 nm |
| | Composite particles | Yes | |
| | Other | | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes  No | Yes if optical contrast of shell is not much smaller than that of core and structure is known OR in diffraction domain |
| | Multishell particles | Yes  No | Yes if optical contrast of coatings is similar to that of core OR in diffraction domain |
| | Particles with inclusions | No | |

| | | | |
|---|---|-------|--|
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No | <100 nm not really accessible |
| | 2 (e.g. diameter of nanofibres) | No | |
| | 3 | Yes ⚠ | <100 nm not really accessible, yet monodisperse equiaxial particles >70 nm may be measurable |
| Shape of particles | Sphere or similar | Yes ⚠ | <100 nm not really accessible, yet monodisperse equiaxial particles > 0 nm may be measurable. Measurement technique can measure objects of different shapes, but provides an equivalent diameter |
| | Equiaxial | Yes ⚠ | <100 nm not really accessible, yet monodisperse equiaxial particles >70 nm may be measurable |
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | <100 nm not really accessible |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | No | |
| | Other | No | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: -40 - 100 °C) | Above 0 °C | No | |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: -40 - 100 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |

| | | | |
|---|---|-------|--|
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes ⚠ | Difficult for sub- μm |
| | Monomodal | Yes | |
| | Multimodal | Yes ⚠ | Difficult for sub- μm |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes ⚠ | Only aggregate size, and <100 nm not really accessible |
| | Nanoparticles are not aggregated | No | <100 nm not really accessible |
| | Nanoparticles are agglomerated | Yes ⚠ | Only agglomerate size, and <100 nm not really accessible |
| | Nanoparticles are not agglomerated | No | <100 nm not really accessible |
| Counting, fractionating or ensemble technique | Single particle counting | No | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | Yes | Intrinsically: surface or volume-weighted size distribution |
| | Integral technique | No | |
| | Used in hyphenated methods ⁵ | Yes | E.g. in FFF for measuring scattering intensity and radius of gyration (there named as MALLS) |

⁵ There are 2 independent signals: average track length and spot size, i.e. diffusion coefficient and scattering intensity; a 2 dimensional plot of a monoconstituent sample would therefore show the functional relationship between size and scattering cross section (i.e. a strong, non-linear correlation); for multiconstituent samples we would expect different groups of such functional dependencies, which offers a possibility to couple size analysis with material or shape analysis

| | | | |
|--|--|--------------------------|--|
| Working range | Size range | 70 nm to 10 mm | Range dependent on instrument type, <100 nm only mean size |
| | Concentration range | 0.0001 to 0.1 vol.-% | The concentration should be adjusted so that „obscuration“ lies within 2% to 20% |
| | Minimum needed sample amount | 10 µL to 50 mL | Depends on instrumentation |
| | Linearity/proportionality | Yes No | No, with regard to size Yes with regard to concentration (total intensity) |
| | Limits of detection/quantification | 50 nm to 100 nm | Depends on optical contrast and size |
| | Sensitivity (counting efficiency) as a function of particle size | good | Insensitive to very fine particles (weak scattering signal) and very coarse particles (which mainly scatter in forward direction) |
| Trueness of particle size measurement | Indicate the trueness of this measurement technique in measuring the particle size | average | Size: “falseness” if wrong model parameters (e.g. refractive index, wavelength) or if too a high concentration (multiple scattering) |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions by this measurement technique | average | Intensity weights: “falseness” if multiple scattering or if too high laser intensities |
| Robustness | Specify the robustness of this measurement technique | good | |
| Precision | Specify the precision of the measurement technique | 0.1% to 2% | For $x_{50,3}$ |
| Resolution | Specify the size resolution of this measurement technique | 10% to 50% 10% to 20% | For $x < 1 \mu\text{m}$ For $x > 1 \mu\text{m}$ |
| Size distribution | Is it possible to measure particle size distribution? | Yes | |

| | | | |
|--|--|----------------------|---|
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | Yes ⚠ | <100 nm not really accessible (depends on polydispersity) |
| | Discrimination between NPs and non-NPs of another chemical composition | No | <100 nm not really accessible |
| | Discrimination from NPs of another chemical composition | No | |
| | Impurities | No | Particulate impurities (in the right size range and with sufficient contrast) are detected. Dissolved impurities are ignored |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | No | |
| Counting constituent particles in aggregates | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | lengths of main axes | Needs shape model to be known, otherwise "equivalent ALS diameter" |
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes ⚠ | If dilution is required; otherwise not |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suitable supports? | No | |

5 Fractionating methods

5.1 Field-flow-fractionation (FFF)

5.1.1 Measurement principle

FFF is a fractionation technique that separates particles, e.g. macromolecules or particles in colloidal suspensions, according to their hydrodynamic size. The sample is pumped through a channel in a laminar flow, which coincides with a parabolic velocity profile. Fluid elements close to the wall have a considerably higher residence time than fluid elements at the channel centre.⁶⁷ Perpendicularly to this channel flow, a field is applied. In most of the cases, it is a second flow but the field can be electric, magnetic or centrifugal. The field causes a separation of particles along the lateral axis with respect to their size, which means that they leave the channel after different residence times. Quantification of the amount of particles depends on the sort of detector used and its calibration. FFF is performed in liquids and it is limited to the analysis of the particles which can to be dispersed in liquids⁶⁷.

Figure 5 visualises the FFF technique principles; Figure 5a is a schematic diagram of a FFF-system including commonly used detection systems, and

Figure 5 shows a cross-section of the channel illustrating the separation mechanism.

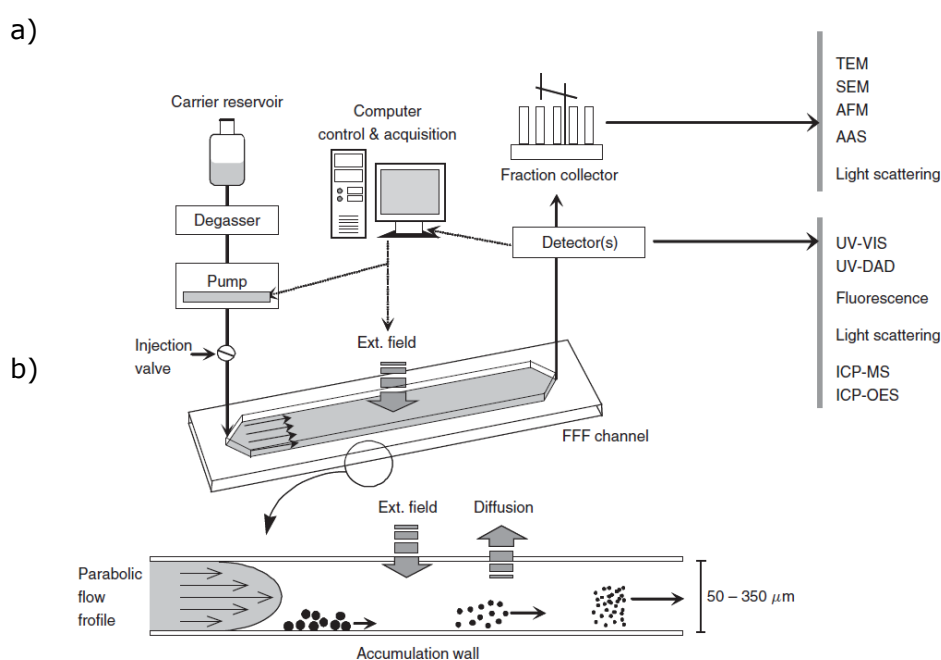


Figure 5: Measurement principle of FFF

a) schematic diagram of a FFF-system including commonly used detection systems; b) cross-section of the channel illustrating the separation mechanism (adapted from v. d. Kammer 2005)⁶⁷

Several FFF techniques have been developed, based on various separation principles such as particle diffusion (e.g. asymmetric flow field-flow-fractionation, AF4, which is based on perpendicular flow field) or buoyant mass (e.g. sedimentation field-flow-fractionation, SedFFF, which is based on centrifugal force). In combination with a suitable detection system, FFF

techniques enable us to derive particle size or molecular mass from sample specific properties, such as particle diffusion. The separation concept of all FFF techniques is similar, as described above.

NanoDefine analysed in particular AF4 separation, during which a force drives the sample (i.e. dissolved and particulate components) towards the accumulation wall that is covered by an ultrafiltration membrane which is permeable for components smaller than the cut-off of the membrane. Components which are retained in the channel, will be distributed in the channel profile according to their diffusional properties (i.e. size), and as described above, depending on these properties the particles will experience different laminar flows resulting in a separation according to their hydrodynamic size. The separated sample is detected online by a suitable detector; the combination studied in NanoDefine was AF4-LS, asymmetric flow field flow fractionation –light scattering.

5.1.2 Performance

FFF separation methods provide a robust technique to separate particles according to their size or molecular mass. Together with a subsequent detection technique, FFF can provide distributions of both physical and chemical properties.

Current limits of application include:

- particle size determined by FFF is always an equivalent spherical particle diameter. Thus, values obtained for non-spherical particles from different sub-techniques will differ from each other,
- in case of ideal conditions in AF4 only diffusional properties of the sample affect the separation. However, in practice, completely ideal conditions cannot be achieved. Therefore, particle sizing might be affected by a number of potentially interfering instrumental factors. It has to be emphasized that optimization of the run conditions for both size standard and sample have to be performed rigorously until behaviour close to ideal elution is achieved,
- for SedFFF no size calibration is required. For size determination the knowledge of buoyant mass of the particle sample is required.

Table **25** and

Table 26 below give the general performance of field-flow-fractionation and the detailed performance table for this method, respectively.

Table 25: General performance of field-flow-fractionation (FFF)

| Main features | |
|----------------------------|--|
| Type of samples | particles (solid, liquid) in liquid dispersion medium |
| Type of sizing | fractionating technique |
| Particle property measured | <p>a) FFF with concentration detectors dependent on applied field, e.g. FlowFFF: particle diffusion coefficient (equivalent diameter : hydrodynamic / mobility diameter)</p> <p>b) FFF with concentration and size detectors dependent on size measurement behind FFF unit e.g. MALLS: radius of gyration, DLS: apparent hydrodynamic diameter</p> |
| Type of quantity | <p>detector dependent: UV/VIS: extinction (turbidity), for transparent nanoparticles ~ squared particle volume, for light-absorbing nanoparticles ~ particle volume fluorescence: particle mass concentration if particle is fluorescing, MALLS → intensity of scattered light; ICP-MS → element mass concentration (element must be constituent of the particle and stoichiometry known); if counting detectors are used, particle number concentrations can be determined (e.g. spICP-MS)</p> |
| Size range | in general 1-1000 nm. Instrumental settings need to be tuned for the size range of interest, dynamic range (ratio of upper to lower measurable diameter) is typically 20 – 40 |
| Concentration range | adjustable, case-specific, the minimum and maximum acceptable concentration depends strongly on the sample characteristics, the size range and applied detector |
| Information content | <p>relatively high, i.e. can well resolve details of the size distribution</p> <ul style="list-style-type: none"> • for AF4: diffusion coefficient, hydrodynamic radius; • for Sedimentation FFF: volumetric radius • further information depending on coupled detector, e.g. <ul style="list-style-type: none"> - light scattering (SLS): root-mean-square (rms)-radius and geometric radius, intensity-weighted size distribution - ICP-MS: chemical composition, mass concentration, mass-based size distribution) - UV/DAD: indicator for chemical composition (element specific wavelength absorption and surface plasmon resonance) |
| Main advantages | |

- physical separation of particles: can operate on complex mixtures and matrices
- element specific: can operate on complex mixtures and matrices
- coupling of FFF with arbitrary techniques for size and concentration measurement principally allows a comprehensive analysis with respect to sample chemical composition and particle shape

Main disadvantages

- typically: number-weighted size distribution Q0 cannot directly be measured.
- does not resolve particle shape, but measures a hydrodynamic/mobility equivalent diameter,
- measures aggregate size rather than size of constituent particles
- lower size limit depends on detectors, design of the FFF unit and parameters of operation
- limited dynamic range (different elution regimes for different particle sizes)
- high efforts for preparing FFF unit and sample
- operators require high level of proficiency

Table 26: Technical characteristics for field-flow-fractionation (FFF)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|------------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes ⚠ | Channels for organic liquids available. Not routinely used |
| | Dispersible in polar liquids other than water | Yes | |
| | Dispersible in material-specific media | Yes ⚠ | Case by case decision |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | Macromolecules and similar are possible |
| | Biological | Yes | |
| | Composite particles | Yes | |
| | Other | | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | Only outer size. Depends on the type of fractionation and the detector |
| | Multishell particles | Yes ⚠ | |
| | Particles with inclusions | Yes ⚠ | |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No | |
| | 2 (e.g. diameter of nanofibres) | No | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | Measurement technique can measure objects of different shapes, but typically provides an equivalent diameter |
| | Equiaxial | Yes | |

| | | | |
|---|--|-----|--|
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | ⚠ Yes in certain cases with highly specialised instrumentation (impractical) |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | No | |
| | Other | | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 10-40 °C) | Above 0 °C | No | |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| | Sensitive above 1000 °C | Yes | |
| Cooling degradation sensitivity (Must be compatible with Measurement Technique working range: 10-40 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | |
| | Monomodal | Yes | |
| | Multimodal | Yes | |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | Only outer size |
| | Not functionalised | Yes | |

| | | | |
|---|---|--|---|
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes | ⚠ Only aggregate size |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes | ⚠ Only agglomerate size |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | No | |
| | Measures or calculates number or number concentration from fractionating techniques | Yes | ⚠ Yes in special FFF-setups with a detection system that counts particles of narrow fractions provided by FFF |
| | Calculates number or number concentration from spectroscopic ensemble techniques | No | |
| | Integral technique | No | |
| | Used in hyphenated methods | Yes | Intrinsically hyphenated as fractionation and detection are locally separated, e.g. FFF+UV/VIS+MALS+spICP-MS |
| Working range | Size range | 1 - 1000 nm | Depending on the detector and type of field, e.g. AF4-MALS: 3 nm to 1000 nm |
| | Concentration range | 20 µg/L - 500 mg/L | Depending on the detector |
| | Minimum needed sample amount | 10 µL | |
| | Linearity/proportionality | Size: No (for diffusion, volume, settling velocity or MALS) Quantity: Yes for light scattering. No for extinction - since measured via transmission | Depending on detector |

| | | | |
|--|--|---------------|--|
| | Limits of detection/quantification | 1-10 nm | Depending on detector |
| | Sensitivity (counting efficiency) as a function of size | good | Detector dependent |
| Trueness of particle size measurement | Indicate the trueness of this measurement technique | average | If reference materials available |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | excellent | Mass quantification if performed by mass specific detector |
| Robustness | Specify the robustness of this measurement technique | good | Important parameter is the membrane quality |
| Precision | Specify the precision of the measurement technique | 1 nm to 10 nm | |
| Resolution | Specify the resolution of this measurement technique | 1 nm to 10 nm | |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | Discrimination between NPs and non-NPs of the same chemical composition | Yes | ⚠ Pre-treatment of the sample is necessary ⁶ |
| | Discrimination between NPs and non-NPs of another chemical composition | Yes ⚠ No | Pre-treatment of the sample is necessary ⁶ . Depends on detection technique |
| | Discrimination from NPs of another chemical composition | Yes | Depends on detection technique |
| | Impurities | ⚠ | Depends on the detector. Dissolved impurities are ignored |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | No | |

⁶ Pretreatment of the sample is necessary because of an steric effect combined with a hydrodynamic lift effect; both phenomena push coarse particles back in the channel centre and cause them to leave the channel together with fine, rapidly diffusing particles; 'coarse' depends on the specific conditions of operation, but typically we talk about particles > 0.5 µm

| | | | |
|--|---|-----------------------|---|
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | N/A | For chemical analysis the FFF needs to be coupled to ICP-MS or ICP-OES |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | Hydrodynamic diameter | Diffusion coefficient (FlowFFF) hydrodynamic diameter can be derived, volumetric diameter (SedFFF or CFFF). In few cases when MALS is applicable also root-mean-square and geometrical diameter, respectively |
| Destructive measurement technique or not | Is it a destructive measurement technique? | No | Fractions can be collected for the case that sample was not diluted |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suited supports? | No | |

5.2 Analytical centrifugation (AC)

5.2.1 Measurement principle

Analytical centrifugation (AC) techniques employ the principle of particle migration in a viscous fluid under influence of a centrifugal force. This leads to variations in the local particle concentration when measured along the settling path and/or over time. Settling distance and settling time correspond to the terminal settling velocity, which in the case of isolated particles solely depends on their individual size, shape, and density. Hence, concentration profiles or time curves reflect the size distribution of the particle system.

Analytical centrifugation is realised in disc-centrifuges, cuvette centrifuges and analytical ultracentrifuge (i.e. cuvette centrifuges with extremely high centrifugal accelerations up to 10^6 g). The sedimentation process can be monitored by means of (spectral) light and X-ray extinction or via the interferometric measurement of the refractive index (RI). Cuvette and ultracentrifuges are operated in the “homogenous” mode, where the particles are homogeneously distributed in the cuvette before measurement. Disc centrifuges also allow this mode of operation, yet are frequently operated in the “line-start” mode, where a thin strap of suspension is added on particle-free solvent with density gradient. This variety of centrifuges, detection systems and modes of operations makes it difficult to provide general answers on the specification. This report is restricted to disc

and cuvette centrifuges with light extinction measurement (discAc-turb, cuvAC-turb) and to ultracentrifuges with refractive index measurement (AUC-RI).

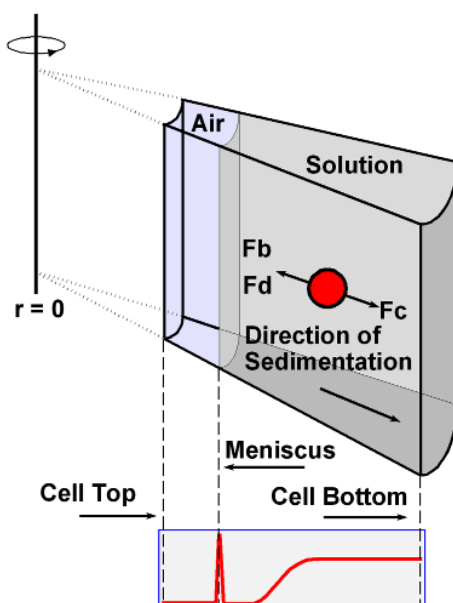


Figure 6: Schematic diagram of an AC cuvette centrifuge system

Disc centrifuges consist of a hollow disc which contains the suspension medium. Upon rotation, the liquid forms a stagnant layer on which a thin layer of the particle system is injected (line-start technique). The particles migrate according to their settling velocity to the outer diameter of the disc. All particles of a certain size (or settling velocity) move in a narrow band with growing distance from the initial position. In the case of multidisperse particle systems, one can observe several of such bands in analogy to chromatographic techniques. The radial concentration profile is, hence, a distorted projection of the density function of the size distribution ($q(x_{\text{Stokes}})$). The line-start technique requires a density gradient in the suspension medium (e.g. by sugar) before the particles are injected; otherwise, there would be a convective transport of particles within strands of the (heavy) suspension layer. The density gradient sets some practical limits to the measurement (e.g. duration) and has to be calibrated before conducting size measurements.

A different set-up and mode of operation is found in cuvette centrifuges, where the particle sedimentation is observed in small cuvettes that are fixed on a rotating table. In this case, the particles are homogeneously suspended in the continuous phase before the centrifugation starts (homogeneous technique which is schematically presented, see Figure 6). During the centrifugation, all particles migrate towards the bottom of the cuvette, which results in the formation of a sediment, in a continuous decline of local particle concentrations above the sediment, and in a monotonous decrease of particle concentration in the direction from the sediment to the meniscus. The two types of variation in particle concentration, the temporal evolution, and the radial profile, can be considered as distorted projections of the cumulative function of the particle size distribution ($Q(x_{\text{Stokes}})$). The cuvette centrifuge was introduced by Svedberg and co-workers^{68,69}. They called the instrument "ultra-centrifuge". Nowadays the term "Analytical Ultracentrifugation" (AUC) is only used for centrifugal accelerations above $100,000\times g$.

Apart from the centrifuge type, instruments differ with regard to the measurement of particle concentration. The most important principles are:

- optical extinction (photocentrifuge), which yields volume-weighted size distributions (q3) for light-absorbing nanoparticles and intensity-weighted size distribution for non-absorbing nanoparticles (q6);
- X-ray absorption (X-ray centrifuge), which always yields volume weighted size distributions (q3);
- refractive index determination by interferometry, which (approximately) yields volume-weighted size distributions for nanoparticles (q3).

Last but not least it should be mentioned that the AUC usually employs a set of different detection systems.

5.2.2 Performance

The AC technique is applicable to both suspensions and powders that can be suspended in liquids. All type pf materials (organic, inorganic) can be measured, however the effective density of particles must be different from the density of the dispersing liquid. Most instruments require that the particle density is higher than the density of water, which can make the measurement of organic particles difficult (note that some instruments also allow measurement of particles with a lower density than the surrounding liquid). The effective density of all particles must be homogenous and known before measurements. Sample preparation and the stability of the suspension are of key importance for the reliability of the measurements. The technique can measure particles below 10 nm but it measures the size of aggregates/agglomerates rather than constituent particles. The technique does not measure actual external dimensions but gives the diameter of an equivalent spheres, i.e. spheres that sediment as fast as the particles measured. It does not provide number-based particle size distribution within acceptable limits of uncertainty.

Table 27 and Table 28 below give the general performance of analytical centrifugation and the detailed performance table for this method, respectively.

Table 27: General Performance of analytical centrifugation (AC)

| Main features | |
|----------------------------|---|
| Type of samples | particles (solid, liquid, gaseous) in liquid dispersion medium |
| Type of sizing | fractionating technique |
| Particle property measured | <ul style="list-style-type: none"> • settling velocity • equivalent diameter: • Stokes diameter determined from settling velocity and skeletal density, or • hydrodynamic diameter from settling velocity and the effective particle density (used for compact aggregates) |
| Type of quantity | <ul style="list-style-type: none"> • dependent on detector • solid volume by X-ray, refractive index or turbidity measurement of light-absorbing nano-particles (q3) • squared solid volume by turbidity measurement non-absorbing nano-particles (q6) • for non-nanoparticles the physical quantity should be stated (e.g. extinction) |

| | |
|--|---|
| Size range | < 5 nm - 10 µm with minimum depending on particle density |
| Concentration range | ≤ 0.1 wt.% |
| Information content | relatively high, i.e. can well resolve details of the size distribution |
| Main advantages | |
| <ul style="list-style-type: none"> • lower size limit < 10 nm • good resolution of particle size distribution (for discAC even high resolution) • in principle wide dynamic range • rapid screening | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • number-weighted size distribution Q0 cannot directly be measured • does not resolve particle shape, but measures a settling velocity equivalent diameter (i.e. Stokes diameter), non-spherical particle geometry will introduce errors • measures aggregate size rather than size of constituent particles • only discAC: limited dynamic range - needs a balance between sedimentation rate (depending on particle size), density of gradient and centrifugal speed. | |

Table 28: Technical characteristics of analytical centrifugation (AC)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characteri- sation (Yes/No) | Notes |
|---|--|-----------------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes ⚠ | Depending on compatibility of liquid with instrument |
| | Dispersible in polar liquids other than water | Yes ⚠ | |
| | Dispersible in material-specific media | Yes ⚠ | Depending on compatibility of liquid with instrument |
| | Can be aerosolized | No | |

| | | | |
|---|--|-------|---|
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes ⚠ | Size-dependent scattering relevant to algorithm converting detector signal to mass value |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes ⚠ | No Only for AUC-RI |
| | Biological | Yes ⚠ | Need for material density, which is not always known for biological samples |
| | Composite particles | Yes ⚠ | No The composition needs to be known in some detail and e.g. just TiO ₂ core with SiO ₂ shell would be not enough |
| | Other | | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | No The composition needs to be known in some detail and e.g. just TiO ₂ core with SiO ₂ shell would be not enough |
| | Multishell particles | | No ⚠ Theoretically possible but in practice very complex |
| | Particles with inclusions | Yes ⚠ | No Not possible with standard AC instrument (single-detector operation) Possible by combining different optical (turbidity, RI, UV) and X-ray detectors |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | | No |
| | 2 (e.g. diameter of nanofibres) | | No |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | Measurement technique can measure objects of different shapes, but provides |
| | Equiaxial | Yes ⚠ | |

| | | | |
|--|---|-------|---|
| | | | an equivalent diameter |
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | No | |
| | Other | | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 5-60 °C) | Above 0 °C | No | |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 5-60 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | |
| | Monomodal | Yes | |
| | Multimodal | Yes | |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes ⚠ | Functionalisation can affect the detected size, if it significantly changes size and density of the particles |
| | Not functionalised | Yes | |

| | | | |
|---|---|---------------------|--|
| Agglomeration / aggregation state | Nanoparticles are aggregated | Yes ⚠ | Measurement technique measures only aggregate size |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Measurement technique measures only agglomerate size |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | No | |
| | Measures or calculates number or number concentration from fractionating techniques | Yes | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | No | |
| | Integral technique | No | |
| | Used in hyphenated methods | No | However AUC can employ multiple detectors to acquire differently weighted size distributions during the same fractionation or to measure UV-vis-spectra of sedimenting samples |
| Working range | Size range | 1 nm-10 µm | Range depends on type of instrument, sample, preparation. Maximum and minimum may not be possible in a single run. 1 nm needs AUC |
| | Concentration range | 0.0001 to 0.3 vol.% | Depending on material, size and detector type |
| | Minimum needed sample amount | 0.1 to 1 mL | |
| | Linearity/proportionality | No | Non-linear linearity in size: No linearity in quantity: No normally, Yes for AC-RI |
| | Limits of detection/quantification | 1 to 50 nm | Strongly depending on material, size and detector type |
| | Sensitivity (counting efficiency) as a function of size | good | Decreasing sensitivity with size for photometric detection |

| | | | |
|--|---|------------------------------------|---|
| Trueness of particle size measurement | Indicate the trueness of this measurement technique | average to good | |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | average to excellent | Depends on material and algorithms used to convert measured signal to weight-% and then to number-%. if “weights” refers to the intrinsic type of quantity (e.g. turbidity/extinction or volume) it is ‘excellent’ for AC, if it refers to “number” then it is “average to good” |
| Robustness | Specify the robustness of this measurement technique | good | Particle density must be reliably known |
| Precision | Specify the precision of the measurement technique | 0.1% to 4% (17%) 0.1 to 2% (8%) | For $x_{50,0}$ ^{70, 53} For $x_{50,ext}$ ^{71, 53} |
| Resolution | Specify the resolution of this measurement technique | 2% | For non-aggregated materials |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | Discrimination between nanoparticles and non-NPs of the same composition | Yes | |
| | Discrimination between nanoparticles and non-NPs nanoparticles of another composition | No | AC probes the settling velocity of particles; particles of different composition (density) may settle equally fast although they differ in size; moreover, a different composition means a different detection sensitivity → interpretation of measured size distributions is hardly possible |
| | Discrimination from nanoparticles of another chemical composition | No | |
| | Impurities | No | Dissolved impurities are ignored |

| | | | |
|--|--|-----------------|---|
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | No | |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | Stokes diameter | i.e. equivalent diameter for settling velocity based on the particles' skeleton density |
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes | |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suited supports? | No | |

5.3 Differential electrical mobility analysis (DEMA)

5.3.1 Measurement principle

The differential electrical mobility analysis is a technique for sizing submicron aerosol particles based on their electrical mobility. For this purpose, the particles need to be electrically charged in a defined manner, classified according to their electrical mobility and finally quantified for each mobility fraction. There are different ways to realise the DEMA principle in technical instruments, for which reasons there is a number of varying terms: MPSS for mobility particle size spectrometer, SMPS for scanning mobility particle sizer, DMPS for differential mobility particle sizer and DMAS for differential mobility analysing system.

DMAS combines a particle classifier (differential mobility analyser DMA or DEMC for differential electrical mobility classifier) that transmits particles within a narrow interval of sizes from an initially polydisperse aerosol, and a detector (for example, a condensation particle counter CPC) that counts the particles within that differential size interval. First, the aerosol passes through an inertial impactor to avoid that particles larger than 1 micrometre enter the DMA column, then the aerosol enters a particle charge conditioner like a charge neutraliser to be conditioned, so, particles that carry several charges lose their charge excess. Once the aerosol is well conditioned, particles are selected using electrical classification inside DMA column: an electric field is created and the

airborne particles drift along the DMA according to their electrical mobility Z_d . It is related to the particle diameter d_p via the expression:

$$Z_d(p, d_p) = \frac{peC_c(d_p)}{3\pi\mu_g d_p}$$

where e is the charge of the electron, μ_g the dynamic gas viscosity and C_c the slip correction factor defined as:

$$C_c(d_p) = 1 + K_n(d_p) \left[\alpha + \beta \exp\left(\frac{\gamma}{K_n(d_p)}\right) \right]$$

$$K_n(d_p) = \frac{2\lambda_m}{d_p}$$

where K_n is the Knudsen number and λ_m is the mean free path of a particle. (α ; β ; γ) are taken from experiments.

Figure 7 presents the fundamental components of DMAS (ISO 15900:2009). The pre-conditioner indicated in this figure serves generally two goals: removing the large particles with impactor the most used and, if necessary, reducing the sample humidity using a dryer. Concerning the aerosol detector, there are two types of detector: a CPC and an aerosol electrometer. Concerning the particle charge conditioner, a bipolar diffusion particle charger (also called aerosol neutralizer) is often used in SMPS. This is often done using a radioactive source like ^{85}Kr or a bipolar ion generator. These chargers establish the equilibrium charge distribution on the aerosol particles. Unipolar Corona chargers may also be used in a DMAS. The DMAS is operated by software controlling the sheath air flow, reading the aerosol flow, reading other system parameters such as T , p , setting the voltage, and reading the CPC output.

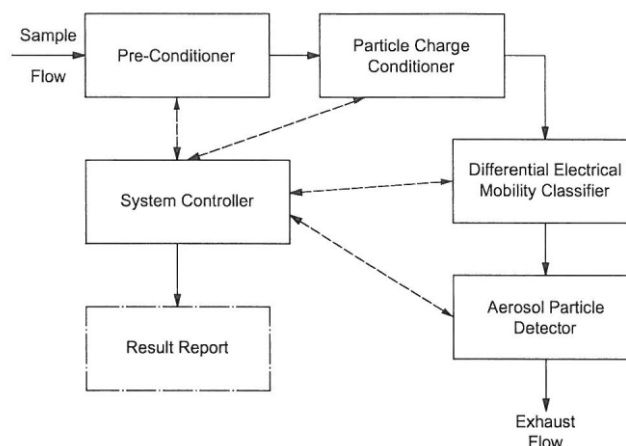


Figure 7: Fundamental components of the differential mobility analysing system (ISO 15900:2009)

5.3.2 Performance

Current limitations of this method include:

- Stability of the aerosol:

The system (DMA + CPC) can only be used when the aerosol is stable during the time of scan. If the aerosol (number size distribution) is unstable below the specified scan time, other systems must be used (DMA + Electrometers detectors like commercial instrument DMS 500, FMPS, EEPS). Nevertheless, it has to be noted that the size resolution for such system is lower compared to the coupled system DMA and CPC.

- Strongly dependent on the physical model used to retrieve the size distribution (charge distribution function, transfer function, etc.)
- When using the system (DMA + CPC), the estimation of the size distribution strongly depends on the type of inversion being implemented in the commercial software.
- System is optimized for particles with spherical shape.

Data analysis, resolution of particle size distribution: The measured electrical mobility distribution is converted to a particle number size distribution employing the charge distribution (see also ISO 15900) and the DMA-transfer probability. Additional corrections could be done for internal particle losses due to diffusion and the size-dependent CPC counting efficiency.

The performances of four SMPS were evaluated by Fissan et al.⁷² under the same conditions for flow rates, flow ratio, input monodisperse aerosols, and transport-line lengths in the 6–50 nm size range. Their results provide a quantitative comparison of the mobility resolution and diffusion loss of the nanometre aerosols in such systems. Moreover, the performance assessment of Fast MPS (FMPS) and ultrafine water-based condensation particle counter (UWCPC) equipped SMPS was performed by Jeong and Evans⁷³ under various conditions on urban ambient particles, urban indoor particles, rural ambient particles, and laboratory-generated particles. Asbach et al.⁷⁴ tested four different mobility particle sizers on NaCl and diesel soot particles measurements. Recently, Wiedensohler et al.⁷⁵ report on harmonization of measurement procedures to facilitate high quality long-term observations of atmospheric particle size number distributions obtained by SMPS. Some results of metrological measurement of NP size and size distribution by SMPS have been carried out recently in the frame of various interlaboratory comparisons specially dedicated to this purpose¹³. Electro-spray-differential mobility analysis (ES-DMA), a technique that exerts electrical and drag forces on clusters, can be used to determine the size and packing of colloidal small clusters (and aggregates more generally) of nanoparticles⁷⁶. NanoDefine studied especially spray-DEMA.

Table 29 and Table 30 below give the general performance of differential electrical mobility analysis and the detailed performance table for this method, respectively.

Table 29: General performance of differential electrical mobility analysis (DEMA)

| Main features | |
|----------------------------|--|
| Type of samples | airborne submicrometre particles; either prepared by dispersing powders in a gas phase or by spray-drying of dilute suspensions, or taken from environment |
| Type of sizing | fractionating technique |
| Particle property measured | electrical mobility equivalent diameter: mobility diameter |
| Type of quantity | particle number |
| Size range | 2.5 nm to 1 µm (range varies in dependence on instrument type like DMA and CPC and the parameter used (flow rate,...)) |

| | |
|---|---|
| Concentration range | 1 to 10 ⁸ particles/cm ³ (10 ⁸ can be achieved by counting with electrometers, whereas CPC maximum concentration counting is 10 ⁷ particles/cm ³) |
| Information content | relatively high, i.e. can well resolve details of the size distribution |
| Main advantages | |
| <ul style="list-style-type: none"> • intrinsically measures number-weighted size distributions • applicable to polydisperse population between 3 nm to 1 µm • good resolution of particle size distributions • provides number concentration of the analysed sample • quick measurement (a few min) | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • does not resolve particle shape, but measures a mobility equivalent diameter, • measures aggregate size rather than size of the constituent particles • particles > 1 µm are physically removed from aerosol sample; they are thus not measured • spray-dried aerosol samples may contain nanosized contaminant particles generated from previously dissolved species | |


Table 30: Technical characteristics for differential electrical mobility analysis (DEMA)

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes ⚠ | spray-DEMA: Measurement technique measures only aerosolized NPs |
| | Solid particulate form | No ⚠ Yes ⚠ | spray-DEMA: No as a liquid suspension is needed DEMA: Yes; DEMAs is intended to measure aerosols, including ultrafine dust or aerosolised powders |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes ⚠ | Measurement technique measures only aerosolized NPs |
| | Dispersible in non-polar liquids | Yes ⚠ | |
| | Dispersible in polar liquids other than water | Yes ⚠ | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | Yes | Measurement technique measures only aerosolized particles |

| | | | |
|---|--|-------|---|
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite particles | Yes | |
| | Other | Yes | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | Only outer size |
| | Multishell particles | Yes ⚠ | |
| | Particles with inclusions | Yes ⚠ | |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | No | Does not resolve shape |
| | 2 (e.g. diameter of nanofibres) | No | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | Measurement technique can measure objects of different shapes, but only provides an equivalent diameter |
| | Equiaxial | Yes ⚠ | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | No | |
| | Flakes and discs (thickness: lateral extension ≤ 0.25) | No | |
| | Other | | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 10-40 °C) | Above 0 °C | No | |
| | Sensitive above 25 °C | Yes | |
| | Sensitive above 37 °C | Yes | |
| | Sensitive above 50 °C | Yes | |
| | Sensitive above 100 °C | Yes | |
| | Sensitive above 150 °C | Yes | |
| | Sensitive above 500 °C | Yes | |
| | Sensitive above 1000 °C | Yes | |
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 10-40 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |

| | | | |
|---|---|-------|--|
| range: 10-40 °C) | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | |
| | Monomodal | Yes | |
| | Multimodal | Yes | |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes ⚠ | Only aggregate size |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Only agglomerate size. Spray-DEMA: only if agglomerates are small enough to avoid capillary clogging |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | No | |
| | Measures or calculates number or number concentration from fractionating techniques | Yes | Measurement technique measures the number concentration of very narrow particle size fractions |
| | Calculates number or number concentration from spectroscopic ensemble methods | No | |
| | Integral technique | No | |
| | Used in hyphenated methods | Yes | E.g. by coupling DEMA with aerosol centrifuges (not standard) |

| | | | |
|--|--|---|---|
| Working range | Size range | 2 nm to 1 μ m | Range varies in dependence on instrument type like DMA and CPC and the parameter used (flow rate,...) |
| | Concentration range | 10^4 to 10^6 particles/cm ³ | For aerosols and dispersed powders and for CPC For sprayed suspensions: 10^4 to 10^7 particles/cm ³ (suspension) |
| | Minimum needed sample amount |  | For aerosols: approx. 30 L For powders: <10 g For sprayed suspensions: 10 to 100 mL |
| | Linearity/proportionality | Yes No | With regard to concentration With regard to particle size (for small particle diameters the fraction of charged particles is very low) |
| | Limits of detection/quantification | 2 nm to 10 nm | Depending on instrument type and operation |
| | Sensitivity (counting efficiency) as a function of size | good | There is a strong impact of the size particle <10 nm for the counting efficiency (CPC) |
| Trueness of particle size measurement | Indicate the trueness of this measurement technique | good | |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this measurement technique | good | |
| Robustness | Specify the robustness of this measurement technique | good | |
| Precision | Specify the precision of the measurement technique | 0.2 to 5% | For $x_{50,0}$ (NanoDefine) |
| Resolution | Specify the resolution of this measurement technique | 3% | |
| Size distribution | Is it possible to measure size distribution? | Yes | |

| | | | |
|--|--|------------------------------|--|
| Selectivity | Discrimination between nanoparticles and non-NPs of the same composition | Yes | |
| | Discrimination between nanoparticles and non-NPs of another composition | Yes | |
| | Discrimination from nanoparticles of another chemical composition | No | |
| | Impurities | N/A | “impurities” only refer to residual particles (salt, polymers) after aerosolisation from suspension; this entry is irrelevant for aerosol characterisation |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | Yes ⚠ No ⚠ | Yes for typical DEMA setup and low aerosol concentration, No otherwise (including spray-DEMA) |
| Counting constituent particles in aggregations | Is the measurement technique able to characterise single constituent particles in aggregates? | No | |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | Electrical mobility diameter | equivalency with respect to the electrical mobility, based on the particle’s electric charge (which is assumed to be known) |
| Destructive measurement technique or not | Is it a destructive measurement technique? | Yes | |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | No | |
| Sample support | Does this measurement technique need preparation on suited supports? | No | |

6 Integral methods

6.1 BET for determination of volume specific surface area (VSSA)

6.1.1 Measurement principle

The Brunauer-Emmett-Teller (BET) theory was derived in 1938 to explain the physical adsorption of gas molecules on a solid surface⁷⁷. BET serves as the most often applied technique for the measurement of the specific surface of powders. BET explains mono- and multilayer adsorption of gas molecules on a solid and dry material. Nitrogen and argon gas are widely used for measurements. BET is based on three hypotheses:

- 1.) gas molecules physically adsorb in infinite layers,
- 2.) no interactions exist between adsorbed layers, and
- 3.) the Langmuir theory is applicable for each layer of gas molecules.

The resulting BET equation is applied for fitting experimental gas adsorption isotherms and gives the adsorbed monolayer gas quantity. Knowledge of gas quantity, adsorption cross section of the adsorbing gas and the molar gas volume allows calculation of the specific surface area of the material⁷⁸.

The BET equation is

$$\frac{1}{v[(p_0/p) - 1]} = \frac{c - 1}{v_m c} \left(\frac{p}{p_0} \right) + \frac{1}{v_m c}$$

where p and p_0 are the equilibrium and the saturation pressure of adsorbates at the temperature of adsorption, v is the adsorbed gas quantity (for example, in volume units), and v_{mono} is the monolayer adsorbed gas quantity, c is the BET constant.

$$c = \exp\left(\frac{E_1 - E_{L1}}{RT}\right)$$

where E_1 is the heat of adsorption for the first layer, and E_L is that for the second and higher layers and is equal to the heat of liquefaction.

The equation is an adsorption isotherm and can be plotted as a straight line with the y-axis showing $1/v[(p_0/p)-1]$ and $\varphi = p/p_0$ on the x-axis according to experimental results (BET plot). p is the equilibrium pressure and p_0 is the saturation pressure. The value of the slope, A , and the y-intercept, I , of the line are used to calculate the monolayer adsorbed gas quantity v_{mono} and the BET constant c . The following equations are used:

$$v_m = \frac{1}{A+I} \quad \text{and} \quad c = 1 + \frac{A}{I}$$

A total surface area $S_{\text{BET, total}}$ and a specific surface area S_{BET} are estimated by the following equations:

$$S_{\text{BET, Total}} = \frac{v_m N_A S}{V} \quad \text{and} \quad S_{\text{BET}} = \frac{S_{\text{Total}}}{a}$$

where v_m is in units of volume which are also the units of the molar volume of the adsorbate gas, N_A is Avogadro's number, S is the adsorption cross section of the adsorbing species, V is the molar volume of adsorbate gas, a is the mass of adsorbent (in g).

6.1.2 Performance

The BET method is widely used and accepted in industry, academia and (governmental and regulatory) research institutes. For example, the National Institute of Standards and Technology (NIST, USA) and BAM provide a practical guide for its application which is available without charge from NIST⁷⁹. BET can be applied easily and is also standardized by ISO (ISO 9277:2010). The BET theory is based on expansive assumptions (see above), and therefore, the results obtained by BET are method-defined⁸⁰. Also different values for the same material can be obtained if different gases are used. As a consequence, the specific surface area values should be named BET surface area and must often be considered as apparent. Nevertheless, Round-Robin tests for the development of reference materials for BET as performed by BAM proved good accuracy of the BET method⁸¹.

Table 31 and Table 32 below give the general performance of BET for determination of specific surface area and the detailed performance table for this method, respectively.

Table 31: General performance table for BET for determination of specific surface area

| Main features | |
|--|---|
| Type of samples | dry powder |
| Type of sizing | integral sizing technique (yielding a mean size of the particle system) |
| Particle property measured | <ul style="list-style-type: none"> • mass specific surface area, • mean size for non-porous particles of unknown shape: surface-weighted mean of VSSA equivalent diameter • mean size for non-porous particles of known dimensionality: surface-weighted mean of VSSA equivalent smallest external dimension |
| Type of quantity | surface-weighted |
| size range | all size ranges |
| concentration range | not applicable (measurements at dry powders) |
| information content | one piece of information |
| Main advantages | |
| <ul style="list-style-type: none"> • measures size of constituent particles within aggregates • lower size limit < 10 nm • wide dynamic range • certified reference materials are available for a wide range of SSA up to 1300 m²/g, | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • cannot resolve size distribution • mean size is surface-weighted • does not resolve particle shape, application to material classification according to the EC NM Definition requires knowledge of dimensionality • Materials must be free of any volatile compounds, for example, water-free • Measurement times can be in the range of hours and increase with increasing surface area | |

Table 32: Technical characteristics for BET for determination of specific surface area

Colour code: material-related technical characteristics and metrological aspects of the technique

| Criteria (general) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | No | |
| | Solid particulate form | Yes | |
| | Dispersed or embedded in matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | No | |
| | Dispersible in non-polar liquids | No | |
| | Dispersible in polar liquids other than water | No | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent light absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | No | |
| | Composite particles ⁷ | Yes | |
| Composite particles (see Section 2.2.1.3) | Core/shell | Yes ⚠ | Measures only surface accessible by the probe gas |
| | Multishell particles | Yes ⚠ | |
| | Particles with inclusions | Yes ⚠ | |
| Number of nanoscaled dimensions | 1 (e.g. thickness of nanoplates) | Yes | Size derived from measured surfaces as available for gas sorption with pre-knowledge on particle shape (from a descriptive SEM micrograph) |
| | 2 (e.g. diameter of nanofibres) | Yes | |
| | 3 | Yes | Size derived from measured surfaces as available for gas sorption |

⁷ See NanoDefine Manual, part 1 for definition

| | | | |
|---|--|-------|--|
| Shape of particles | Sphere or similar | Yes | Measurement technique can measure objects of different shapes, but provides a mean equivalent thickness |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods (length:diameter ≥ 3) | Yes | |
| | Flakes and discs (thickness:lateral extension ≤ 0.25) | Yes | |
| | Other | | |
| Thermal degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 15-40 °C) | Above 0 °C | No | If the sample preparation includes outgassing by heating, (often at 150 °C), and the sample is sensitive to that temperature, the answer is No |
| | Sensitive above 25 °C | Yes ⚠ | |
| | Sensitive above 37 °C | Yes ⚠ | |
| | Sensitive above 50 °C | Yes ⚠ | |
| | Sensitive above 100 °C | Yes ⚠ | |
| | Sensitive above 150 °C | Yes ⚠ | |
| | Sensitivity above 500 °C | Yes | |
| | Sensitivity above 1000 °C | Yes | |
| Cooling degradation sensitivity (of test material) (Must be compatible with Measurement Technique working range: 15-40 °C) | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| Electron beam sensitivity | Electron beam sensitive | Yes | |
| | Not electron beam sensitive | Yes | |
| Particle size dispersity and modality | Monodisperse | Yes | |
| | Polydisperse | Yes | |
| | Monomodal | Yes | |
| | Multimodal | Yes | |
| Conductivity properties (electrical) | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |

| | | | |
|---|---|----------------|--|
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes ⚠ | Resulting surface will be smaller than the sum of the surfaces of the individual particles |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes | |
| | Nanoparticles are not agglomerated | Yes | |
| Counting, fractionating or ensemble technique | Single particle counting | No | |
| | Measures or calculates number or number concentration from fractionating techniques | No | |
| | Calculates number or number concentration from spectroscopic ensemble techniques | No | |
| | Integral technique | Yes | |
| | Used in hyphenated methods ⁸ | No | |
| | | | |
| Working range | Size range | 1 nm to ~10 µm | |
| | Concentration range | N/A | Measurements on dry powders |
| | Minimum needed sample amount | ~100 mg | |
| | Linearity/proportionality | Yes | |
| | Limits of detection/quantification | 1 nm | |
| | Sensitivity (counting efficiency) as a function of particle size | good | |
| Trueness of particle size measurement | Indicate the trueness of this measurement technique in measuring the particle size | average | The values are often apparent BET surfaces. For large specific surfaces these are not real. The measured surface depends often on the type of gas used for measurement |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions by this measurement technique | average | |
| Robustness | Specify the robustness of this | good | For a defined SOP or the ISO |

⁸ There are 2 independent signals: average track length and spot size, i.e. diffusion coefficient and scattering intensity; a 2-dimensional plot of a monoconstituent sample would therefore show the functional relationship between size and scattering cross section (i.e. a strong, non-linear correlation); for multiconstituent samples we would expect different groups of such functional dependencies, which offers a possibility to couple size analysis with material or shape analysis.

| | measurement technique | | standard ISO 9277:2010 |
|--|--|-----------------|---|
| Precision | Specify the precision of the measurement technique | 0.1% to 5% | |
| Resolution | Specify the size resolution of this measurement technique | N/A | Does not measure size distribution |
| Size distribution | Is it possible to measure particle size distribution? | No | |
| Selectivity | Discrimination between nanoparticles and non-NPs of the same chemical composition | No | |
| | Discrimination between nanoparticles and non-NPs of another chemical composition | No | |
| | Discrimination from nanoparticles of another chemical composition | No | |
| | Impurities | No | |
| Identifies state of aggregation | Does the measurement technique reveal whether the measured particles are aggregated or agglomerated? | No | |
| Measurement of individual particles | Does this measurement technique characterise individual particles? | No | |
| Counting constituent particles in aggregates | Is the measurement technique able to characterise single constituent particles in aggregates? | No | Constituents are <u>not counted</u> , yet their average size is measured, i.e. BET does not reveal $x_{50,0}$ of constituents |
| Chemical composition | Does this measurement technique analyse chemical composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | VSSA equivalent | Surface-weighted mean of VSSA equivalent diameter |
| Destructive measurement technique or not | Is it a destructive measurement technique? | No | As long as the particles are stable in high vacuum |
| Other Specificity | | | |
| Vacuum | Does the measurement technique operate under vacuum? | Yes | |
| Sample support | Does this measurement technique need preparation on suitable supports? | No | |

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- ⁸¹ BAM: Certification Report. Certified Reference Material BAM-P110. BET Specific Surface Area of Titanium Dioxide (Anatase) calculated from the nitrogen adsorption isotherm at 77.3 K, 2017. Available at https://rrr.bam.de/RRR/Content/EN/Downloads/RM-certificates/RM-cert-porous-materials/bam_p110repe.pdf?_blob=publicationFile.

Annex 1 NanoDefine priority materials

| Material | Description |
|---|---|
| IRMM-380 Pigment yellow 83 (transparent grade) | Organic diarylide dye with hydrophobic character. Sub-100 nm particulates |
| IRMM-381 BaSO ₄ (fine grade) | Inorganic hydrophilic metal salt with low water solubility |
| IRMM-382 MWCNT | Highly tangled fibrous carbonaceous materials which are strongly hydrophobic in nature |
| IRMM-383 Nano steel | Highly anisotropic (platelets) particulates with negatively charged surface at neutral pH |
| IRMM-384 CaCO ₃ (fine grade) | Inorganic hydrophilic metal salt with low but non-negligible water solubility |
| IRMM-385 Kaolin | Highly anisotropic ceramic/mineral (platelets) particulates with negatively charged metal-oxide surface at neutral pH |
| IRMM-386 Pigment yellow 83 (opaque grade) | Organic diarylide dye with hydrophobic character. Mainly non-nano (>100 nm) particulates |
| IRMM-387 BaSO ₄ (ultrafine grade) | Inorganic salt/mineral with low water solubility |
| IRMM-388 Coated TiO ₂ (pigment grade) | Inorganic metal oxide with thin hydrophilic coating, code Kronos 2360 |
| IRMM-389 Basic methacrylate copolymer particles (BMC) | Hydrophilic organic particles, insoluble in water and highly soluble in most organic liquids |
| BAM-11 Zeolite powder | Nanoporous ceramic/mineral particulates of irregular shape with negatively charged metal-oxide surface at neutral pH |

Annex 2 International standards on particle sizing

This Annex includes a non-exhaustive list of international standards available for particle sizing techniques.

Sizing

[ISO 9276-1:1998](#), Representation of results of particle size analysis – Part 1: Graphical representation

[ISO 9276-1:1998/Cor 1:2004](#)

[ISO 9276-2:2014](#), Representation of results of particle size analysis – Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions

[ISO 9276-3:2008](#), Representation of results of particle size analysis – Part 3: Adjustment of an experimental curve to a reference model

[ISO 9276-6:2008](#), Representation of results of particle size analysis – Part 6: Descriptive and quantitative representation of particle shape and morphology

[ISO 26824:2013](#), Particle characterization of particulate systems – Vocabulary

[ISO/TS 11888:2011](#), Nanotechnologies – Characterization of multiwall carbon nanotubes – Mesoscopic shape factors

Sampling and sample preparation

[ISO 14887:2000](#), Sample preparation – Dispersing procedures for powders in liquids

[ISO 8780-1:1990](#), Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 1: Introduction

[ISO 8780-2:1990](#), Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 2: Dispersion using an oscillatory shaking machine

[ISO 8780-3:1990](#), Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 3: Dispersion using a high-speed impeller mill

[ISO 8780-4:1990](#), Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 4: Dispersion using a bead mill

[ISO 8780-5:1990](#), Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 5: Dispersion using an automatic muller

[ISO 8780-6:1990](#), Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 6: Dispersion using a triple-roll mill

[ISO 23900-1:2015](#), Pigments and extenders – Methods of dispersion and assessment of dispersibility in plastics – Part 1: General introduction

[ISO/TS 16176:2011](#), Rubber compounding ingredients – Carbon black – Determination of the aggregate-size distribution at ultimate dispersion

Electron microscopy

[ISO 13322-1:2014](#), Particle size analysis – Image analysis methods – Part 1: Static image analysis methods

[ISO/TS 10797:2012](#), Nanotechnologies – Characterization of single-wall carbon nanotubes using transmission electron microscopy

[ISO/TS 10798:2011](#), Nanotechnologies – Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis

[ISO 22493:2014](#), Microbeam analysis – Scanning electron microscopy – Vocabulary

[ISO 15932:2013](#), Microbeam analysis – Analytical electron microscopy – Vocabulary

Particle tracking analysis/ Dynamic ultramicroscopy

[ISO/DIS 19430](#), Determination of particle size distribution – Particle tracking analysis

Tunable resistive pulse sensing / electrical sensing zone / nano-Coulter-counter

[ISO 13319:2007](#), Determination of particle size distributions – Electrical sensing zone method

Inductively coupled plasma – mass spectrometry

[ISO/TS 19590:2017](#), Nanotechnologies - Size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectrometry

[ISO/TS 16965:2013](#), Soil quality - Determination of trace elements using inductively coupled plasma mass spectrometry (ICP-MS)

[ISO 17294-1:2004](#), Water quality - Application of inductively coupled plasma mass spectrometry (ICP-MS) – Part 1: General guidelines

[ISO 17294-2:2003](#), Water quality: 'Application of inductively coupled plasma mass spectroscopy (ICP-MS) – Part 2: Determination of 62 elements

[ISO 30011:2010](#), Workplace air – Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma mass spectrometry

[ISO/TR 17276:2014](#), Cosmetics – Analytical approach for screening and quantification methods for heavy metals in cosmetics

Differential mobility analysing system / Differential electrical mobility analysis

[ISO 15900:2009](#), Determination of particle size distribution – Differential electrical mobility analysis for aerosol particles

[ISO 27891:2015](#), Aerosol particle number concentration – Calibration of condensation particle counters

[ISO 12025:2012](#), Nanomaterials -- Quantification of nano-object release from powders by generation of aerosols

Analytical centrifugation

[ISO 13318-1:2001](#), Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 1: General principles and guidelines

[ISO 13318-2:2007](#), Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 2: Photocentrifuge method

[ISO 13318-3:2004](#), Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 3: Centrifugal X-ray method

[ISO 15825:2017](#), Rubber compounding ingredients – Carbon black – Determination of aggregate size distribution by disc centrifuge photosedimentometry

Dynamic light scattering

[ISO 13321:1996](#), Particle Size Analysis – Photon Correlation Spectroscopy

[ISO 22412:2017](#), Particle size analysis – Dynamic light scattering (DLS)

Angular light scattering – laser diffraction

[ISO 13320:2009](#), *Particle size analysis - Laser diffraction methods.*

[ISO 24235:2007](#), Fine ceramics (advanced ceramics, advanced technical ceramics) – Determination of particle size distribution of ceramic powders by laser diffraction method

[ISO 8130-13:2001](#), Coating powders – Part 13: Particle size analysis by laser diffraction

Small angle light scattering

[ISO 17867:2015](#), Particle size analysis – Small-angle X-ray scattering

Ultrasonic spectroscopy

[ISO 20998-1:2006](#), Measurement and characterization of particles by acoustic methods — Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy

[ISO 20998-2:2013](#), Measurement and characterization of particles by acoustic methods — Part 2: Guidelines for linear theory

[ISO/CD 20998-3](#), Measurement and characterization of particles by acoustic methods — Part 3: Guidelines for non-linear theory

BET, Brunauer-Emmett-Teller

[ISO 9277:2010](#), Determination of the specific surface area of solids by gas adsorption – BET method

[ISO 18757:2003](#), Fine ceramics (advanced ceramics, advanced technical ceramics) – Determination of specific surface area of ceramic powders by gas adsorption using the BET method

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