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Nanotechnologies — Measurement technique matrix for the characterization of nano-objects

Nanotechnologies — Matrice de méthodes de mesure pour les nano-objets manufacturés

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Foreword

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

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

The committee responsible for this document is ISO/TC 229, *Nanotechnologies*.

Introduction

This document connects the nano-object parameters that most commonly need to be measured with corresponding measurement techniques. This document will be a useful tool for nanotechnology interested parties to rapidly identify relevant information for measuring nano-objects. The common nano-object parameters are listed along the top row of the Quick-Use-Matrix (see Table 1). If a measurement technique listed in the first column of the matrix is applicable, the box in the matrix will be marked. Once a measurement technique of interest is identified, it is recommended that the reader then enter this document's body of text (see Clause 5), where you will find an alphabetical listing of the measurement techniques and descriptions of the advantages, limitations, relevant standards, measurand(s), and applicable nano-object parameters of each technique.

As scientific advances are made and additional commercial measurement techniques become available, this document will be periodically reviewed and updated to maintain its relevance.

Many of the techniques listed in this document have not been validated through round-robin testing or any other means for the measurement of nano-objects. This document is intended as a starting point and resource to help identify potentially useful and relevant techniques; it is not an exhaustive or primary source. It is recommended that once a technique has been identified, the reader refers to relevant international standards and conducts a literature search for similar or comparable applications. Other sources of information include instrument manufacturer's applications notes and technical literature.

Nanotechnologies — Measurement technique matrix for the characterization of nano-objects

1 Scope

This document provides a matrix that guides users to commercially available techniques relevant to the measurements of common physiochemical parameters for nano-objects. Some techniques are also applicable to nanostructured materials.

NOTE Guidance on sample separation and preparation is given in Annex A.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

3.1 General terms

3.1.1
dispersion
heterogeneous system in which a finely divided material is distributed in another material

[SOURCE: ISO 472:2013, 2.288]

3.1.2
measurand
quantity intended to be measured

[SOURCE: ISO Guide 99:2007, 2.3]

3.1.3
nano-object
discrete piece of material with one, two or three external dimensions in the nanoscale

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-2:2015, 2.2]

3.1.4
nanostructured material
material having internal or surface structure in the nanoscale

Note 1 to entry: If external dimensions are in the nanoscale, the term *nano-object* (3.1.3) is recommended.

[SOURCE: ISO/TS 80004-4:2011, 2.11]

3.2 Nano-object parameters

3.2.1

chemical composition

identity and possible quantification of a material's composite parts and its impurities

3.2.2

concentration

content of nano-objects in a sample, quantified as number, area, volume or mass content

Note 1 to entry: Transformation calculation from one type of quantity to another (e.g. number to volume) requires the knowledge of the complete particle size distribution and the assumption of a size-independent shape, porosity or density[6].

3.2.3

crystal property

effect due to the presence of three-dimensional order at the level of molecular dimensions

Note 1 to entry: See Reference [1].

3.2.4

electrokinetic potential

difference in electric potential between that at the slipping plane and that of the bulk liquid

Note 1 to entry: See Reference [7].

Note 2 to entry: Electrokinetic potential is expressed in volts.

Note 3 to entry: Often called "zeta potential".

3.2.5

shape

external geometric form of a particle

Note 1 to entry: See Reference [8].

Note 2 to entry: For quantitative description by macroshape, mesoshape or microshape descriptors, see Reference [9].

3.2.6

size

linear dimensions of a particle determined by a specified measurement method and under specified measurement conditions

Note 1 to entry: See Reference [10].

Note 2 to entry: Different methods of analysis are based on the measurement of different physical properties. Independent of the particle property actually measured, the particle size is reported as a linear dimension, e.g. as the equivalent spherical diameter.

Note 3 to entry: Examples of size descriptors are those based at the opening of a sieve or a statistical diameter, e.g. the Feret diameter, measured by image analysis.

Note 4 to entry: In ISO 9276-1, the symbol x is used to denote the particle size. However, it is recognized that the symbol d is also widely used to designate these values. Therefore, the symbol x can be replaced by d.

3.2.7

particle size distribution

cumulative distribution of particle concentration as a function of particle size

[SOURCE: ISO 14644-1:2015, 3.2.4]

Note 1 to entry: Particle size distribution is of statistical nature so it can be expressed as a function of particle size[11].

Note 2 to entry: The type of quantity (number, area, volume or mass) as well as the measurand, the measured equivalent spherical diameter, shall be indicated.

Note 3 to entry: Particle size distribution can be expressed as cumulative distribution or a distribution density (distribution of the fraction of material in a size class, divided by the width of that class).

3.2.8

surface area

extent of available surface area as determined by given method under stated conditions

Note 1 to entry: See Reference [12].

Note 2 to entry: Surface area is the quantity of accessible surface of a sample when exposed to either gaseous or liquid adsorbate phase. Surface area is conventionally expressed as a mass-specific surface area or as volume-specific surface area where the total quantity of area has been normalized either to the sample's mass or volume[13].

4 Parameters included in the matrix

Several comprehensive literature searches, input from ISO/TC 229 experts, and a review of current ISO work have identified frequently sought nano-object parameters that are listed across the top of the Quick-Use-Matrix (see Table 1). The parameters listed from left to right are in alphabetical order. A single technique alone often cannot provide sufficient information about all parameters of interest for a particular nano-object under study, nor is one technique likely to fully capture relevant information for a single parameter. Therefore, when available, it is recommended that more than one technique should be used for any parameter investigation.

Table 1 — Quick-Use-Matrix[4]

Technique									
	Acronym	Chemical composition	Concentration	Crystal properties	Electrokinetic potential	Shape	Size	Size distribution	Surface area
Acoustic spectroscopy			+				+	+	
Analytical centrifugation	AC							+	
Aerosol particle mass analyser	AMS		+				+		
Auger electron spectroscopy (scanning)	AES	+	+			+	+		
Brunauer-Emmett-Teller	BET								+
Condensation particle counter	CPC		+						
Differential mobility analysis system[a]	DMAS		+				+	+	
Differential scanning calorimetry	DSC	+							
Dynamic light scattering[b]	DLS						+	+	
Electroacoustic spectroscopy					+				

[a] Requires a special detector or additional instrumentation to obtain the desired parameter.

[b] Light scattering technique.

Table 1 *(continued)*

Technique									
	Acronym	Chemical composition	Concentration	Crystal properties	Electrokinetic potential	Shape	Size	Size distribution	Surface area
Electron energy loss spectroscopy[a]	EELS	+							
Electrophoresis/ capillary electrophoresis					+				
EM based X-ray spectrometry[a]	EDX/ EDS/WDS	+							
Field flow fractionation[a]	FFF	+	+				+	+	
Fluorescence spectroscopy	FL		+						
Fourier transform infrared spectroscopy/ imaging	FTIR	+							
Induced grating method	IG						+	+	
Inductively coupled plasma–mass spectrometry and single particle ICP-MS	ICP-MS	+	+				+	+	
Laser diffraction[b]							+	+	
Liquid chromatography–mass spectrometry	LC-MS	+	+						
Particle tracking analysis	PTA				+		+	+	
Optical absorption spectroscopy	UV/Vis/ NIR	+	+				+		
Quartz microbalances	QCM						+		
Raman spectroscopy/ imaging		+		+					
Resonant mass measurement	RMM						+	+	
Scanning electron microscopy[a]	SEM	+		+		+	+	+	
Scanning probe microscopy	SPM/AFM					+	+	+	
Secondary ion mass spectrometry	SIMS		+						
Small angle X-ray scattering	SAXS					+	+	+	

[a] Requires a special detector or additional instrumentation to obtain the desired parameter.

[b] Light scattering technique.

Table 1 *(continued)*

Technique									
	Acronym	Chemical composition	Concentration	Crystal properties	Electrokinetic potential	Shape	Size	Size distribution	Surface area
Static light scattering[b]	SLS/SMLS		+				+		
Single particle light interaction methods[b]			+				+	+	
Thermogravimetric analysis	TGA	+							
Transmission electron microscopy[a]	TEM	+		+		+	+	+	
X-ray diffraction	XRD			+			+		
X-ray photoelectron spectroscopy	XPS	+	+						

[a] Requires a special detector or additional instrumentation to obtain the desired parameter.

[b] Light scattering technique.

5 Measurement techniques included in the matrix

5.1 General

In the following subclauses, measurement techniques are listed in alphabetical order (with some exceptions where similar techniques are grouped together). Included under each technique are the nano-object parameters that can be measured by the technique and a listing of the technique's advantages, limitations, measurands, and relevant standards. Generally, a single technique cannot provide characterization of all nano-object parameters of interest. Also, a technique may be useful to determine other parameters not listed. For comprehensive analysis of a single parameter, use of multiple techniques, where applicable, is highly recommended.

In some cases, there are multiple techniques that share a similar underlying theory of operation or physical phenomenon, but which have differences that make them uniquely applicable to different types of materials and measurement conditions. For example, different electromagnetic scattering techniques are better suited for the analysis of different materials (e.g. metals, polymers or refractories) or materials in different states (e.g. suspended in a liquid or incorporated into a solid matrix).

5.2 Acoustic spectroscopy

5.2.1 Description

The basic concept is to measure the frequency-dependent attenuation or velocity of ultrasound as it propagates through a heterogeneous sample, e.g. colloids, dispersions and emulsions. There are two main types of vibrations: free and forced. Free vibrations are the natural or normal modes of vibration for a substance. Forced vibrations are caused by some sort of excitation to make the analyte resonate beyond its normal modes.

Both acoustic and electroacoustic spectroscopies (see 5.4) are linked to a sound propagation through a heterogeneous system such as a suspension or an emulsion. An acoustic spectrometer measures only the changes in the properties of the sound wave, whereas an electroacoustic spectrometer deals with connection between electrodynamic phenomena and the sound wave pressure field. Acoustic and electroacoustic spectroscopies are independent methods because the attenuation has little effect on the electroacoustic spectra and conversely, electrokinetic phenomena have negligible effect on the attenuation spectra.

5.2.2 Nano-object parameters

Concentration, size, and size distribution.

5.2.3 Advantages

5.2.3.1 Does not require sample dilution; applicable for concentrated samples.

5.2.3.2 Method is suitable for characterizing aggregated and structured systems.

5.2.3.3 Measurement is not affected by stirring and/or pumping of the sample.

5.2.3.4 Absolute method; does not require size calibration.

5.2.3.5 Instrument verification can be conducted by measurement of water with known acoustic properties.

5.2.3.6 Solvent-based samples (non-aqueous) can be analysed.

5.2.4 Limitations

5.2.4.1 Applicable only to liquid-borne particles.

5.2.4.2 Lower size limit ≈10 nm; upper size limit ≈3 mm.

5.2.4.3 Lower volume fraction limit is ≈0,1 %; upper volume fraction limit is ≈50 %.

5.2.5 Measurand

Equivalent spherical particle size distribution on a volume basis calculated from the ultrasound attenuation frequency spectrum.

5.2.6 Relevant standards

- ISO 20998-1, *Measurement and characterization of particles by acoustic methods — Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy*
- ISO 20998-2, *Measurement and characterization of particles by acoustic methods — Part 2: Guidelines for linear theory*

5.3 Analytical centrifugation (AC)

5.3.1 Description

Analytical centrifugation (AC) is chosen here as the generic term for a number of centrifugal sedimentation techniques, (some of which are) also known as centrifugal liquid sedimentation (CLS), analytical ultracentrifugation (AUC) or differential centrifugal sedimentation (DCS). Together, this is a versatile family of methods with broad diameter range (1 nm to 5 000 nm) and high resolution. The nanoparticles are forced to move through a predominantly aqueous medium inside an AC measuring cell under a centrifugal force of 500 to 500 000 × g (rotor speed 100 r/min to 60 000 r/min). Sedimentation causes fractionation to take place, because the larger particles move faster. The sedimentation velocities of the different fractions are measured with refractive index, ultraviolet (UV)-absorption or light scattering/turbidity detectors. The diameters of the different fractions can be calculated from the difference in sedimentation velocity, i.e. the complete particle size distribution, according to Stokes' law. The resolution of the AC method is so high because, according to Stokes, velocity is proportional to the square of particle diameter.

5.3.2 Nano-object parameters

Size distribution.

5.3.3 Advantages

5.3.3.1 High diameter resolution.

5.3.3.2 Broad diameter range (1 nm to 5 000 nm).

5.3.4 Limitations

The AUC particle size distribution method can give spurious results if particle size is too high, i.e. if the particles form a sediment too quickly or if they display a broad chemical heterogeneity (i.e. no uniform particle density).

5.3.5 Measurand

5.3.5.1 Settling velocity in centrifugal field.

5.3.5.2 Refractive index, ultraviolet (UV)-absorption or light scattering/turbidity.

5.3.6 Relevant standards

- ISO 13318-1, *Determination of particle size distribution by centrifugal liquid sedimentation methods — Part 1: General principles and guidelines*
- ISO 13318-2, *Determination of particle size distribution by centrifugal liquid sedimentation methods — Part 2: Photocentrifuge method*
- ISO 13318-3, *Determination of particle size distribution by centrifugal liquid sedimentation methods — Part 3: Centrifugal X-ray method*

5.4 Electroacoustic spectroscopy

5.4.1 Description

Electroacoustic phenomena arise from the coupling between the ultrasound field and electric field in a liquid that contains ions. Either of these fields can be a primary driving force. The liquid might be a simple Newtonian liquid or a complex heterogeneous dispersion, emulsion or even a porous body. There are several different electroacoustic effects, depending on the nature of the liquid and the type of driving force. Electroacoustic instruments are built as a probe, which includes elements for generating (measuring) ultrasound field and elements for generating (measuring) electric field (current).

5.4.2 Nano-object parameters

Electrokinetic potential.

5.4.3 Advantages

5.4.3.1 Does not require sample dilution; applicable for concentrated samples.

5.4.3.2 Method is suitable for characterizing aggregated and structured systems.

5.4.3.3 Mixing and pumping of the sample are allowed.

5.4.3.4 Sensor design is a probe that can be inserted into the static or into the mixed or pumped sample.

5.4.3.5 Measurement time is under 1 min.

5.4.3.6 Sample volume can be as little as 0,1 ml.

5.4.3.7 Applicable for high ionic strength up to several mole per litre.

5.4.3.8 Solvent-based samples (non-aqueous) are allowed.

5.4.3.9 Well suited for pH and surfactant titrations of the intact concentrated samples.

5.4.4 Limitations

5.4.4.1 Applicable only to liquid-borne nanoparticles.

5.4.4.2 There is no restriction on the lower size limit; upper size limit ≈0,1 mm.

5.4.4.3 Lower volume fraction limit is ≈1 %; upper volume fraction limit is ≈50 %.

5.4.5 Measurand

Electrokinetic potential.

5.4.6 Relevant standards

- ISO 13099-1, *Colloidal systems — Methods for zeta potential determination — Part 1: Electroacoustic and electrokinetic phenomena*
- ISO 13099-3, *Colloidal systems — Methods for zeta potential determination — Part 3: Acoustic methods*

5.5 Aerosol particle mass analyser (AMS)

5.5.1 Description

This technique classifies aerosol particles into fractions based on the mass-to-charge ratio. The basic concept is to flow aerosol with a Boltzmann charge distribution through an annulus in a cylindrical centrifuge with a DC voltage applied. It is the principle of the force equation where the centrifugal acceleration of the particles is counterbalanced by the electrostatic force on a particle.

The aerosol shall first pass through a neutralizing section. At the outlet of the device, an aerosol detection system is required to determine the particle number size distribution. The applied voltage and rotational velocity can be varied to obtain size fractions selected by particle mass.

5.5.2 Nano-object parameters

Concentration and size.

NOTE Size is calculated from particle mass.

5.5.3 Advantages

5.5.3.1 High mass resolution.

5.5.3.2 No mass distribution assumptions required.

5.5.3.3 Near real-time measurement.

5.5.3.4 Effective density information when coupled to differential mobility analysis (see 5.9).

5.5.3.5 Direct measurement of particle mass.

5.5.3.6 Determination of number concentration by mass basis.

5.5.4 Limitations

5.5.4.1 Applicable only to airborne nanoparticles (suspensions or powders are required to be aerosolized).

5.5.4.2 Lower mass limit about 0,01 fg; upper limit about 100 fg (from about 30 nm to 500 nm in size assuming that the shape of particles is spherical with density of 1 g/cm^3).

5.5.4.3 Requires an aerosol particle charger and a radioactive charge neutralizer (beta or alpha emitter).

5.5.5 Measurand

Particle number concentration as function of mass-to-charge ratio of the particles.

5.5.6 Relevant standards

No standards were identified; however, there are papers describing the technique[14][15][16].

5.6 Auger electron spectroscopy (AES)

5.6.1 Description

Auger electron spectroscopy (AES) is an electron beam technique that is commonly used to determine the chemical composition of surfaces. Excitation of the sample with an energetic electron beam results in the generation of a core hole. Relaxation from this excited state occurs when this core hole is filled by an electron from a higher energy state with the simultaneous emission of either an X-ray photon or an Auger electron. Due to the relatively low energies (<3 keV) of the emitted Auger electrons, its mean free path within the sample is limited to less than 10 nm, making AES inherently surface sensitive.

5.6.2 Nano-object parameters

Concentration (secondary electron imaging or elemental mapping), size (secondary electron imaging or elemental mapping), chemical composition and shape.

5.6.3 Advantages

5.6.3.1 High spatial (<1 µm) and surface (≈0,1 nm) resolution [spatial in this context refers to horizontal plane of analysis (*x*-*y* direction) and surface refers to depth].

5.6.3.2 Elemental mapping possible when employing a scanning electron beam.

5.6.3.3 Depth profiling capability when combined with ion sputtering.

5.5.3.4 High detection sensitivity; capable of analysing a fraction of a surface monolayer.

5.6.4 Limitations

5.6.4.1 Analysis of non-conducting samples can be problematic due to surface charging effect.

5.6.4.2 Surface contamination can complicate data analysis.

5.6.5 Measurand

5.6.5.1 Electron energies (eV) and signal intensity (counts).

5.6.5.2 Length and width (A. or nm) measured from secondary electron image or elemental mapping.

5.6.5.3 Depth (A. or nm) using ion sputtering and measured against a standard of known thickness.

5.6.6 Relevant standards

- ISO 16531, *Surface chemical analysis — Depth profiling — Methods for ion beam alignment and the associated measurement of current or current density for depth profiling in AES and XPS*
- ISO 20903, *Surface chemical analysis — Auger electron spectroscopy and X-ray photoelectron spectroscopy — Methods used to determine peak intensities and information required when reporting results*
- ISO/TR 14187, *Surface chemical analysis — Characterization of nanostructured materials*
- ASTM E827-08, *Standard practice for identifying elements by the peaks in Auger Electron Spectroscopy*
- ASTM E984-12, *Standard guide for identifying chemical effects and matrix effects in Auger Electron Spectroscopy*
- ASTM E996-10 *Standard practice for reporting data in Auger Electron Spectroscopy and X-ray Photoelectron Spectroscopy*
- ASTM E1217-11, *Standard practice for determination of the specimen area contributing to the detected signal in Auger Electron Spectrometers and some X-Ray Photoelectron Spectrometers*

5.7 Brunauer-Emmett-Teller (BET) method for physical adsorption — Surface area determination

5.7.1 Description

A technique for the determination of the total specific surface area of finely divided and porous solids by measuring the amount of physically adsorbed gas utilizing the model developed by Brunauer, Emmet and Teller for interpreting gas adsorption isotherms[17].

BET is applicable only to adsorption isotherms of type II (nonporous or macroporous solids) and type IV (mesoporous solids) according to the IUPAC classification of adsorption isotherms. For these cases, the BET area can be regarded as the "probe accessible area" (i.e. the effective area available for the adsorption of the chosen adsorptive).

5.7.2 Nano-object parameters

Surface area.

5.7.3 Advantages

5.7.3.1 Well-defined techniques and ISO standards available.

5.7.3.2 Automated analysis.

5.7.3.3 High precision.

5.7.4 Limitations

5.7.4.1 Generally restricted to dry powders and porous solids.

5.7.4.2 Prior to analysis, the sample is usually outgassed (aggregation might occur) at elevated temperatures.

5.7.4.3 The BET method cannot reliably be applied to solids which absorb the measuring gas.

5.7.5 Measurand

Specific surface area in m^2/g (from BET analysis).

5.7.6 Relevant standards

- ISO 9277, *Determination of the specific surface area of solids by gas adsorption — BET method*
- ISO 15901-2, *Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption — Part 2: Analysis of mesopores and macropores by gas adsorption*
- ISO 18757, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Determination of specific surface area of ceramic powders by gas adsorption using the BET method*
- ASTM C1274-12, *Standard test method for advanced ceramic specific surface area by physical adsorption*
- ASTM D 1993-03, *Standard test method for precipitated silica — Surface area by multipoint BET nitrogen adsorption*
- ASTM B922-10, *Standard test method for metal powder specific surface area by physical adsorption*
- ASTM C1069-09, *Standard test method for specific surface area of alumina or quartz by nitrogen adsorption*

5.8 Condensation particle counter (CPC)

5.8.1 Description

The common principle of all different CPC types is that condensation of supersaturated vapours is used to grow ultra-fine and nanoparticles to droplets of sizes that can be detected optically.

5.8.2 Nano-object parameters

Concentration.

5.8.3 Advantages

5.8.3.1 Real-time measurement.

5.8.3.2 Capable of time-dependent measurement.

5.8.3.3 Well-established quality assurance procedures.

5.8.3.4 Measurement is generally independent of particle properties over a wide size range.

5.8.4 Limitations

5.8.4.1 Applicable only to airborne nanoparticles.

5.8.4.2 Lower size limit ≈1 nm; upper size limit ≈5 µm.

5.8.4.3 Upper number concentration limit subject to the onset of coincidence.

5.8.4.4 Lower size limit is dependent on particle composition.

5.8.5 Measurand

Particle number concentration.

5.8.6 Relevant standards

— ISO 27891, *Aerosol particle number concentration — Calibration of condensation particle counters*

5.9 Differential mobility analysis system (DMAS)

5.9.1 Description

A technique to measure the size distribution of submicrometre aerosols[18].

The differential mobility analysis system (DMAS) consists of charge neutralization, differential electrical mobility classifier (DEMC), particle detector, plumbing, pump and electronics. The DEMC consists of channel with an opening in the wall at a downstream location perpendicular to the flow. An aerosol with a Boltzmann charge distribution flows down the channel with a DC voltage applied to the walls of the channel. The applied voltage gradient will cause the charged particles to drift with a migration velocity according to the mobility of the particle to the wall with the opening. The size of the particles classified by passing through the side wall opening depends on the particle size, particle charge, voltage gradient, width of the channel, length along the channel to the opening and flow velocity[19][20]. This is a first principles measurement.

A condensation particle counter (CPC) is one possible detector suitable for use with a differential mobility analysis system (see 5.8). The common principle of CPC instrument types is that condensation of supersaturated vapour is used to grow ultra-fine and nanoparticles to droplets of sizes that can be detected optically.

NOTE A CPC does not discriminate by size; it is a detector that provides a count of particles within the operational size range over a period of time. If coupled with a size classifier, then the combination technique can provide a number size distribution.

5.9.2 Nano-object parameters

Concentration, size and size distribution.

NOTE The size is an electrical mobility spherical equivalent size.

5.9.3 Advantages

5.9.3.1 High size resolution (<2 % uncertainty at 2,5 nm for commercial instruments).

5.9.3.2 No size distribution assumptions required.

5.9.3.3 Near real-time measurement.

5.9.3.4 Capable of time-dependent measurement.

5.9.3.5 Well-established quality assurance procedures.

5.9.4 Limitations

5.9.4.1 Applicable only to airborne nanoparticles (suspensions or powders are required to be aerosolized).

NOTE An electrospray device is commonly used in conjunction with a DMAS to generate predominantly singly-charged aerosolized material from a particle suspension.

5.9.4.2 Lower size limit ≈2 nm; upper size limit ≈1 000 nm (upper size limit is ≈5 µm with CPC).

5.9.4.3 Lower number concentration limit ≈10^2 cm^{-3}; upper number concentration limit ≈10^7 cm^{-3} (without dilution).

5.9.5 Measurand

Particle number concentration as function of electrical-mobility equivalent diameter.

5.9.6 Relevant standards

- ISO 15900, *Determination of particle size distribution — Differential electrical mobility analysis for aerosol particles*

5.10 Differential scanning calorimetry (DSC)

5.10.1 Description

A technique in which the difference in the energy inputs into a substance and a reference material is measured as a function of temperature while the substance and reference material are subjected to a controlled temperature program.

5.10.2 Nano-object parameters

Chemical composition.

5.10.3 Advantages

5.10.3.1 Minimal sample preparation required.

5.10.3.2 Fast scanning allows suppression of material decomposition at higher temperatures (scan at 400 °C/min and faster).

5.10.3.3 Wide temperature ranges.

5.10.3.4 Measurements can be conducted in different atmospheres.

5.10.3.5 Can characterize qualitative bond strength between nano-filler and epoxy in nanocomposites (strong or weak) and examines rigid amorphous fraction (RAF) of nanocomposite system[21][22].

5.10.4 Limitations

Many DSC instruments cannot scan at 400 °C/min or collect data at those speeds.

5.10.5 Measurand

5.10.5.1 J/g: Energy input per mass in endothermic or exothermic reactions.

5.10.5.2 J/(g. K): heat capacity, specific heat.

5.10.6 Relevant standards

- ISO 11357-1, *Plastics — Differential scanning calorimetry (DSC) — Part 1: General principles*
- ISO 11357-2, *Plastics — Differential scanning calorimetry (DSC) — Part 2: Determination of glass transition temperature and glass transition step height*
- ISO 11357-3, *Plastics — Differential scanning calorimetry (DSC) — Part 3: Determination of temperature and enthalpy of melting and crystallization*
- ISO 11357-4, *Plastics — Differential scanning calorimetry (DSC) — Part 4: Determination of specific heat capacity*
- ISO 11357-5, *Plastics — Differential scanning calorimetry (DSC) — Part 5: Determination of characteristic reaction-curve temperatures and times, enthalpy of reaction and degree of conversion*
- ISO 11357-6, *Plastics — Differential scanning calorimetry (DSC) — Part 6: Determination of oxidation induction time (isothermal OIT) and oxidation induction temperature (dynamic OIT)*
- ISO 11357-7, *Plastics — Differential scanning calorimetry (DSC) — Part 7: Determination of crystallization kinetics*

5.11 Dynamic light scattering (DLS)

5.11.1 Description

DLS, also referred to as quasi-elastic light scattering (QELS), is an ensemble technique for measuring the size of particles undergoing Brownian motion in liquid suspension. DLS includes photon correlation spectroscopy (PCS), photon cross-correlation spectroscopy (PCCS) and frequency analysis, although PCS is by far the most common sub-technique. The random motion of the particles results in a fluctuating photon count rate measured over very small increments of time (order of microseconds) and a small shift in the frequency of light. The rate of fluctuation or the magnitude of the frequency shift is related to the speed of particle motion, which is related to their hydrodynamic size.

NOTE Correlation analysis of the time-dependent intensity of the scattered light, or analysis of the frequency shift of the scattered light in frequency domain, can yield the translational diffusion coefficient and hence the equivalent spherical hydrodynamic diameter via the Stokes-Einstein relationship.

5.11.2 Nano-object parameters

Size and size distribution (polydispersity).

5.11.3 Advantages

5.11.3.1 Rapid measurement (seconds to minutes).

5.11.3.2 Provides robust mean size for particles in dilute dispersion if polydispersity is low.

5.11.3.3 Can provide reproducible results in agreement with other methods for spherical particles with a narrow size distribution.

5.11.3.4 Some optical setups allow measurement of concentrated samples, where multiple scattering would otherwise interfere with analysis.

5.11.3.5 Statistically sound; measures signals from millions of particles.

5.11.3.6 First principles; no calibration required; traceability to the SI unit, the metre, is possible through knowledge of the incident laser wavelength.

5.11.3.7 Non-destructive and non-invasive.

5.11.4 Limitations

5.11.4.1 Broad distributions cannot be determined accurately because scattering efficiency increases strongly with particle size.

5.11.4.2 Lower limit ≈1 nm; upper limit is a few micrometres depending on detection scheme (1 nm to 6 000 nm). The upper limit is usually limited by precipitation and particle number fluctuations sensitive to large particles (In the <100 nm range, scattering is proportional to radius raised to the 6th power of the diameter).

5.11.4.3 Size distributions are sensitive to data quality and the degree of polydispersity, and require use of non-standardized inversion algorithms with user adjustable parameters that impact the resolution and noise apparent in the resulting distribution.

5.11.4.4 Deviations from sphericity and high concentrations of the dispersed particles negatively affect the measurements accuracy.

5.11.5 Measurand

Equivalent spherical hydrodynamic diameter.

NOTE Cumulants analysis of autocorrelation function delivers a robust average — the harmonic mean of intensity weighed size distribution function, i.e. the z-average diameter (ZD).

5.11.6 Relevant standards

- ISO 13321, *Particle size analysis — Photon correlation spectroscopy*
- ISO 22412, *Particle size analysis — Dynamic light scattering (DLS)*
- ASTM E2490, *Standard guide for measurement of particle size distribution of nanomaterials in suspension by Photon Correlation Spectroscopy (PCS)*

5.12 Electron energy loss spectroscopy (transmission EELS)

5.12.1 Description

A technique in which an electron spectrometer measures the energy spectrum of electrons (from a nominally monoenergetic source) while they undergo inelastic interactions with the sample, often exhibiting features due to specific inelastic loss processes. Transmission EELS is performed with a TEM instrument (see 5.34)[23].

NOTE 1 The spectrum obtained using an incident-electron beam of about the same energy as an auger electron spectroscopy (AES) or X-ray photoelectron spectroscopy (XPS) peak approximates to the energy loss spectrum associated with that peak.

NOTE 2 The electron energy loss spectrum, measured with an incident-electron beam, is a function of the beam energy, the angle of incidence of the beam, the angle of emission and the electronic properties of the sample.

5.12.2 Nano-object parameters

Chemical composition.

5.12.3 Advantages

5.12.3.1 Spatial resolution of the order of the electron beam size.

5.12.3.2 Any solid can be analysed.

5.12.3.3 Quantitative analysis is possible.

5.12.3.4 Signal includes chemical information.

5.12.3.5 Direct information can be obtained on the structure of solids and oxidative state of the elements.

5.12.4 Limitations

5.12.4.1 Need very thin specimens, <30 nm.

5.12.4.2 Intensity weak for energy losses >300 eV.

5.12.4.3 Can only be done using transmission electron microscopy (TEM) (see 5.34).

5.12.5 Measurand

The changes in the energy distribution of an electron beam transmitted through a thin specimen (eV).

5.12.6 Relevant standards

Standard being drafted in ISO/TC 202.

5.13 Electrophoresis/capillary electrophoresis

5.13.1 Description

Electrophoresis is a separation technique based on the differential transportation velocities of charged species in an electric field through a conductive medium. The electrokinetic separation methods are performed in sub-millimetre capillaries and in micro- and nanofluidic channels. Electrophoresis is sometimes used with phase analysis light scattering or correlation spectroscopy for determining the electrokinetic potential.

5.13.2 Nano-object parameters

Electrokinetic potential.

5.13.3 Advantages

5.13.3.1 Many types of electrophoresis exist (affinity, capillary, gel, etc.) which can be used to tailor to the separation of analytes.

5.13.3.2 Some electrophoresis techniques can be coupled to other techniques.

5.13.3.3 Quantitative analysis can be done if calibration standards are available.

5.13.3.4 Doppler and phase analysis optical-based techniques for determination of electrophoretic mobility are first principle and do not require calibration; however, zeta potential calculation is model dependent.

5.13.4 Limitations

5.13.4.1 Liquids/dispersions only. No solid or gas analysis.

5.13.4.2 Requires optimization for best separation.

5.13.4.3 Might require labelling of analyte for detection.

5.13.5 Measurands

Electrophoretic velocity per electric field strength (electrophoretic mobility).

5.13.6 Relevant standards

- ISO 13099-1, *Colloidal systems — Methods for zeta-potential determination — Part 1: Electroacoustic and electrokinetic phenomena*
- ISO 13099-2, *Colloidal systems — Methods for zeta-potential determination — Part 2: Optical methods*
- ASTM E2865-12, *Standard Guide for Measurement of Electrophoretic Mobility and Zeta Potential of Nanosized Biological Materials*

5.14 Energy dispersive X-ray spectrometry (EDS/EDX and WDS)

5.14.1 Description

X-ray spectrometry is a technique that uses an electron beam and the surface of the sample to produce individual photons that are measured by a parallel detector. One electron is removed to create a vacancy into which another can “fall” from an outer orbit. The X-rays result from transitions between inner orbitals, which are normally full. This is then used to build up a histogram representing the distribution of X-ray emission energies.

WDS (wavelength-dispersive X-ray spectroscopy) and EDS/EDX (energy-dispersive X-ray spectroscopy) systems are commonly used with scanning or transmission electron microscopes[24].

5.14.2 Nano-object parameters

Chemical composition.

5.14.3 Advantages

5.14.3.1 EDS is capable of semiquantitative or with the appropriate controls and sample preparation quantitative analysis.

5.14.3.2 EDS can be used to generate complex multi-dimensional elemental composition maps.

5.14.3.3 WDS is more sensitive to low atomic number elements than EDS and has the capacity for quantitative analysis.

5.14.4 Limitations

5.14.4.1 Samples should be well-polished and representative of the bulk material. It may be necessary to coat non-conductive samples with a thin layer of carbon, gold, or platinum.

5.14.4.2 Standards might be required for high-accuracy quantification.

5.14.5 Measurand

Elemental composition of a specific point or area or volume, including the type, distribution and relative quantity of elements.

5.14.6 Relevant standards

- ISO 15632, *Microbeam analysis — Selected instrumental performance parameters for the specification and checking of energy-dispersive X-ray spectrometers for use in electron probe microanalysis*
- ISO 22309, *Microbeam analysis — Quantitative analysis using energy-dispersive spectrometry (EDS) for elements with an atomic number of 11 (Na) or above*

5.15 Field flow fractionation (FFF)

5.15.1 Description

A separation technique where a field is applied to a liquid suspension typically passed along a thin and flat channel in order to cause separation of the particles present in the liquid, dependent on their different interactions with the applied field. Hollow fibre separations are also commercially available[25].

NOTE 1 The field can be, for example, gravitational, centrifugal, flow, electrical or magnetic; flow FFF is the most widely used and commercialized FFF sub-technique. Flow FFF separates according to hydrodynamic size.

NOTE 2 Using a suitable detector after or during separation allows determination of the size, size distribution and concentration of nano-objects.

NOTE 3 Separation by FFF can be sensitive to size, length, and shape of the analyte.

NOTE 4 Channel flow is parabolic under operational conditions, with particles moving at different rates depending on the location within the parabolic flow profile.

5.15.2 Nano-object parameters

Size, size distribution, concentration (with an additional detector), chemical composition (with an additional detector).

5.15.3 Advantages

5.15.3.1 Can fractionate highly polydisperse samples into individual populations.

5.15.3.2 Can employ a variety of detectors, e.g. differential refractometer, UV/Vis absorbance, fluorescence, dynamic light scattering, multi-angle static light scattering, or an inductively coupled plasma–mass spectrometer.

5.15.4 Limitations

5.15.4.1 Lower size limit depends on particle density and the FFF method used but is typically 2 nm to 5 nm. Size can only be determined accurately by calibration, retention time, or by secondary detection.

5.15.4.2 Distributions that range from the nm scale to greater than 1 µm will not be properly separated, as the elution order inverts above roughly 1 µm.

5.15.4.3 Sample required to be dispersed in liquid.

5.15.5 Measurand

5.15.5.1 Dependent on detectors used: volume, intensity or mass weighted size or size distribution (nm).

5.15.5.2 Concentration via UV/Vis absorbance or fluorescence intensity.

4.14.5.3 Hydrodynamic diameter based on retention time; greater accuracy is obtained if calibrated with particles of known size under identical elution conditions.

5.15.6 Relevant standards

Standards being drafted in ISO/TC 229[25].

5.16 Fluorescence spectroscopy

5.16.1 Description

A type of electromagnetic spectroscopy which analyses fluorescence from a sample. It involves illuminating the sample, typically with ultraviolet or visible light, to generate an electronically excited state which then emits light (fluorescence) of longer wavelength.

5.16.2 Nano-object parameters

Concentration.

5.16.3 Advantages

5.16.3.1 Sensitive technique, down to single fluorophore level.

5.16.3.2 Rapid technique for qualitative measurements.

5.16.3.3 Compatible with various sample formats.

5.16.3.4 Sensitive to changes in nano-object size and shape.

5.16.4 Limitations

5.16.4.1 Interference from background fluorescence for some samples.

5.16.4.2 Quantitative measurements are time-consuming and require calibration standards.

5.16.4.3 Corrections for scattering are frequently required for quantitative measurements for nano-objects.

5.16.5 Measurand

5.16.5.1 Fluorescence spectrum, intensity vs. wavelength (nm).

5.16.5.2 Quantum yield/efficiency.

5.16.6 Relevant standards

- ASTM E578-07, *Standard Test Method for Linearity of Fluorescence Measuring Systems*
- ASTM E579-04, *Standard Test Method for Limit of Detection of Fluorescence of Quinine Sulfate in Solution*
- ASTM E388-04, *Standard Test Method for Wavelength Accuracy and Spectral Bandwidth of Fluorescence Spectrometers*
- Resch-Genger U., DeRose P. C. Classification, terminology, and recommendations on their selection, use, and production (IUPAC Technical Report). *Pure Appl. Chem.* 2010, **82** pp. 2315–2335

5.17 Fourier transform infrared (FT-IR) spectroscopy and FT-IR imaging

5.17.1 Description

A technique in which a sample is subjected to excitation of molecular bonds by broad-band infra-red radiation, and the Fourier transform mathematical technique is used to obtain an absorption spectrum. Spectra can be obtained as a function of position x and y within a sample and is called FT-IR imaging.

5.17.2 Nano-object parameters

Chemical composition.

5.17.3 Advantages

5.17.3.1 Can determine chemical structure and compositional analysis of chemical mixtures in solid or gas phase.

5.17.3.2 High throughput, high signal-to-noise ratio and high wavelength accuracy.

5.17.3.3 Samples can be run under either ambient temperature (in purged air) or extreme environmental conditions such as ultra-high vacuum, cryo-temperature, high pressure, high temperature.

5.17.3.4 Typically non-destructive and requires little sample preparation.

5.17.3.5 Insensitive to stray light.

5.17.3.6 Large penetration depth; can acquire spectra through low bandgap semiconductors.

5.17.4 Limitations

5.17.4.1 Not suitable for aqueous-phased characterizations due to its sensitivity to water.

5.17.4.2 Sensitive to CO_2 and requires the instrumentation to be purged to remove both H_2O and CO_2.

5.17.4.3 Spectral complexity requires experienced users or access to spectral library for proper band assignment.

5.17.5 Measurand

5.17.5.1 Changes in dipole moment of the excited vibrational modes (complementary technique to Raman spectroscopy).

5.17.5.2 Chemical vibrations are measured in wave number (cm^{-1}).

5.17.5.3 Coupled with IR-microscope or scanning tip, it might be capable of size determination.

5.17.5.4 Coupled with attenuated total reflection (ATR) crystals, it can detect ultra-thin (monolayer thickness) film or molecular species in aqueous phase (special setup needed).

5.17.6 Relevant standards for FT-IR

— ISO/TS 14101, *Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method*

5.18 Induced grating method (IG)

5.18.1 Description

A technique based on a principle for measuring the size of nanoparticles using the phenomenon of dielectrophoresis and diffracted light.

5.18.2 Nano-object parameters

Size, size distribution.

5.18.3 Advantages

5.18.3.1 Simple, rapid measurement.

5.18.3.2 Small disturbance from larger particles.

5.18.3.3 Sensitive to size measurements under 10 nm.

5.18.4 Limitations

5.18.4.1 Lower limit 0,5 nm to 1 nm; upper limit 200 nm to 500 nm.

5.18.4.2 Sample required to be dispersed in liquid.

5.18.4.3 Dispersion liquid required to be dielectric and not conductive.

5.18.5 Measurand

Hydrodynamic equivalent diameter.

5.18.6 Relevant standards

No standards were identified.

5.19 Inductively coupled plasma–mass spectrometry (ICP-MS) and single particle inductively coupled plasma–mass spectrometry (SP-ICP-MS)

5.19.1 Description

An analytical technique used for elemental determinations, in which ions are generated in a high temperature, atmospheric pressure, argon plasma and detected using a mass spectrometer.

ICP-MS systems can be run in single particle mode (SP-ICP-MS) when analysing nano-objects. These particles present in an aqueous solution are introduced to the argon plasma. As the droplets are desolvated in the plasma, the resulting particles are ionized, producing a burst of ions (one ion cloud per particle). The ions then pass into the quadrupole for analysis. The particles are counted using fast data acquisition speeds of up to 100 000 pts/s (data points observed by the detector) and using very short dwell times[26].

5.19.2 Nano-object parameters

Concentration (particle number and ionic), size, size distribution, chemical composition.

5.19.3 Advantages

5.19.3.1 High sensitivity analysis — detection limits of most elements are on the order of ng/l (ng/kg) or lower.

5.19.3.2 Multi-element analysis possible.

5.19.3.3 Can determine element identity and quantity, typically in less than one minute.

5.19.3.4 Wide linear dynamic range with eight orders of magnitude (in conventional mode).

5.19.3.5 Isotopic information possible.

5.19.3.6 Isotope dilution calibration possible.

5.19.3.7 Can differentiate between dissolved and particulate analytes.

5.19.4 Limitations

5.19.4.1 Spectral interference occurs when the instrument cannot distinguish the spectrum of an analyte ion from the spectrum of a concomitant having the same nominal mass-to-charge ratio. Molecular ion interferences can often be mitigated using collision/reaction cells; elemental isobars should be avoided.

5.19.4.2 Contamination in blanks (acids, water, etc.) can sometimes limit detection and quantification.

5.19.4.3 The use of high purity reagents and a clean laboratory environment is necessary, if ultra-trace levels are required.

5.19.4.4 ICP-MS in single particle mode might not be an adequate technique to determine particle number and mass concentration in the event that the ENM size distribution extends below 10 nm for monoisotopic metal nanoparticles. Coupling ICP-MS with field flow fractionation (FFF) or another separation technique will resolve this limitation.

5.19.4.5 Sample required to be dispersed in liquid.

5.19.4.6 Particles with sizes smaller than the particle size detection limit will be quantified as ionic.

5.19.4.7 Multiple analyses are required if no preliminary information on the particle size and number concentration in a sample is available.

5.19.5 Measurand

5.19.5.1 Trace elemental mass concentration (μg/l).

5.19.5.2 Trace elemental mass fraction (μg/kg).

5.19.5.3 Number concentration of nano-objects in suspension (1/ml).

5.19.5.4 Mass concentration of nano-objects in suspension (μg/l).

5.19.5.5 Elemental composition of nano-objects (kg/kg).

5.19.5.6 Nano-object mass/size (nm).

5.19.5.7 Size distribution.

5.19.6 Relevant standards

- ISO/TS 13278, *Nanotechnologies — Determination of elemental impurities in samples of carbon nanotubes using inductively coupled plasma mass spectrometry*
- ISO/TS 19590, *Nanotechnologies — Size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectrometry*

5.19.7 Nano-hyphenated ICP/MS techniques

FFF (field flow fractionation) — Often used in combination with an ICP-MS. Field flow fractionation (FFF) consists of a suite of high-resolution elution techniques which can size separate nanoparticles in the 1 nm to 100 nm range and colloids up to one micron. By use of either FFF theory or calibration with size standards, the technique can be utilized to determine particle size. The separation process is similar to chromatography except that the separation is based on physical forces as opposed to chemical interactions[27].

See 5.15 for more information.

5.20 Laser diffraction

5.20.1 Description

The laser diffraction technique is based on the phenomenon that particles scatter light elastically in all directions with an angular intensity pattern that is dependent on particle size. All commercially available instruments analyse scattering data using an equivalent sphere approximation.

NOTE 1 Laser diffraction instruments have evolved from strictly low angle detection and use of the Fraunhofer approximation, to utilization of a wide range of detector angles, multiple wavelengths and the Mie scattering model. As a result, high-end laser diffraction instruments can yield size data from tens of nanometre to several millimetre.

NOTE 2 Technically, laser diffraction is a form of static light scattering (see 5.31) implemented for size distribution analysis of particles over a wide size range. Optical properties of the particles and medium (principally the refractive indices) are needed for application of the Mie theory. The Fraunhofer approximation is limited to opaque micrometre size particles.

5.20.2 Nano-object parameters

Size, size distribution.

5.20.3 Advantages

5.20.3.1 Can analyse dry powders or particles suspended in a liquid.

5.20.3.2 Broad size range available by combining low angle and high angle detection, as well as multiple wavelength lasers.

5.20.3.3 Well-established technique.

5.20.3.4 Not limited by sedimentation effects.

5.20.3.5 Can characterize samples containing both primary particles and agglomerates.

5.20.4 Limitations

5.20.4.1 Not appropriate for very low particle concentrations or very high concentrations where multiple scattering is significant.

5.20.4.2 Accurate size for sub-micrometre particles requires a prior knowledge of the complex refractive index of the particles.

5.20.5 Measurand

Spherical equivalent differential volume weighted size (nm or µm).

5.20.6 Relevant standards

— ISO 13320, *Particle size analysis — Laser diffraction methods*

5.21 Liquid chromatography–mass spectrometry (LC-MS)

5.21.1 Description

Liquid chromatography (LC) is a technique by which substances in liquid mixtures can be separated. It uses a stationary phase (a column packed with very small particles) and a mobile phase (a system of solvents). The chemistry of solvents flowing through and around the stationary phase particles facilitates the separation of sample constituents. Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of substances in the form of gas phase ions. MS is used for identifying substances in mixtures, for determining the elemental composition of sample constituents, and for elucidating the chemical structures of molecules over a wide range of molecular weights. LC/MS provides a means of separating organic sample constituents and analysing them. Mass spectrometers vary in their mass accuracy and mass resolution — the highest performance instruments in this regard permit structure elucidation. LC/MS allows for the analysis of some organic ligands attached to nano-objects or carbon-based nanomaterials.

NOTE It is applicable to molecular species only, generally organics. This technique will not detect nanoparticles individually but might be used to analyse liquids that include nanoparticles.

5.21.2 Nano-object parameters

Concentration and chemical composition.

5.21.3 Advantages

5.21.3.1 Commonly used in many laboratories for a wide variety of applications.

5.21.3.2 There are multiple MS detectors available depending on application needs.

5.21.4 Limitations

5.21.4.1 Will only detect organic materials.

5.21.4.2 Liquids/dispersions only; no solid or gas analysis.

5.21.4.3 Requires optimization for best separation.

5.21.5 Measurand

Specific identification and mass structure of an organic compound using the MS and the associated concentration of that compound (if calibrated for that compound) using the separation of the LC.

5.21.6 Relevant standards

No standards for nano-object analysis were identified.

5.22 Particle tracking analysis (PTA)

5.22.1 Description

A method where particles undergoing Brownian motion in a liquid suspension are illuminated by a laser and the change in position of individual particles is used to determine particle size.

NOTE 1 Analysis of the time-dependent position of individual particles by means of scattered light can yield the translational diffusion coefficient and hence the particle size as the hydrodynamic diameter using the Stokes-Einstein relationship.

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

5.22.2 Nano-object parameters

Size, size distribution, electrokinetic potential.

5.22.3 Advantages

5.22.3.1 First principles calculation of particle hydrodynamic diameter that does not require calibration other than that of the video-image scale.

5.22.3.2 Allows measurements of particle number concentration.

5.22.3.3 Reduced interference from larger scatters compared to ensemble or batch light scattering techniques.

5.22.3.4 Small sample volume required (≈0,5 ml) which can be recovered after measurement.

5.22.3.5 Quick data acquisition (order of minutes).

5.22.3.6 Ability to measure time-dependent processes (e.g. agglomeration, dissolution).

5.22.3.7 Direct visualization of results of particle number size distribution.

5.22.3.8 Small size (table top) equipment.

5.22.4 Limitations

5.22.4.1 This technique can only be applied to sample dispersed in liquid.

5.22.4.2 Sample required to be diluted (≈10^6 to 10^8 particles per millilitre).

5.22.4.3 Size range limited to a minimum of ≈10 nm due to limited signal-to-noise ratio of the scatter intensity and to a maximum of ≈2 000 nm due to sedimentation interfering with the Brownian motion. Upper limit of particle size is set by sedimentation in the liquid and is of the order of a few micrometres.

5.22.4.4 Particles in suspension and the liquid should have a different refractive index for laser light scattering to take place. Index-matched systems produce low or no signal at all.

5.22.5 Measurand

5.22.5.1 Diffusion parameters of individual nano-objects.

5.22.5.2 Hydrodynamic diameter.

5.22.5.3 Particle concentration.

5.22.5.4 Particle fluorescence when suitably excited by illuminating source.

5.22.5.5 Particle electrophoretic mobility when subject to applied electric field.

5.22.6 Relevant standards

- ISO 13321, *Particle size analysis — Photon correlation spectroscopy*
- ISO 19430, *Determination of particle size distribution — Particle tracking analysis*
- ASTM E2834, *Standard guide for measurement of particle size distribution of nanomaterials in suspension by nanoparticle tracking analysis (NTA)*

5.23 Optical absorption spectroscopy (UV/Vis/NIR)

5.23.1 Description

This technique measures absorbance of electromagnetic radiation with wavelengths in the ultraviolet and/or visible and/or near infrared regions.

There is a wide range of UV/Vis (ultraviolet–visible spectroscopy) instruments available from inexpensive single beam systems to very high performance UV/Vis/NIR (near-infrared) platforms. The following specifications should be considered to provide accurate repeatable data. A scanning double beam instrument should be used to provide a stable system. The wavelength range should be 190 nm to 1 100 nm. It is typically desired to have a minimum spectral resolution of 1 nm or less and the resolution should be selectable between 0,5 nm to 5 nm. Absorbance range minimum of 3A, stray light at 1A (specified absorbance value) is recommended to be 0,010 %T (direct transmittance measurement), accuracy of wavelength measurement +/− 0,1 nm, Photometric accuracy at 1A +/− 0,003A.

5.23.2 Nano-object parameters

Concentration, size, chemical composition.

5.23.3 Advantages

5.23.3.1 UV/Vis analysis requires only a few seconds to provide a response.

5.23.3.2 Wide concentration range of analysis by varying sample path length.

5.23.3.3 Quantification is available with higher end instruments.

5.23.3.4 Little to no sample preparation required.

5.23.3.5 Little maintenance required.

5.23.3.6 Wide range of measurement accessories available to measure characteristics in both liquid and solid form including angular dependent scattered measurements.

5.23.3.7 Absorption properties for some nano-objects provide information on sizes.

5.23.4 Limitations

5.23.4.1 Corrections for scattering might be required.

5.23.4.2 Solid samples require reflectance accessory. There is a wide array of accessories available to allow multiple modes of reflectance measurements to be made. These include relative, absolute and diffuse reflectance. The actual mode of reflectance required is to be determined by the specific analysis.

5.23.5 Measurand

5.23.5.1 Absorption spectrum.

5.23.5.2 Absorbance vs. wavelength (nm).

5.23.6 Relevant standards for

- ISO/TS 10868, *Nanotechnologies — Characterization of single-wall carbon nanotubes using ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectroscopy*
- ISO/TS 17466, *Use of UV-Vis absorption spectroscopy in the characterization of cadmium chalcogenide colloidal quantum dots*

5.24 Quartz crystal microbalance (QCM)

5.24.1 Description

A technique in which the change in frequency of a quartz crystal resonator is measured to determine a change in mass.

NOTE Can be used under vacuum, in gas phase or in liquid environments.

5.24.2 Nano-object parameters

Size.

5.24.3 Advantages

5.24.3.1 Small sample sizes (below 10 µg).

5.24.3.2 Direct correlation between mass/frequency.

5.24.3.3 Liquids, gas, and solids can be used.

5.24.4 Limitations

5.24.4.1 Computation of mass could be difficult with more viscoelastic sample.

5.24.4.2 Quartz microbalances can be extremely sensitive to environmental conditions.

5.24.5 Measurand

5.24.5.1 Frequency change of piezoelectric quartz (Hz).

5.24.5.2 Frequency change can be correlated to mass deposited.

5.24.5.3 Total mass of collected particles.

5.24.6 Relevant standards

No standards were identified.

5.25 Raman spectroscopy/Raman imaging

5.25.1 Description

Spectroscopy in which the radiation emitted from a sample illuminated with monochromatic radiation is characterized by an energy loss or gain arising from rotational, vibrational or phonon excitations[34].

NOTE Various enhanced Raman techniques are now widely used and all exist in microscopy form through coupling with AFM or other optical microscopes. Some examples are surface-enhanced Raman scattering (SERS), resonance Raman scattering, coherent anti-Stokes Raman scattering (CARS), and tip-enhanced Raman scattering (TERS).

5.25.2 Nano-object parameters

Crystal properties, chemical composition.

5.25.3 Advantages

5.25.3.1 Capable of chemical analysis of complex mixture.

5.25.3.2 Insensitive to water, thus suitable for the characterization of aqueous-phased nano-objects.

5.25.3.3 Suitable for in situ measurements in liquid, vapour and solid state and under both ambient or extreme environmental conditions such as ultra-high vacuum, cryo-temperature, high pressure, high temperature or under electrical bias, in a high magnetic field.

5.25.3.4 Typically non-destructive and requires little sample preparation.

5.25.3.5 Small foot-print fibre-optics-based field portable instrumentations are commercially available.

5.25.3.6 Spectra can be collected from very small volume, e.g. in ≈1 μm^3 in confocal Raman microscope.

5.25.4 Limitations

5.25.4.1 Raman scattering is very weak and can be swamped by fluorescence from trace impurities in the sample.

5.25.4.2 Raman spectral complexity often requires even experienced users to access a spectral library for proper band assignment.

5.25.5 Measurand

5.25.5.1 Changes in molecular polarizability of the excited vibrational modes (a complementary technique to FT-IR spectroscopy), measured in frequency difference between scattered and incident excitation light in the unit of wave number difference (cm^{-1}).

5.25.5.2 Hydrodynamic particle size in nanometre (for asymmetric-flow FFF if calibrated).

5.25.5.3 Coupled with AFM or confocal optical microscope, Raman microscopy can be used for nano-object size and chemical composition determination.

5.25.6 Relevant standards for Raman

No standards were identified.

5.26 Resonant mass measurement (RMM)

5.26.1 Description

Resonant mass measurement (RMM) detects and counts particles in the size range of 50 nm to 5 µm, and measures their buoyant mass, dry mass and size. RMM uses a microfluidic channel passing through a resonant cantilever to detect, count and measure the mass of the particles in the liquid passing through the channel. The mass of the particle (or bubble) changes the resonant frequency of the cantilever. Such a change is directly related to the buoyant mass of the particle. The technique also allows to accurately count particles or fine bubbles in the sample.

5.26.2 Nano-object parameters

Size (buoyant mass), size distribution.

5.26.3 Advantages

5.26.3.1 Applicable to characterization of protein aggregates in formulation or buffer.

5.26.3.2 Able to distinguish between proteinaceous material and contaminants such as silicone oil by means of comparing their relative resonant frequencies and buoyant masses.

5.26.3.3 Able to distinguish between nanoparticles and fine bubbles in a fine bubble mixture.

5.26.4 Limitations

5.26.4.1 Size range of 50 nm to 5 µm. The size range depends on the particle density.

5.26.4.2 Cannot measure particles with the same density as the liquid.

5.26.5 Measurand

5.26.5.1 Buoyant mass.

5.26.5.2 Dry mass.

5.26.5.3 Size.

5.26.6 Relevant standards

No standards were identified.

5.27 Scanning electron microscopy (SEM)

5.27.1 Description

A technique that examines and analyses the surface of samples by scanning with a primary electron beam, which causes the ejection of secondary electrons, backscattered electrons, absorbed electrons and X-ray radiation. These signals can be used to determine the structure, composition and topography of the sample[28].

Chemical composition can be determined if additional instrumentation is available, such as energy-dispersive X-ray spectroscopy (EDX) or wavelength dispersive spectrometry (WDX) and it is also possible to generate maps of elemental composition. This technique provides a two-dimensional image of the material. Size, size distribution, and shape measurements can be done using commercial software that analyses images (see 5.14).

Electron back-scatter diffraction (EBSD) is a process that arises between the backscattered electrons and the atomic planes of a highly tilted crystalline specimen when illuminated by a stationary incident electron beam. The measurement takes place within a scanning electron microscope (SEM)[29].

NOTE For a limited field of view that contains all deposited material, the local concentration of nano-objects may be obtained, but this approach is not commonly used to obtain this parameter.

5.27.2 Nano-objects parameters

Size, size distribution, shape, chemical composition, crystal properties (with EBSD).

5.27.3 Advantages

5.27.3.1 Wide range of magnification.

5.27.3.2 Large depth of field with best resolution under 1 nm.

5.27.3.3 Parfocal, i.e. the focus does not change with changing magnification.

5.27.3.4 Two- and three-dimensional and topographical imaging with rich details.

5.27.3.5 Fast, completing imaging and analysis often possible within a few minutes.

5.27.3.6 Most samples require no or minimal preparation.

5.27.3.7 Low landing energy operation or low pressure gas around a specimen allows for imaging and measurement of electrically non-conductive specimens.

5.27.3.8 Low pressure gas around a specimen allows for nanometre-scale etching and deposition of materials.

5.27.3.9 With specially equipped instruments hydrated, even live specimens can be examined in pressure greater than 600 Pa. The sample temperature can vary from 70 K to above 1 000 K and specimen nano-manipulation is possible.

5.27.3.10 Different imaging modes giving different information, e.g. backscattered electron imaging sensitive to composition, secondary electron imaging sensitive to topography.

5.27.3.11 Commercial calibration standards available for size in gold and silver.

5.27.3.12 When using electron back-scatter diffraction, it is highly sensitive to orientation.

5.27.3.13 When using electron back-scatter diffraction, phase identification is through crystal type.

5.27.4 Limitations

5.27.4.1 It may be necessary to coat non-conductive samples with a thin layer of carbon before examination.

5.27.4.2 The instrument may be large in size and required to be housed in an area free of significant electro-magnetic or mechanical interference and might require circulation of cooling water.

5.27.4.3 Special training is required to operate the microscope and to learn the processes of sample preparation, and to recognize and minimize preparation-related artefacts.

5.27.4.4 Works in vacuum, which requires solid samples small enough to fit inside the vacuum chamber.

5.27.4.5 Electron irradiation can alter the sample.

5.27.4.6 Measurements on small particles might have high measurement uncertainties.

5.27.4.7 Distinguishing between agglomerated and aggregated particles might be difficult.

5.27.4.8 When using electron back-scatter diffraction, only polished surfaces can be examined.

5.27.4.9 When using electron back-scatter diffraction, crystallinity determination of phases with resolution is down to 10 nm size (when polished, cross-section of sample including embedded nano-objects is examined).

5.27.4.10 For particle size distribution, a relatively large number of independent images should be obtained in order to have a statistically relevant sample representation.

5.27.5 Measurand

The size, size distribution, shape and location of nano-objects using images generated by electrons, X-ray and light; typical imaging modes for nanometre-scale imaging and measurements use secondary or transmitted electrons. Commercial software for analysing particles reports a number of measurands, such as area-equivalent diameter, maximum Feret diameter, minimum Feret diameter, etc. These measurands are determined by the pixels associated with the image; calibration near the desired size range of the nano-object is suggested.

Electron back-scatter diffraction:

- local orientation of crystalline phase with 10 nm resolution;
- information on local deformation.

5.27.6 Relevant standards

- ISO 13067, *Microbeam analysis — Electron backscatter diffraction — Measurement of average grain size*
- ISO 16700, *Microbeam analysis — Scanning electron microscopy — Guidelines for calibrating image magnification*
- ISO 22493, *Microbeam analysis — Scanning electron microscopy — Vocabulary*
- ISO 24173, *Microbeam analysis — Guidelines for orientation measurement using electron backscatter diffraction*
- ISO/TS 10798, *Nanotechnologies — Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis*

5.28 Scanning probe microscopy (SPM)

5.28.1 Description

A technique that uses a scanning probe microscopy mode for imaging conductive surfaces by mechanically scanning a sharp, voltage-biased, conducting probe tip over their surface, in which the data of the tunnelling current and the tip-surface separation are used in generating the image. The most commonly used SPM technique is atomic force microscopy (AFM). This is a technique for imaging surfaces by mechanically scanning their surface contours, in which the deflection of a sharp tip sensing the surface forces or its vibration amplitude, mounted on a compliant cantilever, is monitored.

Two other common SPM techniques are the following:

- Scanning tunnelling microscopy (STM) measures a weak electrical current flowing between tip and sample as they held a very small distance apart[30].
- Near-field scanning optical microscopy (NSOM) is a method of imaging surfaces optically in transmission or reflection by mechanically scanning an optically active probe much smaller than the wavelength of light over the surface while monitoring the transmitted or reflected light or an associated signal in the near-field regime.

5.28.2 Nano-object parameters

Size, size distribution, shape.

5.28.3 Advantages

5.28.3.1 Direct topographic information from the sample surface.

5.28.3.2 Sample morphology can be correlated with mechanical, electrical, magnetic, and some chemical composition.

5.28.3.3 Compatible with imaging in vacuum, liquid, air or a controlled atmosphere.

5.28.3.4 Minimal sample preparation.

5.28.3.5 Can be calibrated to obtain length measurements traceable to the SI unit.

5.28.3.6 Measurements can be made in atmosphere and at ambient temperatures.

5.28.3.7 It is possible to use accessories for measurements with sample temperature change or in a controlled atmosphere.

5.28.4 Limitations

5.28.4.1 Scanning speed drives the measurement time.

5.28.4.2 Sample immobilization might be required for nano-sized objects.

5.28.4.3 Deconvolution required for accurate width measurements below ≈50 nm. However, if the size measurement is based on *z*-displacement (height measurements), then tip convolution effects are not relevant. The ASTM E2859 technique provides additional guidance on the size measurements.

5.28.4.4 AFM is a surface measurement and cannot determine if the particles are porous or not.

5.28.4.5 Agglomeration of nano-objects can occur during sample preparation.

5.28.4.6 Distinguishing between agglomerated and aggregated particles can be difficult.

5.28.4.7 SPM is a technique for nano-objects surface characterization, so the differences in height on the sample surface should be in the range of a few microns.

5.28.5 Measurand(s)

5.28.5.1 Length, width, height, depth.

5.28.5.2 Electrical field charge (eV)[31].

5.28.5.3 Elastic modulus.

5.28.6 Relevant standards

- ISO 11039, *Surface chemical analysis — Scanning-probe microscopy — Measurement of drift rate*
- ASTM E 2530-06, *Standard practice for calibrating the z-magnification of an atomic force microscope at subnanometer displacement levels using Si(111) monatomic steps*
- ASTM E 2859-11, *Standard guide for size measurement of nanoparticles using atomic force microscopy*

5.29 Secondary ion mass spectrometry (SIMS) and Time of Flight SIMS (TOF-SIMS)

5.29.1 Description

Secondary ion mass spectroscopy is a surface-sensitive technique capable of very low detections limits and 2D and 3D profiling. Extremely high spatial resolution analysis can be obtained when using a finely focused ion beam.

NOTE SIMS involves the bombardment of a sample surface with ions to produce the emission of secondary ions. These secondary ions are then subjected to mass filtering and detection. SIMS instruments come in a variety of configurations, depending on the ion source and mass analysers required.

5.29.2 Nano-object parameters

Concentration (surface element concentration).

5.29.3 Advantages

5.29.3.1 Identification of trace elements up to 2 000 Da with transmission >10 %, with better than 1 parts per million sensitivity.

5.29.3.2 3D elemental distribution with 10 nm depth resolution in depth profiling mode.

5.29.3.3 Detailed chemical isotope ratio information, used for exact quantification [spot to spot reproducibility (1s): 0,4 per mille; mean internal error (1s): 0,3 per mille].

5.29.3.4 Chemical imaging of surfaces with ≈500 nm spatial resolution.

5.29.3.5 Depth profiling of inorganic with ≈10 nm depth resolution.

5.29.3.6 NanoSIMS is a term used to denote the use of an ion beam with an extremely small spot size (down to 50 nm) to provide high spatial resolution analysis.

5.29.3.7 TOF-SIMS Advantages

5.29.3.7.1 Structural information.

5.29.3.7.2 Cryo-conditions for sensitive organic/biological samples available, thus sample analysis in natural biological state is possible.

5.29.3.7.3 No additional matrix (resins/embedding) necessary.

5.29.3.7.4 Simultaneous analysis of a multitude of compounds.

5.29.3.7.5 Chemical imaging of surfaces with ≈9 nm spatial resolution.

5.29.4 Limitations

5.29.4.1 Samples analysed under vacuum.

5.29.4.2 Sample preparation (embedding in resins and polishing).

5.29.4.3 Sample preparation is not necessarily required when running TOF-SIMS.

5.29.4.4 Number of simultaneous monitored elements can be limited but a TOF analyser is capable of monitoring ions of m/e into the hundreds and thousands.

5.29.4.5 Not considered a bulk analysis technique.

5.29.4.6 Homogeneous material is needed to obtain nano-shape information.

5.29.4.7 Extreme care should be taken during sample handling to avoid contamination.

5.29.5 Measurand

Unified atomic mass units (u).

5.29.6 Relevant standards

No standards were identified.

5.30 Small angle X-ray scattering (SAXS)

5.30.1 Description

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

NOTE 1 The angular scattering is usually measured within the range of 0,1° to 10°. This provides structural information on nanoparticles as well as periodicity on length scales typically larger than 5 nm and less than 200 nm for ordered or partially ordered systems[23].

An estimate of nano-concentration may be obtained if the intensity is calibrated and the analyte is of known composition. Shape should be known *a priori*, assumed or estimated by fitting with an appropriate scattering model.

5.30.2 Nano-object parameters

Size, size distribution, shape.

5.30.3 Advantages

5.30.3.1 Non-destructive analysis.

5.30.3.2 Robust analysis based on the model fitting.

5.30.3.3 Can analyse optically opaque suspensions, powders, composites.

5.30.3.4 Broad concentration range possible.

5.30.3.5 Relatively short measurement times compared with small angle neutron scattering.

5.30.3.6 Mass or volume weighted size distributions obtained.

5.30.3.7 Structural information can be extracted under some circumstances.

5.30.4 Limitations

5.30.4.1 Homogeneous and single phase material (monodisperse) is preferred for the convergence of analysis.

5.30.4.2 High polydispersity samples might not be appropriate.

5.30.4.3 Typically performed at large user facilities, but commercial instruments are also available.

5.30.4.4 Sensitivity is dependent on electron density of scatters and contrast with the continuous phase (air, liquid or solid).

5.30.4.5 Less useful for low electron density materials (e.g. soft organic materials, fullerenes).

5.30.4.6 Corrections for X-ray absorbance required to be made.

5.30.4.7 Size determination is model dependent.

5.30.4.8 For quantitative measurements, intensity calibration using a standard is required for a pin-hole detection configuration; primary standardless calibration of X-ray scattering cross-sections is also obtainable using a Bonse-Hart type camera typically found at synchrotron facilities.

5.30.5 Measurand

5.30.5.1 Particle shape.

5.30.5.2 Particle size and distribution (nm).

5.30.5.3 Aggregation and/or agglomeration state of particles.

5.30.5.4 Angular dependent scattered intensity (arbitrary units).

5.30.5.5 Differential scattering cross-section (cm^{-1}).

5.30.6 Relevant standards

— ISO 17867, *Particle size analysis — Small-angle X-ray scattering*

5.31 Static light scattering (SLS) and static multiple light scattering (SMLS)

5.31.1 Description

SLS is also commonly referred to as multi-angle light scattering (MALS) or multi-angle static light scattering (MALSS). As typically implemented, SLS measures time-average intensities of elastically scattered light (i.e. no change in frequency) at angles defined by the direction of light propagation and the position of the detector. Typically, a vertically polarized laser source is used, and the horizontal plane of detection is perpendicular to the direction of polarization. SLS is an ensemble technique (i.e. many particles contribute to the scattering signal) as opposed to a single particle technique (see 5.32).

NOTE 1 The angular dependence of scattered light is analysed using the Rayleigh-Gans-Debye (RGD) theory or the Guinier equation to obtain the radius of gyration (R_g — also called the root mean square radius) of the scatterers. Similarly, the angular dependence might yield the fractal coefficient for nanostructured materials if the combination of structural size, angle and wavelength is suitable.

NOTE 2 The dependence of scattered intensity on concentration in SLS can be used to obtain the average molar mass of macromolecules, if the refractive index dependence on concentration (Δn/Δc) is also known. Both average molar mass and R_g can be obtained from a Zimm plot (simultaneous extrapolation of scattered intensity to zero angle and zero concentration).

SMLS is based on SLS applied to concentrated media, where multiple scattering is dominant. In sufficiently concentrated samples, the incident light is scattered successively by multiple particles before reaching the detector, thereby losing all phase information. The intensity of the multiply scattered light depends on the incident light wavelength and intensity, the particle concentration, the particle size, the refractive index of continuous and dispersed phases, the absorbance of light by the particles, and the detector position.

NOTE 3 SMLS can be used in a backscatter configuration to extract a mean particle size by application of the appropriate theory. Alternatively, the transmission of light can be detected as a function of time and/or position (measured from the suspension/air interface) and used to monitor relative changes in agglomeration state or sedimentation rates; in this case, particle size information cannot be determined. Combining vertical scanning and multiple acquisitions over the sample height enables SMLS to record local sample heterogeneities, the state of dispersion and its time evolution (colloidal stability).

5.31.2 Nano-object parameters

Size, concentration.

5.31.3 Advantages

5.31.3.1 Useful for aerosols (SLS only) as well as particles dispersed in liquid.

5.31.3.2 Very wide dynamic range, from tens of nanometres to several millimetres.

5.31.3.3 Real-time measurement.

5.31.3.4 Capable of time-dependent measurement.

5.31.3.5 Well-established quality assurance procedures.

5.31.3.6 Time-dependent analysis is possible.

5.31.3.7 For SMLS, no sample preparation is typically required (i.e. no dilution required for highly concentrated samples).

5.31.3.8 For SMLS, sensitive to changes in dispersion state (e.g. agglomeration, aggregation, and sedimentation) when coupled to scanning technology.

5.31.4 Limitations

5.31.4.1 Optical properties (refractive index) of the particles and suspending medium might be required for application of specific theoretical analyses (e.g. RGD). Strongly absorbing particles (e.g. metal nanoparticles associated with surface plasmon resonance effects) might not be amenable to analysis by SLS.

5.31.4.2 Calculation of size assumes spherical particle shape. If shape factor is known, it can be incorporated into RGD analysis or used to convert R_g to a defined shape model (e.g. ellipsoid or cylinder).

5.31.4.3 For SLS, size range is limited by the source wavelength and the angular range of detectors. Using RGD or Guinier analysis with the wavelength of a He-Ne laser (633 nm), R_g smaller than about 60 nm can be extracted.

5.31.4.4 Upper number concentration limit subject to the onset of coincidence.

5.31.4.5 For SMLS, volume fraction and refractive indexes are required for mean particle size calculation.

5.31.5 Measurands (SLS)

5.31.5.1 Radius of gyration (nm).

5.31.5.2 Fractal coefficient (unitless).

5.31.6 Measurands (SMLS)

5.31.6.1 Mean equivalent (spherical) diameter in nm.

5.31.6.2 Percentage of backscattering/transmission intensity for dispersion state analysis.

5.31.6.3 Percentage of backscattering/transmission intensity versus time for dispersion stability analysis.

5.31.7 Relevant standards

— ISO 13320, *Particle size analysis — Laser diffraction methods*

— ISO/TR 13097, *Guidelines for the characterization of dispersion stability*

There are also papers describing the technique[32][33].

5.32 Single particle light interaction methods

5.32.1 Description

The scattering of light by a single particle is caused by diffraction, refraction and reflection. The polarization plane of the scattered light wave is also affected. The intensity of the light scattered from a single particle depends on the incident light intensity, the polarization angle, the detection angle of the scattered light, the refractive index, the light wavelength and the particle diameter. Since particles are analysed individually, particle concentration and number-weighted size distributions can be obtained under appropriate conditions. Single particle scattering methods differ from ensemble scattering methods (see 5.20 and 5.31) that measure the signal produced by many scatterers in the sampling volume.

5.32.2 Nano-object parameters

Concentration, size and size distribution.

5.32.3 Advantages

5.32.3.1 High size resolution possible.

5.32.3.2 No size distribution assumptions required.

5.32.3.3 Real-time measurement.

5.32.3.4 Capable of time-dependent measurement.

5.32.3.5 Well-established quality assurance procedures.

5.32.4 Limitations

5.32.4.1 Applicable either to airborne or liquid-borne nanoparticles.

5.32.4.2 Lower size limit ≈60 nm; upper size limit ≈100 µm.

5.32.4.3 Upper number concentration limit subject to the onset of coincidence.

5.32.5 Measurand

Particle number concentration as function of light-scattering or light-extinction equivalent diameter.

5.32.6 Relevant standards

- ISO 21501 (all parts), *Determination of particle size distribution — Single particle light interaction methods*

5.33 Thermogravimetric analysis (TGA)

5.33.1 Description

A technique in which the change in mass of a sample is measured as a function of temperature or time while the sample is subjected to a controlled temperature program.

5.33.2 Nano-object parameters

Chemical composition.

5.33.3 Advantages

5.33.3.1 Various atmospheres available.

5.33.3.2 Scanning: sub-ambient to 1 000 °C.

5.33.3.3 Isothermal studies at a constant temperature for times of seconds to hours.

5.33.4 Limitations

5.33.4.1 Samples over 1,5 mg required.

5.33.4.2 Samples that are wet are required to be let dry before running.

5.33.5 Measurand

5.33.5.1 Mass of volatile components (g).

5.33.5.2 Loss of sample (g).

5.33.5.3 Derivative of mass with temperature (g/K).

5.33.6 Relevant standards

- ISO/TS 11308, *Nanotechnologies — Characterization of single-wall carbon nanotubes using thermogravimetric analysis*

5.33.7 Hyphenated TGA techniques

5.33.7.1 TGA-GC-MS — Thermogravimetric analysis–gas chromatography–mass spectrometry

5.33.7.2 TGA-MS — Thermogravimetric analysis–mass spectrometry

5.33.7.3 TGA-FTIR — Thermogravimetric analysis–Fourier transform infrared spectroscopy

5.34 Transmission electron microscopy (TEM)

5.34.1 Description

A technique that produces images and diffraction patterns with a resolution in atomic scale by using an electron beam which passes through the sample and interacts with the sample to a detector. With this technique, it is also possible to experiment in regions as small as tens of nanometres to obtain chemical (EDX and/or EELS), morphological and crystallographic information [selected area electron diffraction (SAED), micro-diffraction and convergent beam electron diffraction]. It is the most important technique to investigate samples in nanoscale. The images produced by TEM are the projections on a plane of the components in the sample.

5.34.2 Nano-object parameters

Size, size distribution, shape, chemical composition (using EELS; see 5.12), and crystal properties (using SAD, micro diffraction and CBED).

NOTE 1 Chemical composition can be determined if additional instrumentation is available, such as energy-dispersive X-ray spectroscopy.

NOTE 2 In image mode, this technique provides a two-dimensional projection of the sample. Size, size distribution, and shape measurements can be performed manually or using image analysis software.

5.34.3 Advantages

5.34.3.1 70 pm imaging resolution with best instruments.

5.34.3.2 Chemical information with EDS and EELS.

5.34.3.3 Tomography for full 3D shape analysis.

5.34.3.4 Imaging at the sub-nanometre scale.

5.34.3.5 Automated image analysis for improvement of statistical relevance and work-flow.

5.34.3.6 Commercial calibration standards available for size in gold and silver.

5.34.4 Limitations

5.34.4.1 Long-time consumption is required to get good statistics for size measurements.

5.34.4.2 Time and expense of equipment and maintenance.

5.34.4.3 Limited primarily to electron-dense materials; soft materials lack sufficient contrast unless dyed.

5.34.4.4 Electron beam damage or modification of sample.

5.34.4.5 Magnification calibration required for accurate size measurements.

5.34.4.6 Generally unable to differentiate between agglomerates, aggregates and artefacts.

5.34.4.7 Sample preparation is essential to good results.

5.34.4.8 Calibration might change as the lenses system is changed.

5.34.5 Measurand

5.34.5.1 Particle size (Feret diameter).

5.34.5.2 Shape.

5.34.5.3 Size distribution.

5.34.5.4 Equivalent circular diameter.

5.34.5.5 Electron diffraction spectra.

5.34.6 Relevant standards

- ISO 25498, *Microbeam analysis — Analytical electron microscopy — Selected-area electron diffraction analysis using a transmission electron microscope*
- ISO 29301, *Microbeam analysis — Analytical transmission electron microscopy — Methods for calibrating image magnification by using reference materials having periodic structures*