European
Commission

JRC REFERENCE REPORTS

Requirements on measurements for the implementation of the European Commission definition of the term “nanomaterial”

Linsinger T., Roebben G., Gilliland D.,
Calzolai L., Rossi F., Gibson N., Klein C.

2012

Report EUR 25404 EN

Joint
Research
Centre

European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

Contact information
T. P. J. Linsinger
Address: Joint Research Centre,
Institute for Reference Materials and Measurements,
Retieseweg 111,
2440 Geel,
Belgium
E-mail: jrc-irmm-info@ec.europa.eu
Tel.: +32 (0)14 57 1211
Fax: +32 (0)14 58 4273

http://irmm.jrc.ec.europa.eu
http://www.jrc.ec.europa.eu

This publication is a Reference Report by the Joint Research Centre
of the European Commission.

JRC73260

EUR 25404 EN

ISBN 978-92-79-25602-8 (pdf)
ISBN 978-92-79-25603-5 (print)

ISSN 1831-9424 (online)
ISSN 1018-5593 (print)

doi:10.2787/63490

Luxembourg: Publications Office of the European Union, 2012

JRC Reference Report

Requirements on measurements for the implementation of the European Commission definition of the term 'nanomaterial'

T.P.J. Linsinger ([1,*]), G. Roebben ([1]), D. Gilliland ([2]), L. Calzolai ([2]),
F. Rossi ([2]), N. Gibson ([2]), C. Klein ([1])

July 2012

---

[1] European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM)

[2] European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP)

* corresponding author

Contents

Executive Summary

Aims and scope of this report

The European Commission recently published its recommendation on a common definition (1) of the term 'nanomaterial' for regulatory purposes. Proper implementation of this definition requires appropriate tools and methodologies for which measurement aspects are crucial. This report describes the *requirements* for particle size measurements of nanomaterials based on the definition. It discusses the related generic measurement issues and reviews the capabilities of the measurement methods currently available. Moreover, it illustrates with practical examples the measurement issues that remain to be solved.

This report does not cover other related issues, such as the implementation of the definition by means other than through measurements, or methods to detect specific nanomaterials (such as fullerenes and single-wall carbon nanotubes), measurements to assess exposure to or effects of nanomaterials, or the detection and measurement of nanomaterials in consumer products. These will be addressed in a follow-up report.

The Commission definition of 'nanomaterial'

Commission Recommendation 2011/696/EU defined 'nanomaterial' as follows:

1) "Nanomaterial" means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

2) In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

By derogation from point 1, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

For the purposes of point (1), "particle", "agglomerate" and "aggregate" are defined as follows:

(a) "Particle" means a minute piece of matter with defined physical boundaries;

(b) "Agglomerate" means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;

(c) "Aggregate" means a particle comprising of strongly bound or fused particles.

Where technically feasible and requested in specific legislation, compliance with the definition in point (1) may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition in point (1) where the specific surface area by volume of the material is greater than 60 m^2/cm^3. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition in point (1) even if the material has a specific surface area lower than 60 m^2/cm^3.

The European Commission definition is more specific than other existing international definitions (for example the definition by ISO (2)), because it was developed specifically for use in the regulatory field. The intended regulatory use requires consideration of how to best implement the definition relying on the possibility to verify through measurements whether a material meets the definition of 'nanomaterial'. Measurement methods must be available for manufacturers to provide accurate information and for authorities and consumers to verify the accuracy of the information they receive through the envisaged notification and labelling procedures.

Measurement requirements resulting from the definition

Determining whether a material fulfils the recommended definition follows a five-step procedure:

---

[1] COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterial (2011/696/EU). See http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:EN:PDF online.

[2] ISO definitions can be consulted on the ISO Online Browsing Platform. See http://www.iso.org/obp/ui/ online.

1. Determination whether the material consists of fullerenes, graphene flakes or single-wall carbon nanotubes.
   - These materials fall under the definition, regardless of the size of the particles.
2. Determination whether the material consists of particles.
   - Particles may present themselves as dry powders, as a paste or in suspensions.
3. Determination of the constituent particles of the material.
   - The constituent particles are often aggregated or agglomerated.
4. Determination of the external dimensions of the (constituent) particles.
   - Particle size measurements assess the external dimensions of particles.
5. Determination of the median value of the particle size distribution based on the appropriate external dimension.
   - The median value corresponds with the 50 % fraction in the particle number-based particle size distribution mentioned in the definition.

According to the definition, the determination of the specific surface area (SSA) of the material can be used to positively classify a material as a nanomaterial. However, a negative result would not overrule a positive classification from particle size analysis.

The core concepts: particle, aggregate and agglomerate, and particle size

Particles are defined as 'minute pieces of matter with defined physical boundaries'. However, at the nanoscale, the physical boundaries of a particle, especially if it is in a suspension, are affected by dissolution, swelling and adsorption. Furthermore, nanoscale particles often stick to each other, forming aggregates (strongly bound) or agglomerates (weakly bound) of what the definition calls 'constituent particles'. It is important to recognise the dynamic nature of particle boundaries, agglomeration and aggregation.

Generic issues for reliable particle size determination

Sampling: The number of particles counted in a measurement is generally extremely small in comparison with the number of particles in the material under investigation. Therefore, utmost care must be taken to obtain a representative sample for analysis. International standards should be followed to achieve this (see Section 3.1).

Sample preparation: Most size measurement methods necessitate a sample preparation procedure that breaks up agglomerates and aggregates, if the size of their constituent particles must be measured. This and other sample preparation can influence the measured sizes. In practice, strongly-bound aggregates cannot be dispersed into their constituent particles and are therefore often indistinguishable from large particles (see Section 3.2).

Size distributions: Measured particle size distributions are weighted according to the number of particles per size group, the surface area of particles per size group, the volume of particles per size group, or the light-scattering intensity of particles per size group, to name only the most common weighting methods. Most methods produce size distributions that need to be mathematically converted to the number-based size distribution required in the definition. This conversion is based on various assumptions, and becomes increasingly prone to error, difficult or impossible, if the mass fraction of nanoscale particles is not sufficiently large (see Section 3.3.1).

Method-defined size values: Most measurement methods provide a method-defined, *apparent* value for the selected external dimension. Therefore, different size measurement methods may result in significantly different size values (see Section 3.3.2). It is impossible to rank different methods according to trueness or reliability for all possible applications, so any definition of a universal reference method would be arbitrary. On the contrary, the method-dependence of size measurements allows selecting the most appropriate method for the specific application or concern. As results of size measurements are method-defined, standardisation of measurement methods is needed to ensure comparability between different laboratories.

Candidate measurement methods

The methods for measuring the size of nanoparticles can be grouped as follows:

Ensemble methods (methods that measure large numbers of particles simultaneously), like dynamic light scattering (DLS) or small-angle X-ray scattering (SAXS), report intensity-weighted particle sizes. Conversion to number-based size distributions is reliable only for nearly monodisperse, spherical particles, and when sufficient shape information about the particles is available. Other ensemble methods, such as X-ray diffraction (XRD) only measure an average size value and do not provide useful information about size distribution.

Counting methods like particle tracking analysis (PTA) study particle by particle, and assume that the particles have a specific shape. Imaging methods, such as electron microscopy (EM) and atomic force microscopy (AFM), are also counting methods and can deal with non-spherical particles on a surface. However, a high number of particles making up a representative sample must be measured to obtain reliable size distributions for industrial, polydisperse materials.

Fractionation methods like centrifugal liquid sedimentation (CLS), field-flow fractionation (FFF), size-exclusion chromatography (SEC) or hydrodynamic chromatography (HDC) separate the sample into monodisperse fractions prior to quantifying the particles. This eliminates several of the problems associated with measurements on polydisperse samples. Some of these *fractionation* methods can be coupled on-line or off-line with *ensemble* or *counting* methods, and are expected to become a crucial component of size measurement approaches to be developed for the implementation of the definition.

Across the above mentioned categories of measurement methods, three major issues are identified:

Dealing with agglomerates/aggregates: No method is available that can reliably distinguish whether a large particle is an agglomerate, aggregate or a single particle, and at the same time measure the size of large numbers of individual constituent particles. For some materials a distinction is possible using EM, but DLS, CLS, SAXS, AFM and PTA regard each agglomerate/aggregate as a single, large particle.

Working range: No single method alone can cover, in a single measurement, for all materials the complete size range from lower than 1 nm to well above 100 nm, as it would be required for a universal assessment according to the definition. In particular, it is expected that working ranges, when expressed in terms of the measurable particle number fractions (which must include the 50 % or median value), will strongly depend on particle mass fractions.

Method validation: None of the mentioned measurement methods have been specifically validated for their use in the implementation of the nanomaterial definition.

Conclusion and outlook

There are various scientific-technical challenges related to the measurement of materials in the implementation of the recommended nanomaterial definition. Particular challenges are the requirement of measuring the constituent particles inside aggregates, regardless of the strength with which the individual particles are bound, the difficulty to convert the experimentally measured signals to accurate number size distributions for polydisperse materials, and to detect and count particles at the lower size range of the definition (smaller than 10 nm). These measurement issues would be aggravated when a lower threshold (instead of 50 %) would be chosen. Also, most current methods have a detection limit higher than 1 nm or a lower sensitivity for smaller particles. Therefore, they can only be used for a positive test to prove that a material is a nanomaterial, but not for a negative test to prove that a material is not a nanomaterial. Summarising the current technical limitations, none of the currently available methods can determine for all kinds of potential nanomaterials whether they fulfil the definition or not. Therefore, a range of measurement methods is required to investigate whether nanomaterials fulfil the regulatory definition. Implementation of the definition via measurements poses significant difficulties for polydisperse materials and is currently usually not possible for aggregated materials if the size distribution of their constituent primary particles must be determined, unless the aggregates as particulate material themselves fulfil the nanomaterial definition.

The future improvement of measurement technology, development of analytical methods and standardised sample preparation protocols may partly resolve the mentioned limitations. If rapid implementation of the definition through measurements is needed, dedicated guidance documents will have to be provided for specific materials and sectors, with clear and justified indication of the relevant particle size measurement methods and test conditions. A combination of several methods, ideally supported by information on the manufacturing process of the material under investigation, will have to be employed for robust assessments. The reliability of each of the measurement methods used in such combined, tiered processes will need to be thoroughly checked in dedicated method validation and interlaboratory comparison studies. Such technical developments and experiences should be taken into account for a future revision of the definition stipulated by the recommendation.

Glossary

AFM	Atomic force microscopy (or atomic force microscope)
ASTM	ASTM International (former American Society for Testing and Materials)
BET	Brunauer-Emmett-Teller (as in 'BET method for surface area analysis')
CCD	Charge-coupled device
CLS	Centrifugal liquid sedimentation
CRM	Certified reference material
DLS	Dynamic light scattering
D50	Diameter at which 50 % of the particles signal was measured with CLS
EM	Electron microscopy (or electron microscope)
ERM®	European Reference Material
EU	European Union
FFF	Field flow fractionation
HDC	Hydrodynamic chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
IHCP	Institute for Health and Consumer Protection of the JRC
IRMM	Institute for Reference Materials and Measurements of the JRC
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
NIST	US National Institute of Standards and Technology
OECD	Organisation for Economic Co-operation and Development
PTA	Particle tracking analysis
SAXS	Small-angle X-ray scattering
SC	Subcommittee
SEC	Size-exclusion chromatography
SEM	Scanning electron microscopy (or scanning electron microscope)
SSA	Specific surface area
TC	Technical Committee
TEM	Transmission electron microscopy (or transmission electron microscope)
TS	Technical Specification
VSSA	Volume-specific surface area
WPMN	Working Party on Manufactured Nanomaterials of OECD
XRD	X-ray diffraction (or X-ray diffractometry or X-ray diffractometer)
2D	Two-dimensional

1. Introduction

1.1 The Commission definition of the term nanomaterial

In 2009, the European Parliament urged the European Commission (EC) to review all relevant legislation to ensure safety for all applications of nanomaterials in products with potential health, environmental or safety impacts over their life-cycle and to adopt a science-based common definition of the term 'nanomaterial' to be used in all European Union (EU) legislation [1]. Based on input from the Commission's Joint Research Centre (JRC) [2], the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) [3], a public consultation on a draft definition and a consultation among its services, the Commission issued a common definition for the term nanomaterial [4] on 18 October 2011, which will further be referred to in this report as 'the *definition*'.

1) "Nanomaterial" means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

2) In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

By derogation from point 1, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

For the purposes of point (1), "particle", "agglomerate" and "aggregate" are defined as follows:

(a) "Particle" means a minute piece of matter with defined physical boundaries;

(b) "Agglomerate" means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;

(c) "Aggregate" means a particle comprising of strongly bound or fused particles.

Where technically feasible and requested in specific legislation, compliance with the definition in point (1) may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition in point (1) where the specific surface area by volume of the material is greater than 60 m^2/cm^3. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition in point (1) even if the material has a specific surface area lower than 60 m^2/cm^3.

1.2 Implementation of the definition

The *definition* is released as a Commission Recommendation, which does not necessitate immediate practical implementation of the *definition*. This, however, will change as soon as the *definition* is used in specific legislation. First legislation already exists [5, 6] which will require that consumers are informed whether ingredients of food and cosmetic products were added in their nanoform. Therefore, producers of materials and regulators are faced with two questions:

a) Does a certain raw material fulfil the *definition*?

b) Does a final product contain nanomaterials?

Questions a) and b) are two very different aspects of the implementation of the *definition* ([3]).

The question 'Is the material a nanomaterial?' targets the raw materials that are used as basic ingredients to produce finished products. As far as the current definition is concerned, the material is at this stage in its particulate form (in other words, it is a dry powder or a suspension in which the particles are free to move).

The question 'Does the product contain nanomaterials?' deals with the enforcement of product legislation. Control authorities may be faced with a final product that may or may not have been labelled as containing nanomaterials, and need to check whether this label was applied in line with current legislation or whether the absence of a label is justified.

Intrinsically, both aspects of the implementation

---

[3] Replacing the term 'contain' with the term 'consist of' in the definition would result in a better distinction between both questions.

of the *definition* are measurement issues. Measurements are needed:

- to allow producers to correctly identify the materials they produce as nanomaterials. In this case, producers themselves perform or commission measurements to determine whether their materials fulfil the *definition*.
- to allow authorities to confirm or refute classifications by producers. In this case, authorities use measurement results to check whether the classification nanomaterial/non-nanomaterial made by the producer is correct.
- to allow authorities and consumer groups to check whether a product has been labelled correctly, i.e. whether a product contains nanomaterials although it is not labelled as such, or vice versa.

1.3 Aims and scope of the report

The European Commission recently published its recommendation on a common definition of the term ‘nanomaterial’ for regulatory purposes. Proper implementation of this definition requires appropriate tools and methodologies for which measurement aspects are crucial. This report describes *requirements* for particle size measurements of nanomaterials based on the *definition*. It discusses the related generic measurement issues, and reviews the capabilities of the measurement methods currently available. Moreover, it illustrates with practical examples the measurement issues that remain to be solved.

This report focuses on general measurement aspects of the *definition*. Details on the testing strategies, aspects of documentation or inclusion of manufacturing information go beyond this report. The scope of this report also does not cover other related issues, such as the implementation of the *definition* by means other than through measurements, or methods to detect specific nanomaterials (such as fullerenes and single-wall carbon nanotubes), measurements to assess exposure to or effects of nanomaterials, or the detection and measurement of nanomaterials in consumer products. Also testing of specific materials to assess whether they fulfil the *definition* goes beyond the scope of this report. A reflection on the latter will be initiated in Section 6 and will be continued in a separate report.

2. Measurement-related elements in the definition

The *definition* is constructed around a number of core terms and concepts with particular measurement aspects. This section highlights these terms and concepts, including potential ambiguities and needs for clarification.

2.1 Particles

The *definition* restricts the term ‘nanomaterial’ to materials containing *particles*. Particles are defined as ‘minute pieces of matter with defined physical boundaries’. This includes not only ‘equiaxial’ (roughly spherical) particles, but also rod-like (fibres or tubes) and plate-like (flakes or platelets) particles. The term ‘particle’ in the *definition* therefore corresponds to the term ‘object’ in the terminology of the International Organization for Standardization (ISO) for nanomaterials (4) [7].

The notion ‘minute piece of matter’ is not without ambiguity. Clearly, large pieces of material (for example sheets of glass, rods of solid metal, plastic foils) do not fulfil the definition of a particle. Nevertheless, depending on the background of the observer, particles up to masses of several kg may be defined as ‘minute’. This issue is relevant for the *definition*, as the *definition* explicitly includes aggregates of smaller nanoparticles, which may be seemingly large, solid materials (see Section 2.2). For practical reasons, and without jeopardising the essence of the definition, this report will assume that the relevant materials for the assessment definition must be a powder or a suspension of particles (5).

Also the notion ‘defined physical boundaries’ may be interpreted in different ways. At the nanoscale, the thickness of the surface layer surrounding each particle is significant (in relative terms), especially in suspensions. Particles are objects that move as one unit. In this sense, the layer of atoms, ions

---

4 The ISO-definition of the term nano-object includes nanoplates (one dimension in the nanoscale), nanorods (two orthogonal dimensions in the nanoscale) and nanoparticles (three orthogonal dimensions in the nanoscale). Throughout this report the term ‘particle’ is used in the sense of the Commission definition, i.e. comprising nanoparticles, as well as both nanorods and nanoplates from the ISO/TS 27687:2008 [7] definitions.

5 This corresponds with the term ‘particulate nanomaterials’ proposed by the JRC in its Reference Report EUR 24403 (2010) [2].

and molecules that moves with the particle is considered as part of the particle.

Conclusion: Implementation of the definition requires testing whether the material in question indeed consists of particles in form of a powder or suspension.

2.2 Aggregates, agglomerates and constituent particles

2.2.1. The difference between aggregates and agglomerates

The *definition* explicitly states that aggregates and agglomerates composed of nanoscale particles qualify as nanomaterials. The ISO Technical Specification (TS) 27687 [7] gives definitions for particles clustered in agglomerates and aggregates. These definitions were prepared in collaboration with the ISO Technical Committee (TC) 24, Subcommittee (SC) 4 - Particle characterisation, and were largely taken over in the *definition*:

Agglomerate: Collection of weakly-bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components.

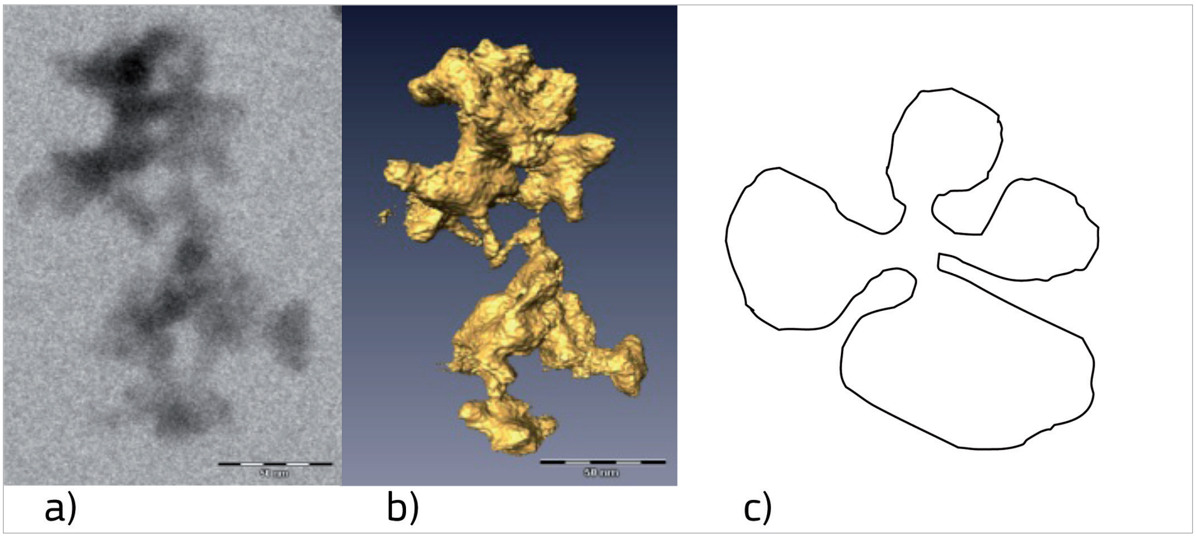
Aggregate: Particle comprising strongly-bonded or fused particles *where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components* (6).

The notes to the respective definitions in ISO/TS 27687 [7] state that the forces holding an agglomerate together are weak forces – for example, van der Waals forces or simple entanglement – whereas aggregates are held together by strong forces – for example, covalent bonds – or by forces resulting from sintering or complex entanglement. In many cases, particles aggregate during production and these aggregates in turn form agglomerates of aggregates. There is no clear distinction between agglomerates and aggregates – there is a continuum of binding strengths. The weakly-bound agglomerates can be dispersed (see Section 3.2.2), whereas the strongly-bound aggregates cannot be de-aggregated without risking destruction of the original, constituent particles.

2.2.2. The detection of constituent particles in aggregates

The *definition* explicitly states that the decisive criterion is the size of the constituent particles, regardless of how large the aggregates or agglomerates are. Figure 1 illustrates that it is not always straightforward to distinguish the constituent particles inside an aggregate particle.

Figure 1: a) electron microscopy image of a nanoparticle; b) transmission electron tomography image of the same nanoparticle as shown in a); a) and b): from Van Doren et al. [8] (reproduction with kind permission from the Journal); c) schematic drawing of the cross-section of a particle that could be an aggregate of four constituent particles or a single branched particle.



---

6 Text in italics not included in the Commission definition.

Figure 1a is a typical electron microscopy (EM) image of a nanoparticle, showing areas of higher and lower electron contrast, which suggest that the particle is an aggregate of smaller particles. Figure 1b is a three-dimensional (3D) reconstruction of the same particle as shown in Figure 1a, using transmission electron tomography. Also, this advanced technique fails to indicate the boundaries of possible constituent particles. Figure 1c depicts the issue in a more schematic way. The image shown may well be a cross-section of an aggregate composed of four constituent particles, or the image may correspond with a cross-section through a single particle with a branched structure. Aggregates are for most practical purposes indistinguishable from single particles.

2.2.3. The distinction between 'aggregate' and 'polycrystalline' particle

In the absence of other indications, this report assumes that the term constituent particle is equivalent to the term primary particle, which is defined in several ISO documents (see http://www.iso.org/obp/ui/). The ISO definition calls agglomerates and aggregates also 'secondary particles' to distinguish them from the original individual particles, named 'primary particles'. Therefore, a constituent particle is seen as a particle that has existed as a discrete, freely moving particle somewhere upstream in the production process, even if only for a short moment.

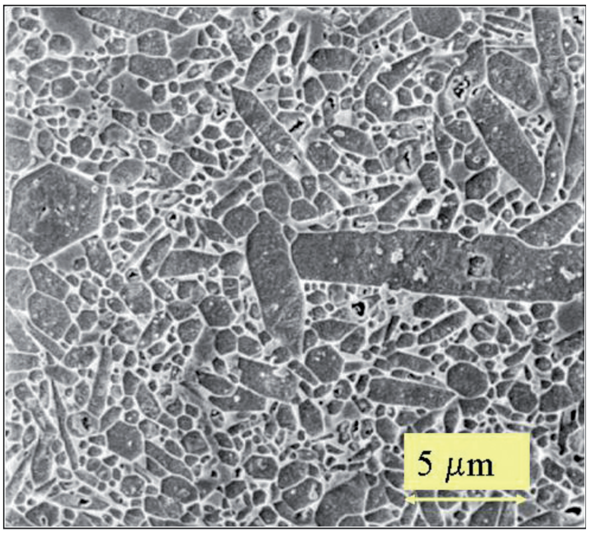


Figure 2: Electron micrograph of a plasma-etched section through a silicon nitride ceramic

Note: During the sintering process, the particles of the original a-Si_3N_4 powder dissolve in a transient liquid phase and re-precipitate as b-Si_3N_4 grains, resulting in a dense, polycrystalline material. The grains in the sintered ceramic never existed as free particles (from [9], with kind permission).

Many solid materials are polycrystalline, i.e. they consist of a more or less dense packing of many small crystals, as shown in Figure 2. The polycrystalline material shown in Figure 2 is not an aggregate of particles. The constituent particles are the result of precipitation and phase transformation processes and have never been freely moving entities.

The boundaries of the crystals in polycrystalline materials are often indistinguishable from boundaries between constituent particles in aggregates. A distinction between the two types of materials, based only on measurements of the size of the constituent particles or crystals, is not possible. Instead, detailed micrographic analysis and a minimum knowledge about the manufacturing process are needed.

Conclusion: Implementation of the definition requires a determination of what the constituent particles of a particulate material are.

2.3 Particle size and external dimensions of one particle

The term *particle size* seems easy to understand, but this is misleading. A *particle size* expressed as one or several length values can only fully capture the geometry for regularly shaped particles. For example, the size of a sphere is completely defined by the particle radius or diameter, and the size of a cuboid by its height, width and depth. This simplicity rapidly breaks down for irregularly shaped bodies, as is the case for most particles. An example of how to deal with sizing of irregularly shaped bodies, although without relevance for nanoparticle size analysis, can be found in the food industry. Rather than using a length value, eggs are sized according to their mass, accounting for the different shapes eggs may have. An alternative option is to use the diameter of a sphere with equivalent properties (see Section 3.3.2) as a measure of particle size.

Instead of the term 'particle size', the *definition* uses the term 'external dimension'. To illustrate the latter term, the irregular particle depicted in Figure 1c is shown again in Figure 3 and is considered here to be a single particle. Figure 3 indicates a number of external dimensions. The most intuitive 'external dimension' would correspond to the calliper distance, or the distance 'between one's thumb and finger', if one could hold the particle between thumb and finger. In scientific terms, this is the so-called Feret diameter. Feret diameters are distances between two parallel tangents on opposite sides of the image of a particle [10]. Figure 3 indicates the maximum Feret diameter, $x_{F,max}$, and the minimum Feret diameter, $x_{F,min}$, of the shown particle. Both diameters have a value in the order of 100 nm. One should note that the Feret diameter is a very useful concept, but it is a dimension that can only be measured by imaging methods.

Moreover, Figure 3 shows the diameter of the heart of the particle, x_i, which is also an external dimension of the particle, and this diameter is much smaller than 100 nm. Another external dimension is the perimeter of a two-dimensional (2D) projection of the particle, which, when measured on the shown image, would be significantly larger than 100 nm. Apart from pure size parameters, a plethora of measures characterising shape and area have been developed. They are all useful in their own right, but they are only peripherally connected to the topic of this report and are therefore not listed here.

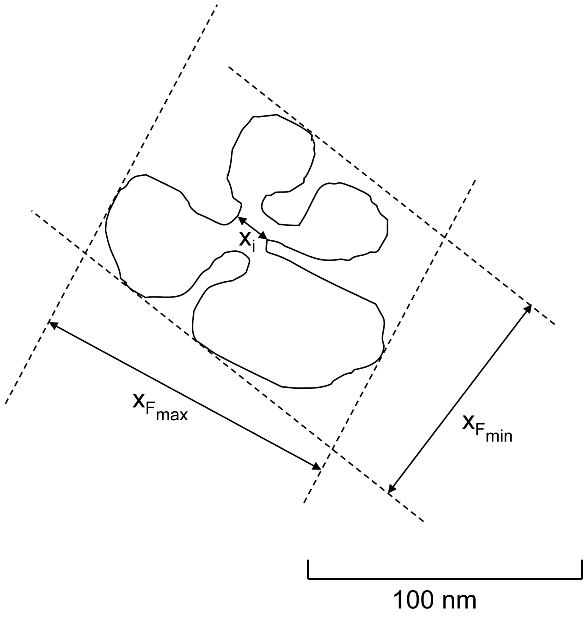


Figure 3: Illustration of a number of possible external dimensions of an irregular particle as apparent from a 2D image of the particle

Note: Indicated are the diameter of the central part of the particle (x_i), the minimum Feret diameter ($x_{F,min}$) and the maximum Feret diameter ($x_{F,max}$).

As discussed in more detail in Section 3.3.2, most sizing methods result in method-defined equivalent diameters, which refer to a certain geometric or physical property of the material. Consequently, a clear choice of the most relevant particle size is necessary to judge the value and applicability of the various existing measurement methods. In fact, the definition of the term 'particle' most recently being discussed at ISO/TC 24/SC4 (the ISO SC responsible for particle characterisation) is the following: 'linear dimension of a particle determined by a specified measurement method and under specified measurement conditions'. This (draft) definition not only indicates that there is a variety of ways to interpret or assess particle size, it also reminds of the impact of the surroundings, in which a particle finds itself, on the measured particle size.

Conclusion: Implementation of the definition requires measuring a suitable, commonly agreed characteristic external particle dimension under defined conditions and comparing the result with the limit values set in the definition (1 nm and 100 nm).

2.4 Particle size distribution

The *definition* implicitly acknowledges that a nanomaterial does not consist of one nanoscale particle but of a collection of particles – hence the importance of the notion 'size distribution'.

2.4.1. Particle size distribution to describe polydispersity

An important characteristic of materials consisting of a collection (or 'population') of particles is their polydispersity. A *monodisperse* material consists only of particles of the same size and shape. A material consisting of particles is, to a certain degree, always *polydisperse*: it contains particles of various sizes. How the sizes of the individual particles vary is described by the *particle size distribution*. The most direct way of determining a particle size distribution is to measure a particular kind of size or dimension of all particles and to draw a diagram showing how many particles have sizes for example, between 0 nm and 10 nm, between 10 nm and 20 nm, and so on. The number of particles in each 'size bin' is depicted as a rectangle with a width equal to the width of the size bin and an area proportional to the number of particles in each size bin. Together, these rectangles form a histogram. The result is a particle number-weighted particle

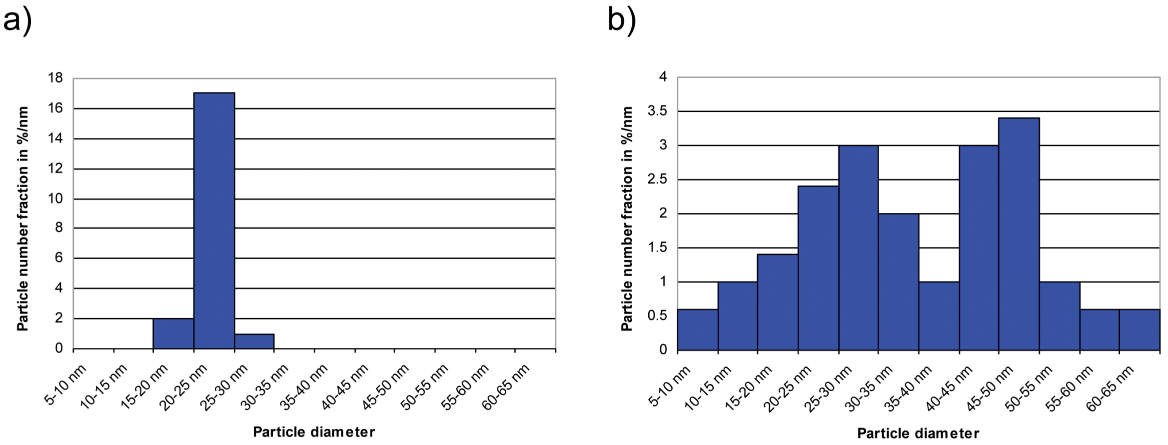


Figure 4: Histograms representing the number-based size distribution of a): a nearly monodisperse and b): a polydisperse material

Note: The column areas correspond with the percentage of particles which have a size (here: diameter) in the given size range. The polydisperse material contains particles with a wide range of diameters, and is also bimodal, i.e. the size distribution has two peaks.

size distribution. Depending on the selected size measuring method, the size distribution can be based on another type of quantity (not the number of particles per size bin, but the sum of the volumes of all particles per size bin, or their surface area, or their scattering intensity; see Section 3.3.1 for more details).

Fictitious examples of number-weighted size distributions of a monodisperse and a polydisperse material, respectively, for which the size (here: diameter) of a certain number of particles was measured, are shown in Figure 4. By making the width of the bins smaller, and by counting more particles, the histograms are turned into smoother, almost continuous particle size distribution curves.

2.4.2. Characteristic parameters of a particle size distribution

A particle size distribution can be characterised by various parameters.

- The modal size value: the most frequently occurring value of the chosen kind of size (i.e. the highest bar in the histogram). In Figure 4b, the modal value would be in the range 45 nm to 50 nm.
- The median size value: the size value for which equal numbers of particles are smaller and larger. The median value in Figure 4b is in the range 30 nm to 35 nm.
- A mean size value, i.e. an average of all measured sizes: several averaging methods exist (see [11]) and the resulting mean values can differ significantly. For example, the geometric mean gives a higher weight to smaller values than the arithmetic mean.

For materials where the particle size distribution is monomodal and symmetric on a linear size axis, the modal, median and arithmetic mean values coincide, but few real-world materials have symmetric particle size distributions, and certainly not on a linear size axis ([7]).

According to the definition, materials where *for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm* are classified as nanomaterials. One can rephrase this definition in terms of the median value: materials with a median particle size between 1 nm and 100 nm are classified as nanomaterials. This rephrasing is only incorrect if the material contains a sizeable fraction of particles with external dimensions < 1 nm. However, in practice, particles are larger than 1 nm. In fact, ISO has introduced the 1 nm limit in its definition of nanoscale to indicate its desire to separate particles from single molecules. Therefore, the discussion about objects smaller than 1 nm is a conceptual issue (are they still particles?) rather

---

[7] Note that broad size distributions are usually represented on a logarithmic size axis.

than a measurement issue (is their size smaller or larger than 1 nm?). In any case, the most relevant parameter of the three mentioned parameters of the size distribution (modal, median and mean value) for the definition is the median value ([8]).

Conclusion: Implementation of the definition requires the determination of the median value of the number-based size distribution of a material. The median value must be determined from the size values of the constituent particles from 1 nm to several µm.

Measurement of the median value of the particle size distribution requires counting all particles with sizes ranging from 1 nm to potentially several mm, and determining the fraction that have an external dimension within the specified size range. In practice, the number of particles on the mm scale will usually not be significant in comparison to the number of particles on the nanoscale. Therefore, the upper size limit that needs to be considered is presumably some µm. On the other hand, as will be illustrated in Section 4, most methods have either a lower measurement limit higher than 1 nm, or at least they have a tendency to be selective and detect a smaller fraction of the smaller particles in a polydisperse material. This creates a bias towards larger median particle size values. While it is possible to use these methods to prove that a material is a nanomaterial, it is not possible to use these methods to prove that the material is not a nanomaterial.

Conclusion: Methods having a detection limit higher than 1 nm or a lower sensitivity for smaller particles can only be used for a positive test (to prove that a material is a nanomaterial) but not for a negative one (to prove that a material is not a nanomaterial).

2.5 Fullerenes, graphene flakes and single-wall carbon nanotubes

Fullerenes, graphene flakes and single-wall carbon nanotubes are carbon or carbon-based materials of various sizes and shapes [12]. The *definition* explicitly includes them as nanomaterials, even if they are often smaller than 1 nm along their most nano-characteristic dimension.

The focus of this report is on the issue of particle size measurement. Therefore, the report does not list or discuss the variety of methods that can identify fullerenes, graphene flakes and single-wall carbon nanotubes. The reader is referred to, for example, the work of ISO/TC 229 Joint Working Group 2, which is developing a series of measurement standards for this group of materials.

Conclusion: Implementation of the definition requires testing whether the material in question consists of fullerenes, graphene flakes or single-wall carbon nanotubes.

2.6 Specific surface area

Small particles have a high specific surface area (SSA). Therefore, as an alternative implementation route, the *definition* specifies that materials with a volume-specific surface area (VSSA) larger than 60 m^2/cm^3 shall be considered as nanomaterials. The proposed threshold value is based on the VSSA of a material consisting of idealised (spherical and non-porous) particles of 100 nm diameter.

In the *definition*, the VSSA can be used as a positive test (to qualify) but not as a negative test (to disqualify a material as nanomaterial): a material with 50 % of constituent particles < 100 nm is a nanomaterial, even if the VSSA is < 60 m^2/cm^3. This is of particular relevance for aggregates of nanoparticles, for which the constituent particles contribute to the nano-fraction in the size distribution, but less to the material's VSSA.

The most frequently used method to determine the SSA is the BET (Brunauer-Emmett-Teller) method, which is used on dry powders (see also Section 4.9). Similar, but less mature methods exist to measure the surface area of suspended particles (see also Section 4.10). Most of these methods, both for dry and suspended powders, share a common approach: they measure an amount of another material (adsorbant) that is adsorbed on the surface of the particles in question. Different adsorbants are used (gases and liquids), with larger or smaller own sizes, and different idealised assumptions have to be made, for example about the thickness of the adsorbed layers, lateral interactions and the reversibility of the adsorption. Therefore, various SSA measurement methods can give different results for the same material and the parameters SSA and VSSA are method-defined, as is particle size (see Section 2.3 and, for more details, Section 3.3.2).

---

[8] Choosing the term 'median value' as short for '50 % threshold in the number base size distribution' may not be misunderstood as a means to avoid the discussion of other threshold values (instead of 50 %). In general, lower threshold values (for example, 10 %) will be (even) more difficult to assess than the median value.

An additional issue is related to the calculation of VSSA from the mass-specific surface area, which is the usual measurand of the available SSA techniques (such as BET). To calculate VSSA from SSA, one needs the value of the density of the measured nanomaterial. In general, the density of the nanoform is different from the density of the bulk form, and this difference can be significant. Unfortunately, the methods for the measurement of nanomaterial density (such as helium or air pycnometry) have similar limitations as the SSA measurements themselves.

Conclusion: the definition states that the volume-specific surface area can be used to positively classify a material as a nanomaterial. A clear definition of the measurement method (preferably an international standard) for the measurement of specific surface area is necessary to implement this aspect of the definition.

3. Generic reliability issues in particle size analysis

Inappropriate measurement procedures can produce incorrect particle size distribution curves: they can be shifted or broadened, or more generally deformed. These effects, which can also occur in combinations, are schematically illustrated in Figure 5. The underlying errors can stem from any of the major steps of a typical measurement procedure (sampling, sample preparation and quantification), which are discussed in this section. All of the effects shown in Figure 5 may result in incorrect classifications of a material as being a nanomaterial or not. A particularly relevant case is e) where particles above or below a certain size are not captured by the method anymore. This is likely to occur in practice, as no single method can reliably cover the complete size range, from 1 nm to several micrometres, certainly not within a single measurement.

This section will also introduce the concepts of 'equivalent particle diameter' and discuss in more detail the previously mentioned issues of particle size distributions and aggregates and agglomerates. Clarification of these issues is necessary to understand what should be measured. After clarification of the goal of the measurement, general quality assurance principles must be followed to ensure reliability of measurement results.

3.1 Sampling

Producers and regulators will have to assess whether a certain production batch, which can consist of tonnes of material, fulfils the *definition*. Subjecting this amount of material to the measurement procedure is impossible. Instead, small samples will be taken to make this assessment. To be meaningful, it is important to ensure that this small sample of for example 1 mg has on average the same properties as the complete production batch of 10 tonnes. Only if this is the case, is the sample representative.

Taking a representative sample ensures that the measurement result obtained on the sample applies to the complete batch. To achieve this, several small portions are usually taken from different positions of the batch and are combined into one sample. This is needed to eliminate, for example, the effect of particle size-related segregation or agglomeration that can be caused by transport. Guidelines for

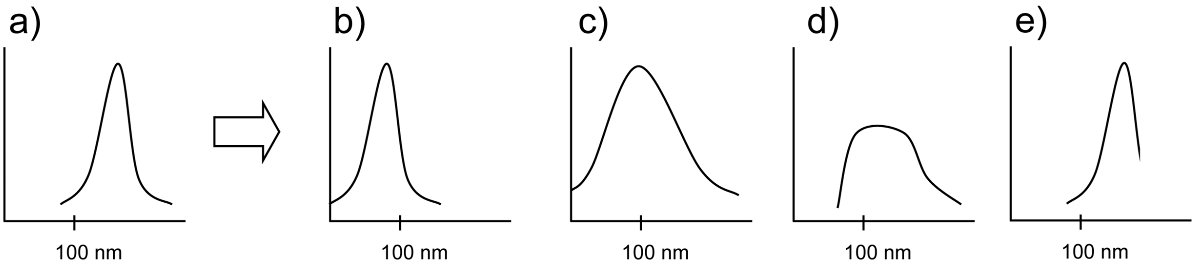


Figure 5: Possible modes of incorrect measurement responses

Note: The real particle size distribution (a) is shifted towards smaller particles (b), broadened (c), deformed or (d) cut at the upper size limit of the method (e). Note that b) and c) mean that the material (which is not a nanomaterial) is incorrectly classified as nanomaterial (the same effect can occur with e). Naturally, the opposite effect is possible as well.

taking representative samples are given, for instance, in ISO 14488:2007 [13].

A second 'sampling' step may occur in the actual measurement or 'quantification' step (see Section 3.3), depending on the type of method. Among the candidate measurement methods listed in Chapter 4, one can distinguish methods that investigate individual particles ('counting methods') and methods that analyse large numbers of particles simultaneously ('ensemble methods'). This means a limited number of the particles in the test sample are selected for counting methods. Conversely, ensemble methods allow the investigation of much larger numbers of particles, many orders of magnitude larger than for the counting methods.

Conclusion: Obtaining a representative sample is critical for meaningful measurement results. Therefore, sampling needs to be done in a well designed and controlled manner, usually according to a prescribed protocol.

3.2 Sample preparation

As indicated in the previous section, and as shown in Chapter 4, the candidate measurement methods are typically 'off-line' methods ([9]). In other words, after sampling, the representative sample (powder or suspension) must be transferred to the laboratory where the measurements are performed. Depending on the chosen measurement method, the sample will require additional treatment before it is fit for analysis. Frequently used treatments in the field of particle size distribution measurements are suspending, diluting, drying and dispersing.

3.2.1. Suspending, diluting and drying

Many size measurement methods cannot analyse powders directly. Therefore, powders may need to be suspended in a suitable medium to make their particle size distribution measurable. This suspension of particles may lead to partial dissolution of particles, or swelling of particles, or both, thus changing the initial size distribution of the sample to be tested.

Also, the working range of many size measurement methods is limited in terms of the suspended particle concentration. Most methods cannot deal with the concentrated particle suspensions typical for industrial production, and dilution is required to decrease this concentration.

Suspension and dilution are both likely to affect the agglomeration state of the powders. This is due to the fact that agglomeration is controlled by surface energies and forces which are extremely sensitive to changes in the particle's environment.

While some measurement methods require suspended particles, other methods require dry particles. Drying a droplet of suspension can lead to changes in the size of the constituent particles, to agglomeration or aggregation. For methods which do not directly assess the size of constituent particles, the latter means that the measured particle size is different from the one in the original sample.

---

[9] Because of the additional challenges associated with on-line measurements, the few existing on-line measurement methods are not discussed in this report. Nevertheless, they are relevant, for example for quality control of production processes.

3.2.2. Dispersing

In their powder form, all (particulate) nanomaterials are agglomerated or aggregated. Free constituent nanoparticles only exist in stabilising suspensions or in very low concentrated aerosols, but not in powders. Most particle size analysis methods measure a size corresponding with the external dimensions of the particles in the sample and disregard the internal particle structure, irrespective of whether the particles are aggregates or agglomerates, or single constituent particles ([10]). As the definition requires size determination of the constituent particles, most measurement methods will require that the aggregates or agglomerates are decomposed into their constituent particles in the sample preparation step prior to measurement.

The process of breaking up the weakly-bound *agglomerates* into smaller particles is called dispersion. To prevent dispersed particles from re-agglomerating, the dispersion process typically is done in a stabilising suspension medium. The dispersion process requires energy input, in the simplest case by shaking the suspension. Generally speaking, the higher the amount of energy put into a suspension, the more agglomerates will break up, but exceptions to this rule have been reported [14]. Currently, the most common way to break up agglomerated particles in an analytical sample preparation step is the application of ultrasound to the particle suspension. The amount of ultrasound (intensity, duration) needed to break up the agglomerates depends on the type of particle in question and on the suspending medium, as well as on other instrumental parameters [15]. Alternatively, dispersion can be achieved through a change of the chemical environment in which the agglomerates are kept (pH, concentration of dispersants, etc.).

The energy of ultrasound is often sufficient to break the weak binding forces of agglomerates, but it is insufficient to break the stronger binding forces of aggregates. Also, it is virtually impossible to de-aggregate aggregates by chemical treatment without affecting the size of the constituent particles. In fact, the term ‘aggregate’ is sometimes defined as ‘smallest dispersible unit’ [16, 17, 18]. This means agglomerates can be dispersed into non-agglomerated aggregates, but the aggregates cannot be dispersed further. Actually, a too high energy input may break up the aggregate’s constituent particles, thus creating nanoparticles which did not exist in the original material.

Conclusion: Sample preparation steps are often essential to obtaining a physical state of the particles suitable for their size measurements, but also have the potential to introduce artefacts that lead to wrong conclusions. Careful control of these steps is therefore necessary to ensure reliable results. Most size measurement methods require a dispersion procedure that results in breaking up agglomerates into the relevant constituent particles. Aggregates cannot be broken up and most size measurement methods count them as one larger particle.

3.3 Quantification

The quantification step in the size measurement process can be realised by various approaches, as illustrated in more detail in Chapter 4. Quantification is possible if the sample creates a signal that is related to the size and number of the particles it contains. This section describes the main generic issues associated with the quantification step, and outlines how counting methods, ensemble methods and fractionation methods are generally affected by these issues.

3.3.1. Differently weighted particle size distributions

The *definition* specifies that the particle-number based particle size distribution of primary/constituent particles is the basis for defining nanomaterials ([11]). This means measurement methods need to be able to determine number size distributions.

Counting methods, creating separate signals for each measured particle, directly produce number size distributions. *Ensemble methods*, however, detect and analyse a collective signal that stems from all particles in the sampled volume. The size distributions deduced from such data rely on a deconvolution step in the analysis algorithm, which constitutes an ill-defined mathematical problem. The distributions obtained by counting methods as well as ensemble methods are not necessarily the desired *size* distributions but may be distributions of properties related to size. This may be an advantage in terms of information content, but it is associated with disadvantages. For example, the contributions from individual particles to the signal may vary strongly with particle size. Typically, the part of the signal intensity produced by the smaller

---

[10] Exceptions are X-ray diffraction and, to some extent, transmission electron microscopy; see Chapter 4.

[11] As in the definition, this report will further use the term ‘number size distribution’ as shorthand for the correct term ‘particle-number based particle size distribution’.

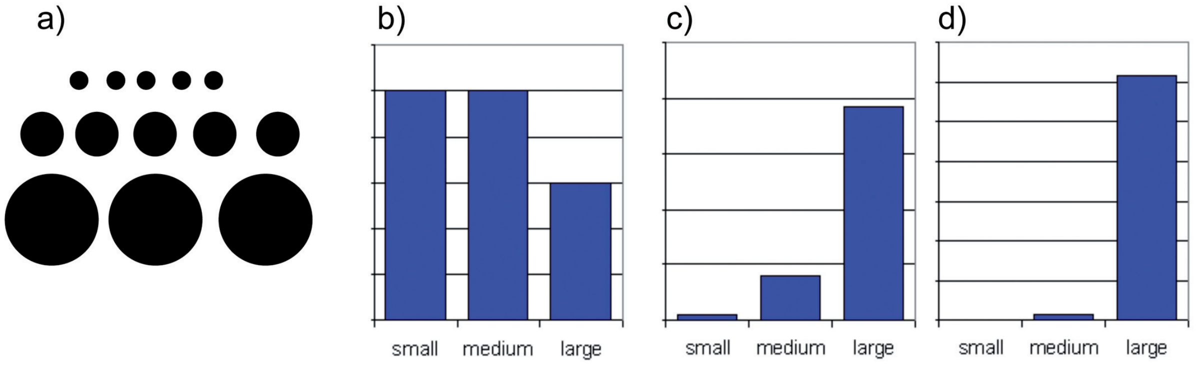
particles in the population will be much smaller, or even negligible, in comparison to the contributions from larger particles. This problem is at least partly solved by the *fractionation methods*, which collect signals corresponding with specific size fractions of the sample. This facilitates the interpretation of the measured results, as the relation between signal intensity and size of a monodisperse sample population is often relatively well understood. This relation may depend on physical properties of the particles and the suspending medium (such as density or refractive index). If the corresponding material data are available, then fractionation methods can produce the required number size distribution.

Nevertheless, for the above mentioned reasons (absence of additional material data such as density or refractive index, or the negligible contribution of smaller particles to the measured signal, effect of shape, etc.), conversion of the raw signals into volume or number size distributions is prone to error. For this reason, the quantitative results of particle size distribution measurements are often expressed as 'intensity-weighted' ([12]) size distributions, which are direct representations of the 'raw' signals and use a minimum of additional assumptions about the particles.

It is not possible to judge a material's compliance with the *definition* from distributions other than number size distributions. It is therefore tempting to develop methods which transform the different kinds of size distribution into the number size distribution. Currently, this is only possible for particles having a perfectly spherical shape, or otherwise simple geometrical and well known shape, and for ideal measurements, which correctly detect the distribution in the respective type of quantity. In all other cases, conversion is cumbersome, and the broader a size distribution, the larger the errors created by conversion.

Depending on the measurement method and the specific size distribution involved, a homogeneous density within and between particles of the same and different sizes may also be required. Assuming that this would be the case, and only for illustrative purposes, Figure 6 shows how the shape of the size distributions may differ depending on the basis of size distribution. For number size distributions, each particle counts equally, whereas for example for volume and mass-based distributions, larger particles have a larger weight. In the example of Figure 6, the raw signal is proportional to the volume of the particle, which is proportional to the cube of the particle diameter. Other methods create distributions weighted by intensity of scattered light, which is proportional to the diameter to the power of 6 (if the diameter of a particle increases by a factor 10, the intensity increases by a factor 1 000 000). This means that number-, mass- and signal intensity-based size distributions of the same material differ significantly, as shown in Figure 6. It is therefore expected that the working ranges of the candidate measurement methods, in terms of particle number fractions, will strongly depend on particle mass fractions: if the mass of one or more fractions in the particle size distribution is not large enough, the particles in this fraction will not be detected, nor counted.

Figure 6: Different size distributions for the highly idealised case of a population of perfectly spherical particles all with the same density; a) population consisting of 5 small (radius = 1), 5 medium (radius = 2) and 3 large particles (radius = 4); b) number-based histogram; c) volume- or mass-based histogram; d) signal intensity-based histogram



---

[12] In this report, the term 'intensity weighted' refers to weighting by light scattering intensity, unless specified otherwise.

3.3.2. Method-defined equivalent diameter

Many of the particle size measurement issues at the nanoscale do not differ from those at the macroscale. While the methods for size measurement are different, the generic problems of defining and measuring a particle size apply in the same way to gravel as they apply to nanoparticles. Several concepts developed for larger particles can immediately be applied to measurements at the nanoscale. One of the crucial concepts is that of the 'equivalent diameter' (see for example [19] for more details and other generic particle size analysis issues).

Equivalent diameter: diameter of the sphere that, for a particular, measurable aspect of its behaviour, has the same property as the particle in question.

Figure 7 illustrates the concept of equivalent diameter. In Figure 7a, particle size is measured from the 2D electron image of the particle (i.e. the projected area or *shadow* of the particle). The equivalent area particle size is the diameter of a circle with the same area as the particle's projected area. In Figure 7b, the particle size is determined from its rotational speed. The equivalent particle size is the size of a particle that has the same moment of rotational inertia or radius of gyration. In Figure 7c, particle size is deduced from the sedimentation velocity of the particle in a certain medium. The equivalent particle size is the diameter of a sphere that sediments at the same velocity. Figure 7 illustrates that various particle size analysis methods will give different equivalent particle diameters, even when measuring the same particle(s).

It has been suggested that electron microscopy (EM) (in particular, transmission electron microscopy (TEM)) provides the most accurate particle size values. This, however, relies on the assumption that a projected area equivalent size is the 'true size'. Unfortunately, this size is deduced from a 2D image and is independent of the thickness of the particle in the third dimension. A second disadvantage is that it does not correspond to common understanding: the area equivalent size of a highly branched particle may be very small, whereas it can extend widely, reaching a large external dimension.

It is important to note that all measurement methods relevant for the implementation of the *definition* produce one or another equivalent diameter. This is not a unique situation and the phenomenon of equivalent or apparent property values is well known in metrology. In metrological terms: particle size measurements produce *method-defined* results. Therefore, the applied measurement method (including critical method parameters and evaluation algorithms) must be stated together with a reported particle size value to make the result meaningful. This recommended practice

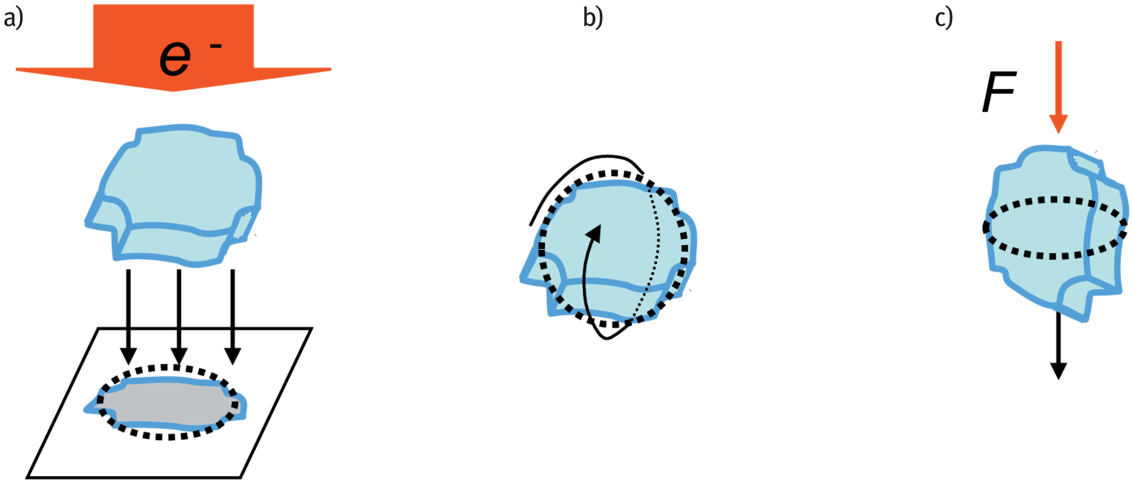


Figure 7: Illustration of the concept of equivalent diameter: a) diameter of a circle causing the equivalent electron-shadow area; b) diameter of a sphere that has the same inertia of rotation; c) diameter of a sphere that would sediment due to a gravitational force F with the same speed as the particle

Note: All three diameters are different as they correspond to different aspects of the particle behaviour and properties.

is not unique to nanomaterials, but applies to all particle size measurements, as already suggested in the previously quoted particle size definition being developed at ISO (see Section 2.3).

Interestingly, for particular aspects of the particle behaviour, one equivalent diameter may be more relevant than the other. Therefore, a measurement method should not only be selected because of its availability or ease-of-use, but also bearing in mind the intended use of the measured values.

Conclusion: Particle size values are method-defined and usually refer to an apparent or equivalent diameter of a virtual spherical particle with similar behaviour as the actual particle. Values of different equivalent diameters obtained on the same particle(s) are likely to diverge.

3.4 Quality assurance and confidence in particle size measurements

The previous Sections 3.1 to 3.3 highlight major generic issues regarding the reliability and comparability of particle size values, with relevance for the implementation of the *definition*. The systematic investigation of the performance characteristics and reliability of a measurement method is called method validation. This cornerstone of metrological quality assurance and confidence in measurements is briefly described in this section.

3.4.1. Method validation

Slight intentional variations of any of the steps of a measurement procedure may result in more or less different results. In fact, even repeating a measurement without intentionally varying the measurement procedure will usually not lead to exactly the same result, even if the sample is the same. In addition, something important may have been overlooked so that the method gives wrong or *biased* results. Methods need to be validated to demonstrate the absence of significant bias and to quantitatively assess the variation of results.

Method validation thus refers to the process of verifying that 'specified requirements are adequate for an intended use' [20], thereby establishing the performance of a method. It is important to recognise the need to be specific about the intended use. For the implementation of the *definition*, this intended use is very specific and the question asked in the method validation is straightforward:

How well can the method determine the number fraction of particles that have at least one dimension in the range from 1 nm to 100 nm for the constituent particles in a powder or suspension of particles?

Usual validation parameters are:

- precision: measure of the variation of results on the same sample;
- trueness: measure of how much the average of many repeated measurements differs from the true value. The difference between the true value and the average is often called bias;
- robustness: measure of the effect of controlled variation in measurement conditions on the result;
- working range: range of possible measured values for which the method has been shown to be reliable. For the implementation of the *definition*, the working range has two aspects: one of measurable size (1 nm to 100 nm) and one of measurable fraction (50 % in the number size distribution);
- limit of detection: smallest signal that a method can still detect;
- limit of quantification: smallest signal that can reliably be quantified.

Until today, none of the available methods that will be mentioned in Chapter 4 have been thoroughly validated specifically for their use in the implementation of the *definition*. This method validation is urgently needed. In particular, it is expected that most methods will reveal limited working ranges, especially near the lower size range, similar to the methods used for aerosol measurements, for which method validations have been performed under regulatory pressure (see for example [21]).

Conclusion: Systematic and documented method validation is a necessary activity to ensure and demonstrate that a method is reliable and adequate.

3.4.2. Measurement uncertainty

With the information collected during method validation, it is possible to estimate the measurement uncertainty for a specific measurement. This measurement uncertainty quantifies the reliability of a given measurement result. The measurement uncertainty gives a range around a measured value in which the true value lies with a defined (usually high) probability, for example:

(30 ± 5) % of particles have a size between 1 nm and 100 nm.

In this case the measured value (here 30 %) has a measurement uncertainty of 5 %. The analyst therefore states that she is sure that between 25 % and 35 % of the particles have a size between 1 nm and 100 nm. These statements are made for a certain degree of sureness ('confidence level'), which is usually set to 95 %, meaning that when doing 100 such assessments, the analyst would be wrong in only 5 % of the cases.

Conclusion: Measurement uncertainty values are the quantitative expression of the reliability of the results of a validated measurement method.

3.4.3. Compliance assessment

Measurement uncertainties are crucial to assess compliance of a material or product with contractually defined specifications or legally required limit values, as shown in Figure 8.

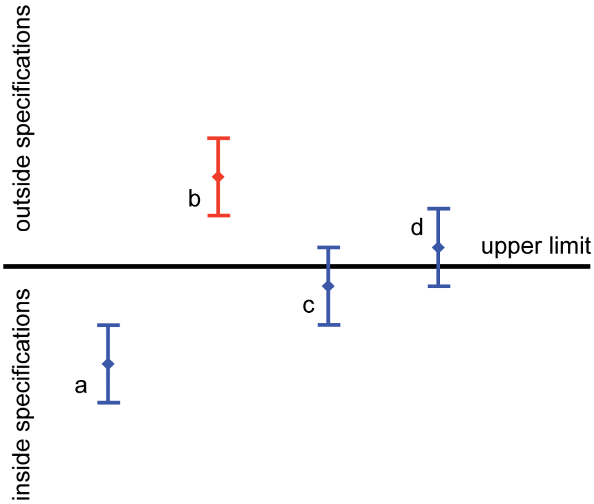


Figure 8: Measurement uncertainty and compliance testing

Note: a) demonstrated to be below the legal limit; b) demonstrated to be above the legal limit; c, d) Measurement gives a certain probability to be above/below the legal limit.

As shown in Figure 8c and Figure 8d, there are cases where it cannot be unequivocally decided whether a result is above or below a legal limit. In this case, the regulator has to specify if compliance or violation has to be demonstrated. In Figure 8, only case a) would demonstrate a level below the legal limit, whereas only case b) would demonstrate a level above the legal limit. The larger the measurement uncertainty, the more the measured value must be above or below a limit to be able to reliably demonstrate compliance or violation. For example, the result '(54 ± 3) % of particles are between 1 nm and 100 nm' demonstrates that a product is a nanomaterial according to the *definition*, but a result '(53 ± 8) %' does not. It follows that, for regulatory limit levels, it is recommended to also specify maximum acceptable measurement uncertainty levels.

Conclusion: Estimation of measurement uncertainty is a prerequisite for the assessment of compliance/non-compliance with the definition. Measurement results without statement of measurement uncertainties are meaningless, certainly in a legal context.

3.4.4. The role of standardisation and reference methods

Standardised methods are essential for method-defined parameters such as particle size. As the parameter 'particle size' is defined by the method, different methods will usually give different results. Standardisation ensures that everybody uses the same method in the same way and hence measures the same physical property. Results obtained by the same standardised method on the same sample should agree within their respective measurement uncertainties. In this way, standardisation ensures comparability of measurement results.

'Commonly agreed methods' are methods that, while not being standardised, have emerged as the usual approach for a certain measurement problem. While such formal or informal agreement solves to some extent the problem of different results obtained by different methods, these methods are often not tested for their reliability in the way standardised methods are. This means the reliability of commonly agreed methods is often not well known and such methods are clearly inferior to standardised methods.

'Reference methods' are either methods of higher accuracy than normally achieved with routine methods, or methods prescribed as reference in a legal context. Results from such methods therefore act as reference for other methods. However, as the parameters for implementing the *definition* are method-defined, reference methods are by definition impossible: any decision to define a method as 'reference' would not improve comparability of measurement results across methods and would exclude the use of other methods.

Conclusion: Because particle size is a method-defined property, standardisation of size measurement methods is a necessity to obtain values that are comparable between different laboratories.

4. Evaluation of specific measurement methods

Several scientific reviews of methods for the characterisation of nanoparticles have been published over the last few years (see for example [22]). The following section of this report is different, as it very specifically addresses the question whether measurement methods currently available to determine the size of particles in the range between 1 nm and 100 nm are suitable for the implementation of the *definition*. For each method, a short description of the measurement principle is given, followed by a discussion on whether it can in principle fulfil the measurement requirements of the *definition* and for which nanomaterials, what the main reliability issues are, whether standard methods are published, the availability and cost of the method, and prospects for further development of the technique.

4.1 Electron Microscopy (EM)

4.1.1. How does it work?

Electron microscopy (EM) uses electron beams to visualise structures that are not visible to the naked eye, or not even with optical microscopy. In scanning electron microscopy (SEM), images are constructed based on electrons coming from the sample surface. In transmission electron microscopy (TEM), images are constructed based on electrons passing through (thin sections of) the sample. Both SEM and TEM, in their basic form, produce 2D projections of a 3D particle. From such images a multitude of particle size values can be obtained, typically giving a value somewhere between that of the diameter of the smallest circle in which the particle image fits, and the diameter of the smallest circle fitting in the particle image. An example of an often used intermediate size parameter is that of the diameter of a circle having the same area as the 2D image of the particle (see ISO 9276 series of documents for details [23]).

4.1.2. Can it measure nanoparticles according to the definition?

EM is a counting method: it produces a size value for each of the particles selected for analysis on the obtained images. Hence, EM can be used to construct the required number-based size distributions. TEM has the required resolution to determine the smallest external dimension of nanoparticles. SEM is limited to particles above some nm.

EM can deal, to a limited extent, with the issue of agglomeration and aggregation because it can reveal the structure of these groups of particles. However, the measurement of the size of the constituent particles inside aggregates or agglomerates necessarily relies on extensive operator intervention and interpretation of the obtained images (see Figure 1). This excludes the use of automated image analysis which is so needed to obtain a sufficient number of particles to construct a reliable particle size distribution.

4.1.3. Which type of nanomaterials can be measured?

Most EM is performed in high-vacuum chambers, implying that the measurements are done on dry powders. Under these conditions, the use of EM is limited to the analysis of particles which are not affected by the combined action of high vacuum and electron beams. This can be a problem for organic particles or particles with an organic surface coating. Advanced EM techniques (for example. cryo-TEM and environmental SEM) are available for these applications.

In theory, EM can deal with polydispersity because it evaluates the size of individual particles, from which the size distribution can be constructed, particle by particle. It is noted, however, that the bandwidth of EM is limited: it is difficult to obtain images that can capture both the larger and the smaller particles in a material with a broad size distribution.

In addition to the size, EM allows assessment of the morphology of particles by the shape of their projections. However, the analysis of flattened particles (platelets, flakes) can be problematic because they preferentially align on the sample substrate or grid: it is very likely that the smallest axis of the particle is hidden, and the measured size is an overestimation of the size relevant for the *definition*.

For reasons of image contrast, EM is most suitable for particles containing heavier atoms. EM has problems detecting low density, organic nanoparticles and measuring particles made of an inorganic core and an organic outer shell. In the latter case, EM may only measure the metallic core and may therefore incorrectly conclude that a particle is in the nanoscale.

For well dispersed materials, the EM measurement process can be automated. For all other materials, EM is a slow technique. Operator intervention is especially required for aggregated materials, if the analysis of the constituent particles in these materials is possible at all (see also Section 2.2.2).

4.1.4. How reliable is it?

Very few laboratories have performed a full validation of EM as a technique to measure the size of nanomaterials. Those that did limited the validation to near-spherical and relatively large particles. As a result, most particle size values obtained with EM are reported without a reliable measurement uncertainty. The main issues in validation of EM for nanomaterial size analysis are the following:

Robustness: to some extent, EM for nanomaterial size analysis is a robust method, and it is possible to study the effect of varying instrument parameters (acceleration voltage, magnification, etc.). However, the image analysis step in EM on aggregated materials is susceptible to operator dependence.

Precision: if sample preparation is performed in accordance with a detailed protocol, then EM measurements of the median value of a particle size distribution have the required precision to be well repeatable in one laboratory over a short period of time.

Reproducibility: EM is a widely used technique, with a large operator community. Nevertheless, reproducibility between laboratories is shown to be acceptable only for spherical, monodisperse nanomaterials [24]. Reproducibility for more polydisperse or non-spherical materials would require a very detailed description of the sample preparation method and of the size parameter to be determined from the images.

Selectivity: it is unlikely that for materials with a high polydispersity the smaller and the larger particles have an equal chance of contributing to the measurement result, considering the full measurement process (from sample preparation to detection and selection of particles for analysis on the images). For example, since large agglomerates tend to detach from the EM grid, the particles that remain attached to the grid are not representative for the test sample [25].

Trueness: as for all other methods for nanomaterial size analysis, it is difficult to judge the trueness of the EM method because of the absence of reference materials. Among the above mentioned validation themes, especially the selectivity issue may affect the trueness.

To avoid the introduction of operator-dependent bias, a routine for systematic random sampling should be applied to select the particles to be measured. Without such a system the outcome of the measurement is somewhat subjective. This may be important, as one operator may focus on the small particles thus incorrectly ‘creating’ a nanomaterial, whereas another may focus on the bigger particles, thus making a nanomaterial ‘non-nano’.

4.1.5. Are standard methods available?

The basic operation of electron microscopes is well described. ISO/TC 202 developed ISO 29301:2010 [26] on the calibration of the image magnification. ISO/TC 24/SC 4 released the already mentioned ISO 9276 [23] series of standards, describing size features of particles, as well as ISO 13322-1:2004 [10], describing how to best obtain values for these size features through static image analysis. There is, however, no standard describing the particularities of preparing samples of nanomaterials for reliable particle size analysis by EM.

4.1.6. Are reference materials available?

There are no reference nanomaterials with a certified EM-based median particle size or particle size distribution in the nanometre range. There are only a number of certified reference materials (CRMs) consisting of monodisperse nearly-spherical particles for which an EM-based modal or mean diameter is certified (for example, ERM-FD100, a CRM of the European Reference Materials (ERM®) series [24]).

4.1.7. Is it widely available at acceptable costs?

SEM instruments are more widely available than TEM instruments, and both of them are significantly more expensive than most other instruments presented in this report. With regards to TEM, sizing of dispersed particles, which can be achieved by a basic instrument, is usually not the core task of a TEM. Therefore, most TEM measurements tend to be performed on instruments that are over sophisticated for this task and therefore complex to operate as well as expensive.

When it comes to the analysis of the required large number (hundreds or thousands) of particles to construct a reliable size distribution, both SEM and TEM are only time and cost efficient if the analysis of the images can be automated.

4.1.8. What are the prospects for further development of the technique in the near future?

Integration of hardware (SEM and TEM control) and software (image analysis) will improve automation of imaging, detection and analysis of particles, and report generation.

Development of larger charge-coupled device (CCD) cameras will increase the useful range of TEM.

The disadvantage of operating in high-vacuum chambers is solved in microscopes allowing creation of a low-vacuum atmosphere around the sample that is compatible with the electron investigations. However, this is achieved only at the expense of instrument resolution.

The use of stereoscopy or even electron tomography can solve the problem of judging 3D particles from 2D images. Whereas the principles of these techniques do exist, they cannot yet produce routine measurements for large numbers of particles. Electron tomography can also produce values for the volumetric specific surface area (SSA).

4.2 Dynamic light scattering (DLS)

4.2.1. How does it work?

Dynamic light scattering (DLS) measures the hydrodynamic diameter of a particle. This is the diameter that determines how fast a particle moves in a suspension through Brownian motion. To measure this, DLS uses a laser beam, which, when passing through the suspension, is scattered by the nanoparticles. The Brownian motion of particles causes fluctuations in the intensity of the scattered light around a mean value. In most DLS instruments, the autocorrelation function of the fluctuations is recorded as a function of time. Size of the particles can be obtained, for example, by fitting the autocorrelation function to an exponential decay function that delivers an average diffusion coefficient for the particles (cumulants method). This diffusion coefficient can be used to calculate an average diameter of the particles via the Stokes-Einstein relationship.

4.2.2. Can it measure nanoparticles according to the definition?

DLS, in its standardised form, does not produce particle size distributions, but a light-scattering intensity-weighted average value. In addition, a well known difficulty of DLS measurements is that the results are strongly biased in the presence of a small fraction of large particles. This is due to the fact that the intensity of the scattered light is inversely proportional to the sixth power of the radius of the nanoparticle. Thus a 50 nm particle will scatter 10^6 as much light as a 5 nm particle. As a result, average particle size values determined by DLS are biased. DLS does not distinguish between constituent particles and aggregates/agglomerates. It gives the size of one diffusing ensemble, regardless of whether this is an individual particle, agglomerate or aggregate.

4.2.3. Which type of nanomaterials can be measured?

DLS is performed in liquids. This limits the use of DLS to the analysis of particles which do not dissolve.

DLS performs well when dealing with monodisperse samples of suspended nanoparticles with a known refractive index and can measure nanoparticles in the 1 nm to 500 nm range, if these are present in a sufficient concentration (but not too high) and if they scatter light effectively. To be able to calculate the hydrodynamic diameter, the temperature and viscosity of the medium are needed.

Attempts have been made to also produce DLS-based particle size distributions. To account for polydispersity in a measured sample, the autocorrelation function must be fitted by a sum of functions, each corresponding to monodisperse nanoparticles of the same properties. Each function is used to capture the contribution of the particles from a particular size range to the signal measured. Even if specific algorithms have been developed, the fitting is an ill-posed mathematical problem since it contains more unknowns than equations. The results of the fitting procedure are strongly algorithm and fitting parameter dependent. In these conditions, DLS can give misleading results; for example it will not resolve particles of sizes that differ less than a factor of 3 in size. Also, it is only possible to convert the scattering-intensity–weighted size distributions to the required number size distribution in the case of spherical particles, with a known refractive index.

DLS cannot determine the size of the constituent particles of aggregates or agglomerates, unless these are dispersed into their constituent particles prior to the DLS measurement.

4.2.4. How reliable is it?

DLS reports scattering-intensity–weighted results. Conversion to number-weighted results requires strong assumptions about material shape and dispersity, which are usually not fulfilled in real samples. Therefore, DLS is only relevant for the implementation of the *definition* if the measurement step is preceded by a fractionation step in which particles of different sizes are separated (see Section 4.5). Within this narrowed-down playing field of monodisperse materials, the DLS cumulants method has been shown to be repeatable, robust and reproducible [24].

4.2.5. Are standard methods available?

An ISO standard (ISO 22412:2008 [27]) is available, but the detailed description of the evaluation algorithms is limited to the cumulants method, the results of which reduce a size distribution to only two descriptors: an average size and a polydispersity index.

4.2.6. Are reference materials available?

There are no reference nanomaterials with a certified DLS-based median particle size or particle size distribution in the nanometre range. Commercially available reference materials for DLS are monodisperse. Some certified materials are available, for example from the Institute for Reference Materials and Measurements of the Joint Research Centre (JRC-IRMM) and from the US National Institute of Standards and Technology (NIST), but the certified values are mean or modal values, rather than median values.

4.2.7. Is it widely available at acceptable costs?

Several instruments exist on the market. These instruments are easy to use and not very expensive.

4.2.8. What are the prospects for further development of the technique in the near future?

Instrument manufacturers are working on the detection scheme to improve the sensitivity and robustness of the measurements. This should allow measurements of nanoparticles at lower and at higher concentrations. The solution for the fundamental problem of deconvoluting the ensemble signal into a reliable particle size distribution, is being looked for in adding a fractionation step in the sample preparation (see also 4.5).

4.3 Centrifugal liquid sedimentation (CLS)

4.3.1. How does it work?

Centrifugal liquid sedimentation (CLS) is based on the simple principle that large particles sediment faster than small particles of the same density. Particulate nanomaterials are actually so small that they do not sediment through simple gravitation. However, their sedimentation can be induced by centrifugal forces, i.e. by rotating the liquid, pushing the suspended particles away from the rotation axis. The relation between measured sedimentation time and particle size is simple for spherical particles of a uniform and known density. The result of the calculation is an equivalent diameter, more specifically the particle's Stokes' diameter.

4.3.2. Can it measure nanoparticles according to the definition?

CLS does not usually produce number-based size distributions, and it does not measure the smallest external dimension of a particle. Therefore, it seems of limited use for the implementation of the *definition*, except for monodisperse, near-spherical particles. However, it has an important advantage over ensemble methods: it separates different size fractions in polydisperse materials prior to the detection/quantification step. This makes the obtained intensity-extinction–based size distributions more similar to the number-based size distributions than the scattering-intensity–based size distribution of, for example, DLS.

4.3.3. Which type of nanomaterials can be measured?

CLS is performed in liquids. This limits the use of CLS to the analysis of particles which do not dissolve.

In theory, CLS can deal with polydispersity because the sedimentation process naturally results in a fractionation of particles of different sizes. However, the bandwidth of CLS is limited: it is difficult to select a rotational velocity which is suitable for both the larger and the smaller particles in a material with a broad size distribution.

Deviation of particle shape from a spherical or equiaxial shape is a major problem: the measured diameters are based on the assumption of a spherical shape. Non-spherical particles can preferentially align in the rotating liquid, resulting in over- or underestimations of the size relevant for the *definition*.

For CLS, the particles need to be of a different (in practically all instruments: higher) density than the liquid. This can be a problem for low-density, organic particles. In addition, the density of the particles must be known and uniform, which is a problem for particles with a surface coating, and for porous particles and irregular particles such as aggregates and agglomerates, where the liquid penetrates the particle.

CLS cannot determine the size of the constituent particles of aggregates or agglomerates, unless these are dispersed into their constituent particles prior to the CLS measurement.

4.3.4. How reliable is it?

Very few laboratories have performed a full validation of CLS as a technique to measure the size of nanomaterials. Those that did, limited the validation to spherical particles [28]. As a result, very often particle size values obtained with CLS are reported without a reliable measurement uncertainty. The main issues in validation of CLS for nanomaterial size analysis are the following:

Robustness: CLS is a robust method for those nanomaterials for which CLS is suitable (spherical, known and uniform density), and for which the particle concentration is at the same time sufficiently high (to detect the particles) and sufficiently low (to avoid collective sedimentation of groups of particles). Deviations from these conditions (for example, samples containing particles of different composition and therefore different density) directly affect the reliability of the results.

Precision: CLS measurements of the median value of a particle size distribution have the required precision to be well repeatable (in one laboratory over a short period of time).

Reproducibility: CLS is a widely used technique, of which several forms exist. The most common types are disc centrifugation (or the line-start method) and analytical ultracentrifugation (or homogeneous sedimentation). So far, reproducibility between laboratories has only been demonstrated within the same type of CLS approach and only for spherical, monodisperse nanomaterials [26, 28].

Trueness: as with all other methods for nanomaterial size analysis, it is difficult to judge the trueness of the CLS method when measuring non-spherical particles. Also, the uncertainty about the particle density may affect the trueness.

4.3.5. Are standard methods available?

The basic principles of CLS methods are described in ISO 13318-1:2001 [29]. Details about the different available CLS methods are described in ISO 13318-2:2007 [30] and ISO 13318-3:2004 [31].

4.3.6. Are reference materials available?

There are no reference nanomaterials with a certified CLS-based median particle size or particle size distribution in the nanometre range. There are only a number of CRMs consisting of monodisperse, nearly-spherical particles for which a CLS-based mean diameter is certified [24].

4.3.7. Is it widely available at acceptable costs?

The operation of a CLS is rather straightforward. Instruments are available and their cost is acceptable as is sample preparation time. Sedimentation times may, however, be very long for light and small particles.

4.3.8. What are the prospects for further development of the technique in the near future?

It is not likely that the CLS method will develop into a method that can measure the smallest external dimension of particles. However, with improved detection systems and temperature control as well as increased rotational speeds, CLS will provide method-defined estimates of the apparent Stokes' diameter in an increasingly broad size and concentration range.

4.4 Small-angle X-ray scattering (SAXS)

4.4.1. How does it work?

SAXS measures the size of particles based on the scattering of X-rays at the particle surface. Light passing through a medium that is not completely homogeneous is forced to deviate from the straight line. The scattering angle, i.e. the degree of deviation from the straight line, depends on the wavelength of light and the size of the particles on which it is scattered. X-rays which have wavelengths roughly between 0.1 nm and 1 nm are scattered by particles in the range between 1 nm and 100 nm. The intensity of the scattered radiation is measured in dependence of the scattering angle. This intensity pattern can give information of the particle size distribution as well as the shape of particles. Size and shape of the particles are obtained via fitting of the measured scattering curve (scattering intensity versus scattering angle). Results are expressed as radii of spheres, cylinders or discs of equivalent scattering properties.

4.4.2. Can it measure nanoparticles according to the definition?

Different algorithms for calculation of particle size distributions from raw SAXS data have been described. For relatively monodisperse samples, oscillations are observed in the scattering curve. The periodicity is directly related to the mean particle diameter. By fitting the curve, number, volume and intensity, weighted mean diameters can be obtained.

Other algorithms like Guinier analysis produce in first instance scattering-intensity–based distributions, which over-represent large particles. By making a number of simplifying assumptions, the data can be converted into particle volume-based distributions and subsequently particle number-based distributions, but at the cost of decreased reliability of the results. As the scattering-intensity–based average diameter by SAXS is often 1.5 or 2 times larger than the particle number-based average diameter, average diameters below 100 nm clearly indicate a particle number-based median diameter of < 100 nm.

Also, the calculation of SSA per volume is in principle possible, but only for monodisperse samples.

4.4.3. Which type of nanomaterials can be measured?

SAXS can be applied to any two-phase system where the phases have different density (13). In such a system, SAXS measures the phase with the smaller volume fraction. This means that in a suspension it will measure the size of the particles. SAXS is applicable to suspensions, but not to powders as the volume fraction has to be below a few % to avoid particle-particle interaction. This limits the use of SAXS to the analysis of particles which do not dissolve.

---

[13] In theory, the two phases have to differ in electronic density, but this translates in most cases to different mass density.

The size and size distributions of particles with basic shapes (spheres, discs or cylinders) can be determined but only for relatively monodisperse samples (width of the size distribution < 20 % of the average particle size). Fitting broader distributions requires prior knowledge of the shape of the size distribution.

Particles themselves should have the same density throughout. SAXS can provide correct external dimensions for core-shell particles but only with higher uncertainties.

SAXS does not distinguish between agglomerates, aggregates and primary particles, hence agglomerates and aggregates need to be broken up to obtain the sizes of the primary particles.

4.4.4. How reliable is it?

SAXS has been shown to be very reproducible for monodisperse and fairly spherical particles [24]. Measurement of polymorphous and/or polydisperse samples is complex and currently algorithm-dependent, resulting in significant differences between instruments for the same samples. ISO/TS 13762 [32] (now withdrawn) had a table with indicative values for repeatability and accuracy.

4.4.5. Are standard methods available?

The withdrawn ISO/TS 13762:2001 [32] is undergoing major revisions for conversion into a full standard. This standard will only address basic shapes (spheres, cylinders, flakes) and monodisperse samples, as the development of methods for more detailed shape analysis and for polydisperse samples is not sufficiently advanced.

4.4.6. Are reference materials available?

There are reference nanomaterials with a certified SAXS-based mean particle size in the nanometre range available, for example from JRC-IRMM and from NIST. Commercially available reference materials for SAXS are monodisperse. As for all ensemble methods, the certified values are mean or modal values, rather than median values.

4.4.7. Is it widely available at acceptable costs?

Several manufacturers of routine instruments exist. These instruments are easy to use and deliver fast results. To improve the results, SAXS can also be applied using synchrotron radiation at selected particle accelerators, obviously at much higher cost.

SAXS measurements can be fast (results are known within a few minutes) and some instruments are fully automated, thus requiring little staff time.

4.4.8. What are the prospects for further development of the technique in the near future?

Improvements in the modelling of the intensity pattern will allow calculation of size distributions of more irregular shaped and broader distributed materials, but some a priori information on either shape or distribution of the particles will remain necessary to allow successful calculation.

4.5 Field flow fractionation (FFF)

4.5.1. How does it work?

Field flow fractionation (FFF) is a fractionation technique: it separates particles based on their hydrodynamic size. The sample (a suspension of particles) is pumped though a narrow channel in a laminar flow, which means that the fluid in the centre moves faster than the fluid at the edges of the channel. A 'field' is applied perpendicular to this flow, which is in most cases a second flow, but this field can also be electric, gravitation, etc., which pushes the particles to the edge of the channel, where they move slower. Because of Brownian motion, the smaller particles diffuse against the applied force field towards the centre of the flow channel, where they move faster. These two effects result in a separation between big and small particles.

FFF separates according to the hydrodynamic diameter of the particles. If an electric field is applied, the charge of the particles also plays a role. It is possible to transform the retention time to a hydrodynamic diameter, requiring either calibration with particle size standards or theoretical calculations using the physical properties of the medium, the particles and the dimensions of the cell.

4.5.2. Can it measure nanoparticles according to the definition?

Size information can be obtained by calibrating the instrument with particle size standards, which is only reliable if the particles have the same properties as the calibration standards. Apart from the uncertainty of the size because of differences between calibration standards and measured particles, quantification of the amount of particles depends on the sort of detector used and its calibration. Also here, the refractive index of the light absorption detector would have to be calibrated with the same particles that are measured, which is in practice impossible.

Therefore, FFF is in reality a separation technique, not a size measurement technique. Most often FFF is coupled to a detector system that performs, for example, on-line size measurements on the fractionated material (see for example [33]). A multitude of detectors exists, exploiting light absorption and refractive index but also static and dynamic light scattering. FFF is especially valuable for coupling to methods which require monodisperse particles that follow a narrow distribution, for example DLS. Different fractions can also be collected and analysed off-line, for example by EM.

4.5.3. Which type of nanomaterials can be measured?

FFF is performed in liquids. This limits the use of FFF to the analysis of particles which do not dissolve.

As a separation technique, it is well suited to deal with polydispersity, but it does not distinguish between primary particles, aggregates and agglomerates. These need to be broken up to obtain information on the primary particles.

The technique can also separate low-density materials, but whether or not the different fractions in the size-separated material can be measured depends on the detection technique coupled to the FFF. The detection method also determines whether non-spherical particles can be correctly measured; the FFF step can assist in this process at the hydrodynamic separation process is not only size-based but also shape-based.

4.5.4. How reliable is it?

FFF exploits a rather complex system, where interactions between particles, the carrier liquid and the channel membrane must be considered. Significant experience is required to develop methods for FFF. In addition, particles build up at the lower channel wall in the course of an analysis, limiting the number of samples that can be analysed in one run.

FFF is prone to interferences by large particles: because of their size, these protrude far into the channel and therefore may start rolling in the laminar flow, thereby being transported as fast as or even faster than small particles. Careful sample preparation which removes large particles is necessary to avoid confusing very large particles with small ones.

The method has the potential to provide very repeatable results (repeatability standard deviations on the diameter < 0.2 nm [34]). If used without a separate sizing instrument, then the main problem is the calibration of the FFF, which requires particles of the same kind in order to deliver unbiased results.

4.5.5. Are standard methods available?

Currently, no standard methods for FFF are available.

4.5.6. Are reference materials available?

There are no CRMs available with specific certified FFF-size values. Nevertheless, other CRMs can be used for FFF calibration. Relevant CRMs exist only for polymer latex and silica, limiting the scope of FFF as a sizing technique. While these standards are available, it must be borne in mind that if the analysed particles behave differently from the standards in the FFF process, the measured sizes are meaningless.

4.5.7. Is it widely available at acceptable costs?

Only a few instrument manufacturers exist that supply FFF instruments. FFF as a separation technique can be coupled with existing chromatography systems, thus reducing the cost of new instrumentation.

Because of the fragile equilibrium particle-carrier-membrane, experience is needed to perform reliable measurements.

4.5.8. What are the prospects for further development of the technique in the near future?

FFF in itself is a separation but not a sizing technique. Increasing recognition that its power lies in its use as a separation technique before sizing with, for example, DLS will most likely make it a very valuable tool for the detection and quantification of nanomaterials in final products. The development of disposable hollow-fibre versions increases the robustness and decreases risks of cross-contamination between samples.

4.6 Particle tracking analysis (PTA)

4.6.1. How does it work?

Particle tracking analysis (PTA) is an ultramicroscopy technique which exploits two important physical phenomena of (nano)particle behaviour when suspended in a liquid: the ability of individual particles to scatter light and the characteristic movement of particles produced by the effect of Brownian motion (diffusion). In practice, a specially designed optical cell containing a dilute solution of particles is illuminated with a laser light source and, using an optical microscope, the pin-points of light scattered by the rapidly moving particles or aggregates are observed and recorded using a highly sensitive CCD video camera. The video images can be analysed so as to allow particles to be located, individually identified and their movements followed on a frame-by-frame basis. Since the velocity of each individual particle is characterised by only the liquid viscosity, the temperature and the particle's hydrodynamic size, it is possible to calculate the particle size through the Stokes-Einstein equation. When the displacements of a statistically relevant number of particles in a population are evaluated over a suitable lapse of time it becomes possible to obtain reliable counting statistics of number size distributions. This method has a number of important advantages including relatively low instrument cost and high sensitivity which can detect nanoparticles at concentrations as low as low as 10^6 particle/cm^3 [35].

4.6.2. Can it measure nanoparticles according to the definition?

This technique calculates particle size on a particle-by-particle basis and is thus effective in overcoming some of the inherent weaknesses of DLS and other ensemble methods when confronted with solutions containing many small particles in the presence of a few larger strongly scattering particles or aggregates.

The particles must be suspended in a fluid of accurately known viscosity which is optically transparent and non-fluorescent at the wavelength corresponding to the laser source used in the instrument. The scattering of light by a particle in solution depends also on the refractive indices of the particle and liquid and most importantly the size of the particles. In practice, the effective lower limit is dependent on a combination of these factors but detecting particles below 25 nm to 35 nm becomes problematic for materials other than those with high refractive indexes such as gold or TiO_2.

4.6.3. Which type of nanomaterials can be measured?

PTA is performed in liquids. This limits the use of PTA to the analysis of particles which do not dissolve.

The method, counting individual particles, is potentially suited to dealing with samples which are polydisperse. PTA has a better size resolution than DLS but is still unable to separate fractions of particles with relative difference much less than 50 %.

The size calculated by PTA is, like in DLS, the equivalent hydrodynamic diameter with all connected issues of particle geometry. PTA does not distinguish particle shape and so measurements of samples of mixed sphericity will result in an average hydrodynamic radius which may differ from the external dimensions measured with other methods.

In mixtures of particles of different optical properties (for example, TiO_2 and SiO_2), the weaker scattering particle is underestimated.

PTA does not distinguish between agglomerates, aggregates and primary particles, hence agglomerates and aggregates need to be broken up to obtain the sizes of the primary particles.

4.6.4. How reliable is it?

The instrument requires the user to adjust some parameters such as brightness, gain and detection threshold to facilitate particle identification and processing. These values can influence the final result following image analysis and data processing and therefore introduce a potential degree of operator bias. For samples in which solvent viscosity and temperature are correctly entered, the diameter of a well prepared, monodisperse polystyrene micro-sphere calibrant can be accurately measured to within 1 % or 2 % of the certified value. Because of the statistical nature of the particle selection process, reproducibility is a function of the number of particles measured over the period of analysis. It is reported that for optimum concentrations of monodisperse samples containing no contaminating aggregates, results reproducible to within 2 % to 3 % can be achieved in a comparatively short time (for example, in 10 seconds to 20 seconds).

4.6.5. Are standard methods available?

At this time no internationally accepted standard methods are known for the operation of this type of instrument.

4.6.6. Are reference materials available?

There are no reference nanomaterials with a certified PTA-based median particle size or particle size distribution in the nanometre range. Typically, calibration is performed using spherical, monodisperse reference materials with certified values obtained by other methods (such as EM or DLS).

4.6.7. Is it widely available at acceptable costs?

This type of instrumentation is available from one main manufacturer and is available in a fairly large number of specialised laboratories. An important advantage of this instrumentation is that it has a relatively low capital cost compared to the majority of the other methods. The instrumentation is compact and needs no dedicated laboratory infrastructure for its operation. Furthermore, it has low running costs since it uses a single re-useable sample cell and other than minimal quantities of cleaning agents it requires no other disposable consumables.

4.6.8. What are the prospects for further development of the technique in the near future?

The scattering of light by a particle in solution depends on a number of factors including the wavelength of the scattering light, the refractive indices of the particle and liquid, and most importantly the size of the particles. The lower detection limit has been reduced by recent advances in laser diode technology which have resulted in the availability of powerful, stable near-ultraviolet light sources which can be used as an alternative to the red-light sources more commonly used in this technology. The use of shorter wavelength light permits more efficient detection of small particles but reaching the 1 nm limit in the *definition* remains beyond the capabilities of this technology.

Another area of ongoing development is in distinguishing particles with different composition in the same size class. In many cases, PTA instrumentation derives information only from the movement of particles as determined by the light they scatter without considering the intensity of light scattered. In recent evolutions of the techniques, this additional information is beginning to be exploited to determine whether particles detected are of a different nature by analysis of the diffracted light intensity.

4.7 Atomic force microscopy (AFM)

4.7.1. How does it work?

In atomic force microscopy (AFM), a sharp tip which is mounted on a cantilever is moved along the surface to be measured and the changes of height are recorded. These height changes can be caused by particles lying on the surface, but can also be the roughness of the surface itself. In its most basic set-up, the instrument works similar to a record player, with the tip being the needle and the record the surface to be measured. In the simplest of set-ups, the bend in the cantilever is kept constant and the instrument measures how much the cantilever needs to be moved up and down to achieve constant bending. Other measurement modes exist, where the cantilever does not touch the surface or where the cantilever is tapping the surface.

AFM images are not only influenced by the substrate surface, but also by the shape of the tip, which has in particular an effect on the accuracy of the lateral (x-y) information. Because of this, the obtained height information is more reliable, especially for very small particles.

4.7.2. Can it measure nanoparticles according to the definition?

Size information can be obtained in two different ways from AFM images. In the first, more frequently used way, the height of the particle is used as particle diameter. This approach can only be applied to well separated particles. The second approach is analysis of the image in the lateral (x-y) dimension similar to other imaging methods. By applying one of the approaches to many particles, one can obtain the required number size distributions.

4.7.3. Which type of nanomaterials can be measured?

To be measured by AFM, particles need to be fixed to the surface to prevent them from being moved by the tip. Images are typically obtained from particles on a dry surface: a droplet of a suspension containing the particles is applied to the holder surface and the remaining liquid is dried off.

AFM is an imaging method. As the image can be used for sizing and counting particle by particle, it can deal with polydispersity and, to some extent, also with non-spherical particles. However, automated image processing software usually requires some general assumptions about the particle shape to allow time-efficient evaluation of images [36].

Fragile, organic particles can be imaged in non-contact scanning modes. It is even possible to image particles in a liquid (as long as they remain adsorbed to the surface of the sample holder), but this approach is not yet sufficiently mature for practical use in the implementation of the *definition*.

Automated AFM image analysis cannot (easily) distinguish whether large particles are single particles or agglomerates of smaller particles. Only extensive operator intervention and interpretation of the obtained images can partly solve this issue.

4.7.4. How reliable is it?

Apart from errors introduced by the shape of the tip, the contact between tip and particle can change the particle, leading to wrong results. These factors contribute to unsatisfactory reliability: a recent publication concluded that 'an improvement in AFM calibration technology using nanometrological standards is required for both AFM manufacturers and AFM users' [37]. However, as implementation of the *definition* only requires deciding whether particles are above or below 100 nm, a 5 % uncertainty on the calibration will not render the method unsuitable.

4.7.5. Are standard methods available?

Two ISO standards for measuring of drift rate (ISO 11039:2012 [38]) and for calibration of the lateral dimension (ISO 27911 [39]) are available and more standards are in development. ASTM International has released a series of standards dealing with calibration (ASTM E2530-06 [40]) and a guide to scanner and tip artefacts (ASTM E2382-04 [41]). ASTM is also preparing a guide for 'Size Measurement of Nanoparticles Using Atomic Force Microscopy (AFM)'.

4.7.6. Are reference materials available?

A sufficient number of step gratings for height calibration as well as stripe patterns for lateral calibration are available, but no CRM allowing performance checks of particle size measurements is currently on the market.

4.7.7. Is it widely available at acceptable costs?

For AFM instruments, there is a clear trade-off between resolution and the roughness of the surface to be investigated. Measuring the number of particles required to obtain a reliable size distribution is time consuming and, therefore, expensive.

4.7.8. What are the prospects for further development of the technique in the near future?

Even more than for EM, the time required to measure a sufficiently large number of particles to obtain a reliable size distribution prohibits the use of AFM for implementation of the *definition*. In addition, there are still problems with sample preparation and characterisation of AFM tips. Therefore, AFM will presumably not become widely used for the characterisation of particle size in the sense of the *definition*. Automation of the image analysis step can in the future improve the statistical reliability.

4.8 X-ray diffraction (XRD)

4.8.1. How does it work?

X-ray diffraction (XRD) is a widely used technique for analysis of the crystalline structure of materials. The principles of XRD have been known for many decades. The fundamental equation on which XRD is based is *Bragg's law*, which links lattice spacing with the observed angles of constructive interference when X-rays scatter from a crystalline material. For nanomaterials, measurements are made on powders containing a very large number of randomly oriented particles. Thus diffraction rings or halos are seen at various angles with respect to the incoming beam, each originating from a subset of particles oriented correctly to satisfy the Bragg equation, and the corresponding technique is known as the *powder method*, or the *Debye-Scherrer method*. Analysis of the position and intensity of the peaks in the powder diffraction pattern allows the identification and quantification of the phase composition of the sample.

Another fundamental equation applied in XRD analysis is *Scherrer's equation*, which relates the broadening of XRD peaks to the (average) finite size of the individual diffracting domains. This finite size can correspond with the crystals within a solid, but it can also correspond with the individual (monocrystalline) particles in a (agglomerated or aggregated) nanomaterial.

A more advanced approach to the fitting of the diffraction spectra is Rietveld Analysis. This analysis combines the contributions of crystal structure, volume fractions, grain sizes, and other material and instrumental parameters to simulate the measured diffraction spectra.

4.8.2. Can it measure nanoparticles according to the definition?

XRD provides a powerful and relatively simple way of determining the average particle size of a nanomaterial, but not the size distribution. The technique is more reliable for particles towards the lower end of the 1 nm to 100 nm size range because the peak broadening is much more pronounced for smaller particles. Modifications of the analysis method are necessary at the very low range and high-resolution instruments are required to measure close to 100 nm.

4.8.3. Which type of nanomaterials can be measured?

The optimum way to measure XRD is without any physical barrier between the sample and the X-ray source and detector system. This eliminates unwanted scattering of X-rays that might complicate peak analysis. It is therefore best to measure a dry powder sample. Nevertheless, measurements can also be made on nanoparticles in suspension if the concentration of nanoparticles is high enough. For very small amounts of material, a glancing angle XRD geometry may be employed with a thin layer of the nanoparticles deposited on, for example, a silicon wafer.

A particulate sample with a fairly broad primary particle size distribution will produce a similar line broadening to a sample with the same average size but a narrow size distribution. Therefore, while XRD is an excellent method for average particle size measurement in certain specific circumstances, it is generally not suitable for measurement of particle size distribution. Also, it does not provide information about the shape of the individual particles.

The method is limited to particles that are crystalline. For amorphous or very poorly crystalline particles, Fourier transform techniques can be used in principle to determine average particle size but these are not widely applied and may not be very accurate. Finally, standard methods for XRD particle size analysis cannot be easily applied to more complex nano-structured particles, such as core-shell particles.

In the case of aggregated or agglomerated primary (single-crystal) particles, the XRD analysis will not give any information about the size of the aggregates or agglomerates, but will give the size information only for the primary particles. This is an important difference with respect to all other techniques mentioned in this report.

4.8.4. How reliable is it?

XRD peak shape analysis is a reliable method for average primary particle size measurement in some very specific cases. However, it is quite unreliable if in-depth knowledge of the crystallinity and nanostructure of the primary particles is lacking (i.e. if the primary particles are single nanocrystals or not, or whether the particles are coated or not, etc.).

4.8.5. Are standard methods available?

At this time no internationally accepted standard methods are known for the operation of XRD instruments with the specific aim of determining average nanocrystallite size. Likewise, no standard procedures are available for sample preparation or peak shape analysis.

4.8.6. Are reference materials available?

There have been efforts towards making a nanocrystallite size reference material, but none are available today.

4.8.7. Is it widely available at acceptable costs?

Many XRD systems are available commercially, most offering the powder diffraction technique and being provided with software for peak shape analysis and deduction of average crystallite size.

XRD instruments vary in several aspects that influence their cost. In general, XRD instruments are quite expensive, but because they provide the standard, fastest and most reliable method of crystalline phase analysis, many research and industrial laboratories are equipped with them. Many instruments also dispose of peak shape analysis software, but, as noted above, it is critical that this should be used by an expert in order to provide reliable information on crystallite sizes.

4.8.8. What are the prospects for further development of the technique in the near future?

XRD is a mature technique that is unlikely to see much further development in terms of the type of data that it provides. The limiting factors in terms of size determination are mainly associated with the samples themselves, since many nanomaterials are simply not suitable for particle size determination by XRD. While it is theoretically possible to improve the information about particle size distributions in very specific circumstances, in practical terms there is little prospect that this will lead to a generally useful method.

4.9 Determination of specific surface area by BET

4.9.1. How does it work?

The *definition* recommends measurement of the SSA by the BET method. This method, named after Brunauer, Emmett and Teller who developed the underlying theory, measures how much gas (usually nitrogen) is adsorbed at a specific temperature and pressure.

The underlying theory for the BET method describes the monolayer adsorption of inert gases such as nitrogen or argon by weak physisorptive interaction with the surface of solids at low temperatures. The BET method itself consists in the determination of the number of adsorbed molecules or atoms on the surface. With this amount and together with the assumed cross-sectional area of one adsorbed molecule or atom, the absolute surface area of the material (here of the powder) can be calculated.

4.9.2. Can it measure nanoparticles according to the definition?

The BET method measures particle surface area. Dividing the absolute surface area by the sample mass gives the so-called mass-specific surface area, commonly reported in the unit square metre per gram (m^2/g). This is not the same unit as used in the *definition*, which refers to the volume-specific surface area (VSSA) with the unit m^2/cm^3 or m^2/m^3. Therefore, the calculation of the VSSA from the common result of a BET measurement requires the knowledge of the particle density (see also Section 2.6).

4.9.3. Which type of nanomaterials can be measured?

Particles must be present as dry powders. Because the interaction between the gas molecules and the particles is too weak to break up agglomerates and aggregates, the result corresponds to the surface area of the aggregate or agglomerate, not of the constituent particles.

4.9.4. How reliable is it?

The method will give the total surface area accessible to the gas used, which includes inner surface such as pores. A multitude of porous materials have been developed that by far exceed the limit of 60 m^2/cm^3 due to their porosity, although their particle size may be as high as 1 mm. In other words, many porous materials with particle sizes of 1 mm and more have SSAs much larger than the one specified in the *definition*.

The density of very fine powders is often not the same as those of larger pieces of the same material. The reason for that is the fact that atoms or molecules located near the surface have different equilibrium positions than those within the solid structure. The percentage of near surface atoms or molecules increases with its resultant effect upon the density when the particle size decreases. For very fine powders, the true particle density is not easy to determine. This means that the transformation of BET results into VSSA may be connected with an increased measurement uncertainty.

4.9.5. Are standard methods available?

Numerous standards for the BET method exist, for example ISO 9277:2010 [42], which provides a basic and general description of the BET method, ISO 18757:2003 [43] and ISO 18852:2005 [44], which describe application of the BET method to ceramics and rubber.

4.9.6. Are reference materials available?

Several CRMs for testing BET equipment are available, amongst them some from JRC-IRMM, BAM (the German Federal Institute for Materials Research and Testing) and NIST.

4.9.7. Is it widely available at acceptable costs?

Instruments are widely available and a large number of private companies perform analyses. Disadvantages of the BET method are the necessity for powder drying prior to measurement and the relatively long measuring time due to the slow adsorption rate of the inert gas molecules at low temperatures.

4.9.8. What are the prospects for further development of the technique in the near future?

The method has been a standard method for many years. No significant further changes are to be expected in the near future.

4.10 Additional particle size and specific surface area measurement methods

In addition to the methods discussed in the previous sections, there are quite a number of other particle size measurement methods which have been used to solve specific particle size measurement problems in the nanoscale. However, for several reasons, they do not show the same potential. In this section some of these methods are listed and briefly discussed.

4.10.1. Hydrodynamic chromatography (HDC) and size-exclusion chromatography (SEC)

Hydrodynamic chromatography (HDC) and size-exclusion chromatography (SEC) are not sizing methods as such but are separation methods. In SEC, the suspension containing the nanoparticles flows along porous particles. As small particles will migrate more often into the pores than larger ones, a separation according to size is achieved. In HDC, the nanoparticle suspension flows along solid particles. Larger nanoparticles cannot get as close to the separation particles and therefore spend more time in the high-flow region of the flow than smaller particles, resulting in separation between particles according to size.

Similar to FFF, the time from sample introduction to arrival at the detector can be calibrated for apparent (equivalent spherical) particle size. In general, the methods have rather poor separation power, i.e. unless particle sizes differ widely, they will leave the instrument as one broad ‘peak’. HDC has the additional drawback that currently only one column is available.

Seeing the poor separation power and the assumptions necessary for calibration, SEC and HDC are not suitable for measuring nanoparticles according to the *definition*. However, the methods are useful to separate the nanoparticles in question from other material components and can therefore play an important role in the determination of nanoparticles in finalised products.

4.10.2. Gas-Phase Electrophoretic Molecular Mobility Analysis (GEMMA)

In Gas-Phase Electrophoretic Molecular Mobility Analysis (GEMMA) the suspension containing the nanoparticles is transformed into an aerosol. The liquid droplets are evaporated and charged with one negative charge. The movement of the particles in an electric field under a simultaneous applied flow of gas is used to obtain particle sizes (a similar principle to FFF).

The method gives requires calibration for obtaining size information. The method is mainly used to determine molar masses, but can potentially be used to size nanoparticles. The main drawback is the need to convert the sample into an aerosol. A second disadvantage is that the method only works at low concentrations as each aerosol droplet must not contain more than one particle.

4.10.3. Single-particle inductively coupled plasma-mass spectrometry (ICP-MS)

Single-particle inductively coupled plasma-mass spectrometry (ICP-MS) is a new technique, which, due to its potential, is included in this report. The method employs the well established and standardised technique of ICP-MS: in this technique, the liquid sample is transformed into an aerosol, which is then transported into a plasma of very high temperature (10 000 K), where the atoms are ionised and quantified in a mass spectrometer. Single-particle ICP-MS splits the total observation time into very small time windows (10 ms and below). Because of this short observation time, usually one particle at most enters the plasma in every time window. Therefore, each individual particle gives rise to a signal that is proportional to the number of atoms of which it consists. If the size of one atom is known and a certain particle shape is assumed, particle sizes can be calculated. As it counts the number of atoms, it cannot distinguish between single particles, agglomerates and aggregates.

The method is particularly suitable to particles consisting of one element only which are not frequent in the environment. Even then, the lowest particle sizes detectable are between 10 nm and 20 nm. Smaller particles are not discernable from the analytical noise.

Ideally, the method is combined with imaging methods to gain information on the shape to assume. Otherwise, long rods with a diameter < 100 nm may well be falsely interpreted as spheres with diameters > 100 nm.

The method is currently still in its development and is applied by only a few laboratories. The ability to obtain chemical information together with some measure of size may make it well suited for a number of specific measurement purposes.

4.10.4. Specific surface area measurements via nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) measurements are most often used to determine molecular structures. NMR can also be used to measure the surface area of suspended particles. The method is based on the measurable difference of the relaxation time of liquid molecules that are adsorbed on a particle surface and the molecules that are in the bulk of the suspending medium. The fact that so many nanomaterials are produced or used as suspensions is a driving force for the development of standard methods, which currently are not yet available.

4.11 Summary table

An overview of the eight particle size measurement methods is given in Table 1. The table indicates the typical measurement range (which may not be achievable for all kinds of nanomaterials), the type of particle size distribution that is initially obtained with the method, the assumptions made in the calculation of the (average) particle size, the method's capacity to deal with particular types of materials, and the availability of internationally agreed standard methods.

Method name (abbreviation)	Measurement range and medium (limiting factors)	Type of size distribution of raw data
Electron microscopy (EM)	1 nm and higher; dry (dynamic range)	number-based
Dynamic light scattering (DLS)	5 nm to 500 nm; suspension (sedimentation, scattering intensity)	(no distribution, or scattering-intensity–based)
Centrifugal liquid sedimentation (CLS)	20 nm and higher; suspension (particle density)	extinction-intensity–based
Small-angle X-ray scattering (SAXS)	5 nm and higher; suspension (dynamic range)	scattering-intensity–based
Field flow fractionation (FFF)	1 nm to 200 nm; suspension (dynamic range)	(depends on detector)
Particle tracking analysis (PTA)	25 nm and higher; suspension (scattering intensity)	number-based
Atomic force microscopy (AFM)	1 nm and higher; dry (dynamic range)	number-based
X-ray diffraction (XRD)	1 nm and higher; dry (only for crystalline materials)	(no distribution measured)

* scale: ++ = very well, + = well, o = moderately, - = not well, -- = not at all.

Table 1: Main characteristics of particle size methods relevant to the nanomaterial definition

Note: Readers shall note that the mentioned characteristics may not apply to all types of nanomaterials. For more details, readers are referred to Sections 4.1 to 4.8 of the report.

Can deal with challenges of particular types of nanomaterials? (scale: ++, +, o, -, --)*				Standards for use of method for size analysis available?
poly-dispersity	non-spherical particles	low-density materials	aggregates	
+	long: + flat: -	-	-	yes
--	--	+	--	yes
+	--	-	--	yes
o	-	o	--	yes
+	-	+	--	no
+	--	o	--	no
+	long: + flat: +	o	-	yes
--	--	-	+	yes

5. Two illustrative examples

5.1 Silica nanoparticle certified reference materials

In 2011 and 2012, JRC-IRMM released two certified nanoparticle reference materials consisting of colloidal silica (SiO_2 in aqueous solution). The measurement results on these materials may serve as a hint to what is possible to achieve, but also highlight the problems which all methods face.

5.1.1. Materials

Two different, commercial silica materials were selected.

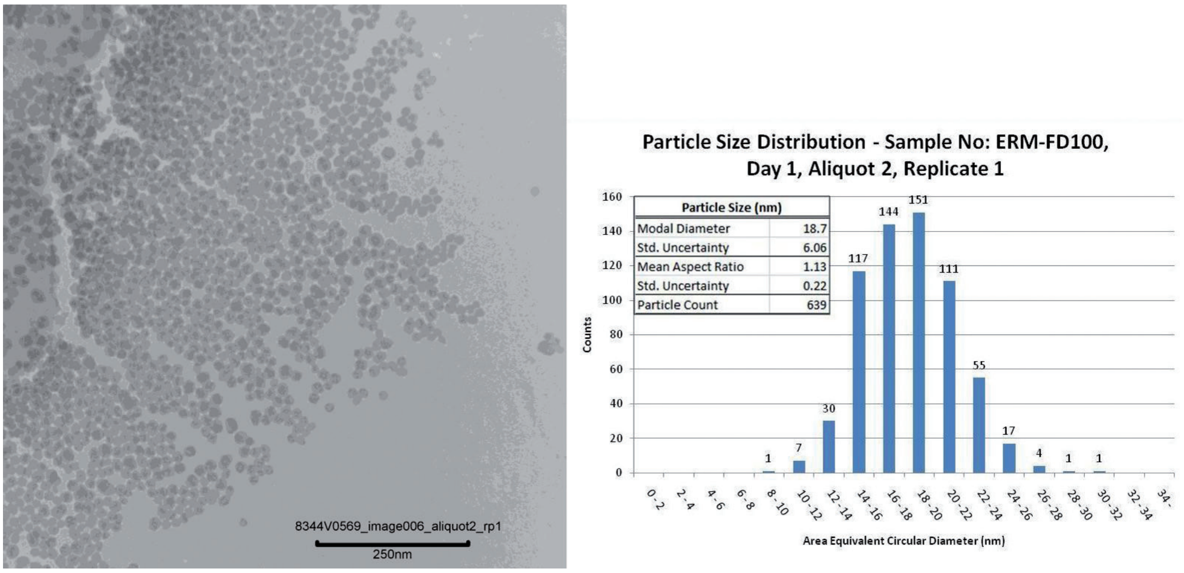
- ERM-FD100 [24] was produced from a silica suspension supplied by Chemiewerk Bad Koestritz GmbH, Germany (marketed under the name Koestrosol 1530). This material was selected as tests showed that the material was very spherical and very monodisperse.
- ERM-FD304 [45] was produced from a silica suspension supplied by Grace Davison GmbH (marketed under the name Ludox TM 50). The material is also spherical, but has a slightly broader particle size distribution. This broader size distribution was explicitly selected as a step closer towards other materials, which often show very broad particle size distributions.

Transmission electron microscopy (TEM) images and particle size distributions obtained by one TEM measurement are shown in Figure 9 and Figure 10.

5.1.2. Measurement methods and laboratories

A first interlaboratory comparison was organised in 2009 [46] to identify laboratories with competence in the field of nanoparticle size analysis. In 2010, 33 selected laboratories from 11 countries in America, Asia and Europe participated in a second interlaboratory comparison to certify the mean particle diameters of ERM-FD100 and ERM-FD304. The laboratories contributed 19 datasets for dynamic light scattering (DLS), 6 for centrifugal liquid sedimentation (CLS), 11 for electron microscopy (EM) and 5 for small-angle X-ray scattering (SAXS). Different weighting methods (intensity-based and volume-based) were applied for DLS. Care was taken that the various laboratories represented a broad range of different instruments, to avoid any instrument-specific bias.

The results on ERM-FD100 and ERM-FD304 were obtained from the same laboratories, in the same measurement series. Differences between the



Particle Size (nm)	
Modal Diameter	18.7
Std. Uncertainty	6.06
Mean Aspect Ratio	1.13
Std. Uncertainty	0.22
Particle Count	639

Figure 9: TEM image and particle size distribution obtained from one laboratory for ERM-FD100

Note: 500 particles were measured with sizes ranging from 8 nm to 32 nm.

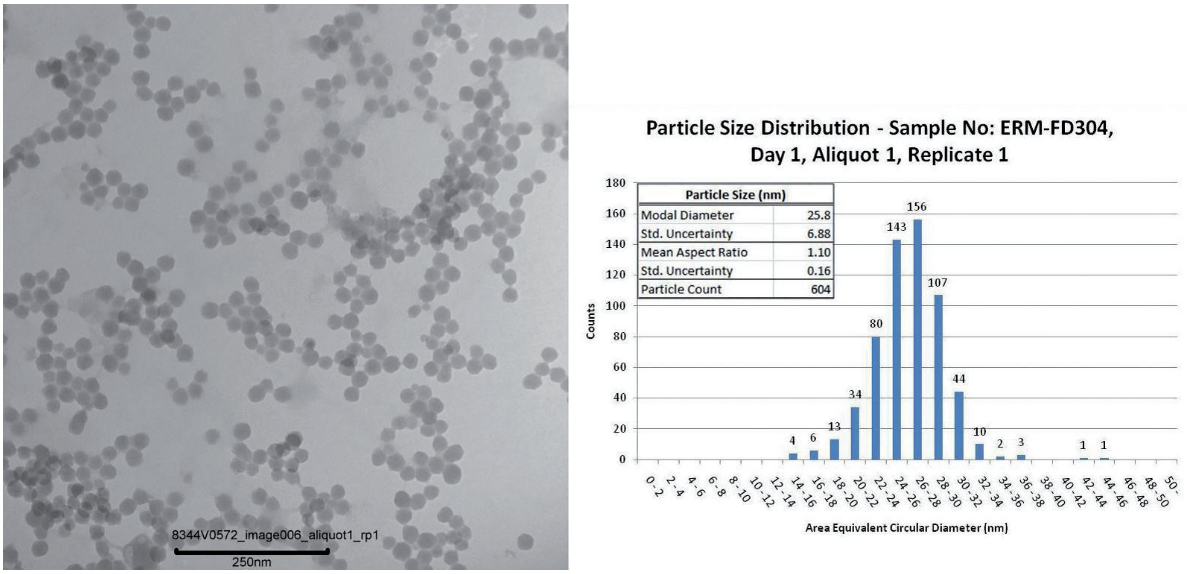


Figure 10: TEM image and particle size distribution obtained from one laboratory for ERM-FD304
Note: 500 particles were measured with sizes ranging from 10 nm to 50 nm.

reliability of results therefore allow a judgement about the influence of material-specific issues on comparability of results.

5.1.3. Results

For DLS, it became apparent that the two dominant modes of data evaluation (called 'correlation analysis' and 'frequency analysis') yielded different results. The evaluation had to be restricted to one of the two modes of evaluations to obtain a consistent set of results. Evaluation was also restricted to an evaluation algorithm that explicitly assumes monodisperse particle distributions. This requirement was by and large fulfilled in this case, but will not be fulfilled for other materials. The study also clearly showed that the conversion from intensity-weighted to volume-weighted distributions introduces significant errors. Figure 11 shows results from the same laboratories

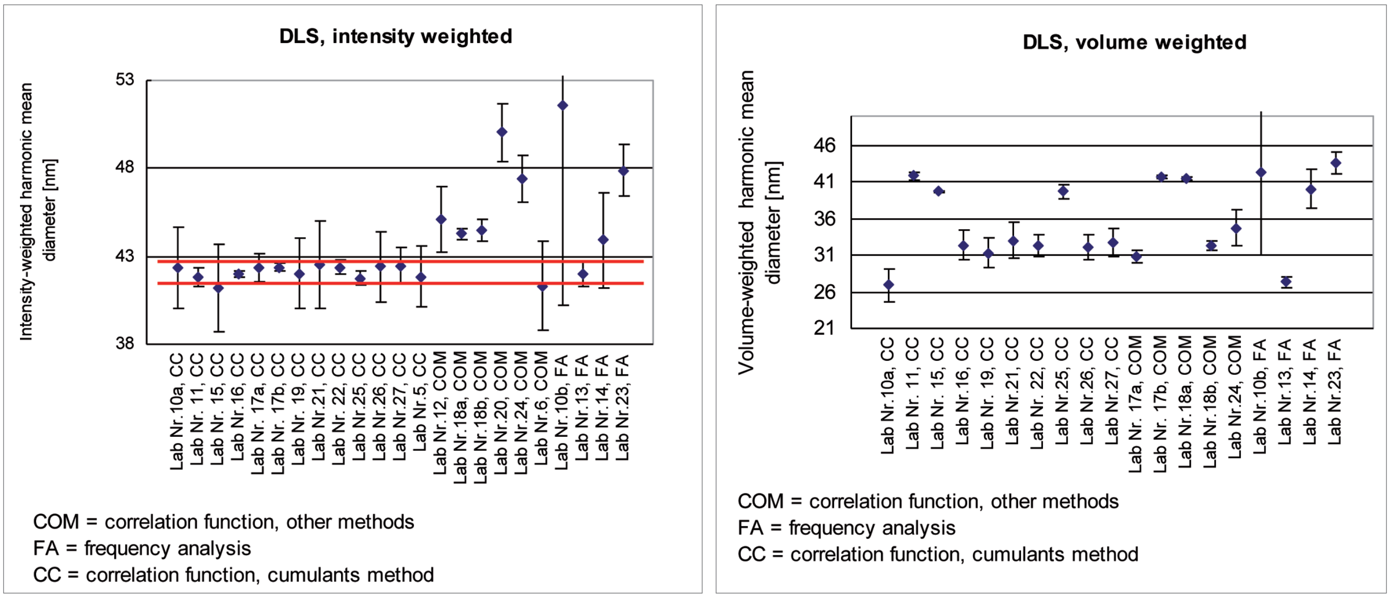


Figure 11: Results on ERM-FD304 obtained by DLS
Note: Only intensity-weighted results (left) labelled with CC were used for certification. Note the much larger variation of the volume-weighted results, which come from exactly the same measurements as the intensity-weighted, highlighting the perils of data transformation.

once evaluated as intensity-weighted, and once evaluated as volume-weighted distribution. While the difference in mean diameter is expected, the larger scatter of the volume-weighted distribution clearly demonstrates that such a conversion is not straightforward. It relies on knowledge about the particle properties (shape, refractive index, etc.) that is not available or can only be assumed.

Similar to DLS, CLS also has different modes of operation and these also gave different results. Also here, evaluation was restricted to one operation mode to obtain a consistent dataset. The standard deviation between laboratories was larger for ERM-FD304, probably caused by the larger degree of polydispersity in the sample. Nevertheless, standard deviations between laboratory means were 0.7 nm (ERM-FD100) and 1.2 nm (ERM-FD304), which is very low. Note that the selected CLS method measures a modal diameter, which intrinsically is a more robust parameter than the mean diameters produced by DLS methods.

For SAXS, exceptionally good agreement was obtained for the very monodisperse material ERM-FD100. The standard deviation between the five intensity-weighted laboratory means was 0.34 nm. This good agreement, however, failed for the slightly more polydisperse ERM-FD304, with one laboratory submitting results differing by 20 nm from the others. No clear reason could be found for this discrepancy: one laboratory claimed that it can measure more large particles, whereas the other laboratory claimed that the deviating laboratory used an outdated evaluation algorithm. As the different laboratories represent different instrument manufacturers, they were reluctant to share all data. However, this finding shows that already slight polydispersity can lead to widely differing results.

For EM, results for ERM-FD100 agreed reasonably well, giving a standard deviation of the 11 laboratory values of 2.0 nm. As for SAXS, agreement was worse for ERM-FD304, with results ranging from 24 nm to 32 nm, giving a standard deviation of 2.3 nm.

These data indicate that in principle, nanoparticles can be sized very accurately as long as the particles are (1) dispersed (not aggregated), (2) near-spherical, and (3) monodisperse. Deviations from monodispersity, however, result in a much wider scatter of data, which makes each individual result less reliable. It should be pointed out that while ERM-FD304 is more polydisperse than ERM-FD100, it is still very monodisperse. Results on many other materials (for example, those of the sponsorship programme of the Organisation for Economic Co-operation and Development (OECD) Working Party on Manufactured Nanomaterials (WPMN)) show a much wider variation of particle sizes than for these two materials, resulting in much larger variation of results.

5.2 Zinc oxide representative nanoparticle test materials

Several zinc oxide (ZnO) materials were studied in a joint effort by the Institute for Health and Consumer Protection of the JRC (JRC-IHCP), a dedicated United Kingdom project (PROSPECT) led by the Nanotechnologies Industries Association (NIA) [25], and a number of European research and industry partners. The ZnO materials were selected by the OECD WPMN steering group 3, 'Safety Testing of a Representative Set of Manufactured Manomaterials'.

Unlike the silica materials from the previous section, which were produced in suspension and thus have fewer agglomeration problems, the ZnO materials were produced as powders. The results show the difficulties in measuring agglomerated and aggregated materials.

5.2.1. Materials and measurement methods

The two commercial ZnO materials discussed here are coded NM-110 and NM-111. Both were produced and supplied by BASF AG, Germany. The materials are representative for commercial uncoated and coated ZnO materials, respectively. Measurements by TEM, Scanning electron microscopy (SEM), X-ray diffraction (XRD), DLS and CLS were performed. Measurements by one laboratory were not replicated by other laboratories at this stage of the study.

5.2.2. Electron microscopy images

The SEM images in Figure 12 also show that the 'as-delivered' powders were highly agglomerated and aggregated. Differences between size measurement results were expected due to the difficulty of distinguishing between agglomerates and single particles.

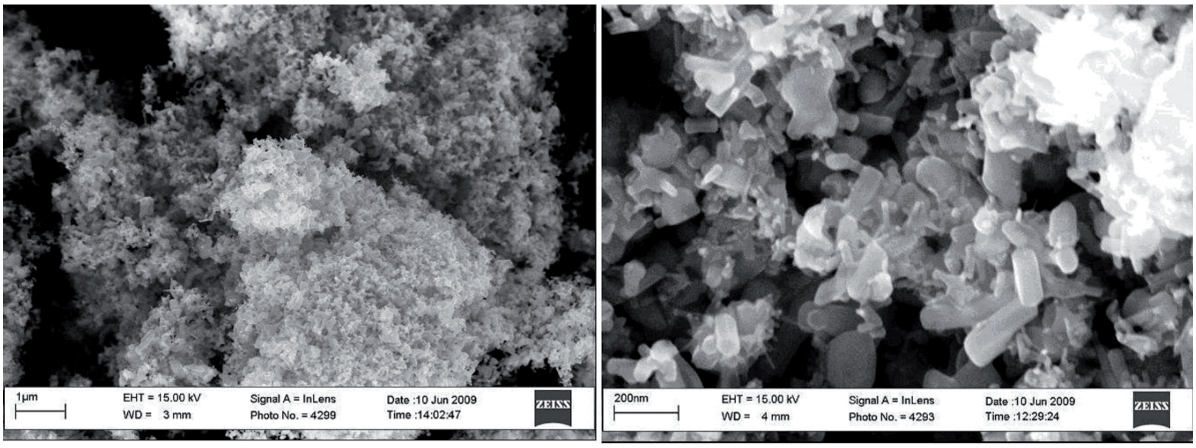
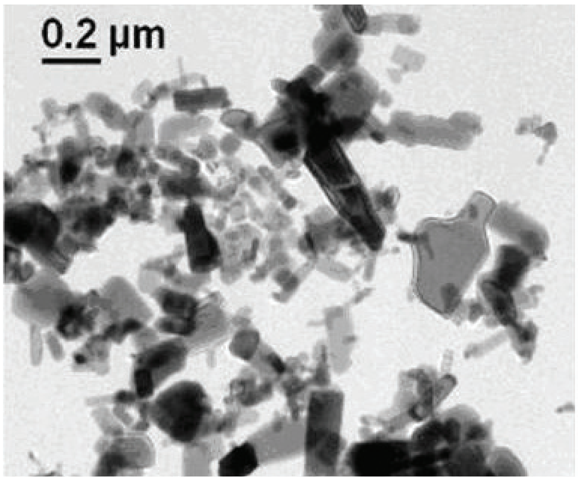


Figure 12: SEM image of NM-110 (left) and NM-111 (right), indicating high agglomeration of particles

Note: Note the different scale of the images.

The TEM images of NM-110 and NM-111 shown in Figures 13 and 14 were obtained in a laboratory with specific expertise in the EM analysis of nanomaterials, following well documented and detailed sample preparation (including suspension and sonication) and imaging protocols. The observed particles are not spherical, aggregated, and much more polydisperse than for the two silica materials discussed above. Since large agglomerates tend to detach from the EM grid relatively easily, the analysts considered it unlikely that the particles that remain attached to the grid are representative for the sample. Therefore, the particle size distributions shown in Figures 12 and 13 were qualified as being qualitative rather than quantitative by the authors of the report [25].



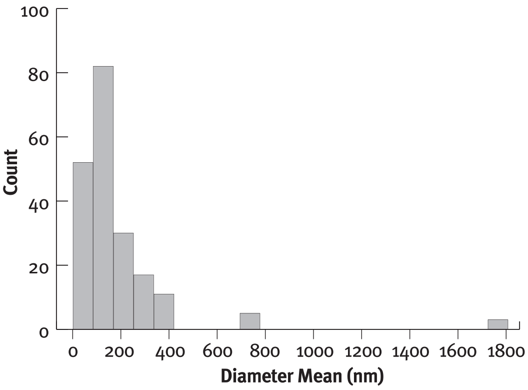
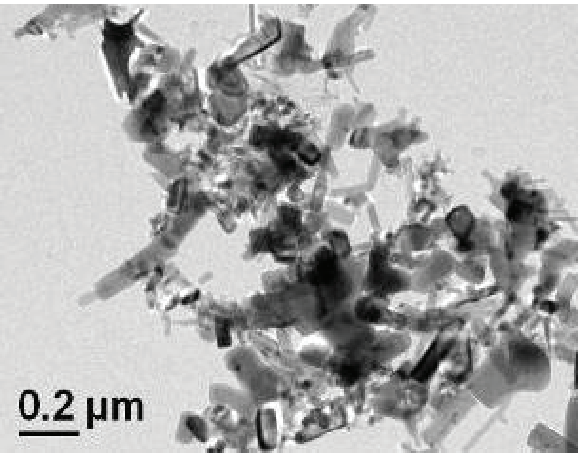


Figure 13: TEM image and corresponding size distribution obtained for NM-110



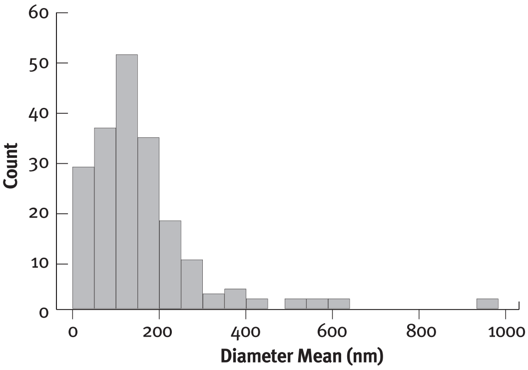


Figure 14: TEM image and corresponding size distribution obtained for NM-111

5.2.3. Size measurements on the dry, as-delivered powders

Particle size was measured for the dry NM-110 and NM-111 powders with several methods. SEM and XRD results are shown in Table 2.

Particle size determination from SEM images for highly aggregated and agglomerated powders requires in-depth analysis of each image. This was done by manually tracing contours of primary particles onto a transparency sheet. Therefore, only a few particles (about 50) were measured, which is insufficient to obtain reliable estimations for the size distributions. The transparency sheet was scanned for further image analysis using ImageJ software, which automatically calculated particle diameter dimensions.

Particle size was also evaluated with the Scherrer analysis of XRD spectra. The values, reported in Table 2, correspond with the crystallite (primary particle) size. The more refined Rietveld evaluation of the XRD spectra also takes into consideration structure and morphology, and yields larger crystallite sizes of NM-110 (> 85.5 nm) and NM-111 (75.5 nm). Nevertheless, for both types of analysis, the crystallite size by XRD is much smaller than the corresponding values as reported from SEM analysis. This is not surprising as each particle may be made up of several different crystallites.

Table 2: Particle size determined by SEM (Feret diameter) and XRD (crystallite size)

Sample Name	Average Feret diameter of about 50 particles imaged with SEM ± standard deviation (nm)	Crystallite diameter from XRD – Scherrer equation (nm)	Crystallite diameter from XRD – Rietveld analysis (nm)
NM-110	151 ± 56	41.5	> 85.5
NM-111	141 ± 66	33.8	75.5

5.2.4. Measurements on suspensions of the as-delivered powders

Several measurement methods require the particles to be suspended in a medium. As noted above, the suspension method and the suspension medium influence the amount of de-agglomeration, whereas the aggregates are not broken up.

DLS: DLS measurements of the particle size of NM-110 and NM-111 were performed on the materials as prepared in distilled water. The mean particle size based on three measurements was approximately 275 nm for NM-110 and 253 nm for NM-111. The polydispersity index (PI), a commonly used parameter to assess the width of a DLS-based size distribution, was 0.15 for NM-110 and 0.40 for NM-111. As a rule of thumb, PI values smaller than about 0.04 are considered monodisperse, so the high PI values confirm the polydispersity of the material and confirm the broad distributions already found by SEM (see Table 2). It should be recalled that DLS results may be of limited value since multimodal particle size distributions are not accurately analysed by this method.

CLS: Measurements of NM-110 and NM-111 by CLS were also performed on the materials as prepared in different media (distilled water, seawater, and 2 aqueous media corresponding with the ecotoxicological tests in simulated fresh water on daphnia and fish). Table 3 shows the D50 value, which is the diameter at which 50 % of the particle's signals (extinguished light) was measured (14). The different results demonstrate the strong influence of the nature of the medium on the measured size. Note also that measurements on NM-111 could not be performed: the material is coated in a way that prevents its ready dispersion in aqueous media.

---

14 This means: the sum of the signals of all particles smaller than D50 equals the sum of the signals of all particles larger than D50.

Table 3: Particle size measurement by CLS

Sample Name	Distilled water (nm)	Fish medium (nm)	Seawater (nm)	Daphnia medium (nm)
NM-110	82.8 ± 1.9	270 ± 20	301 ± 8	285 ± 16

Note: Shown is the D_{50} value (the diameter for which 50 % of the cumulated signal comes from particles smaller than D_{50}) ± standard deviations of three measurements.

5.2.5. Summary of results

The results on the zinc oxides NM-110 and NM-111 are shown in Table 4.

Table 4: Summary of results on ZnO materials NM-110 and NM-111

Method	NM-110 (nm)	NM-111 (nm)
Size measurement on dry powders		
TEM, Feret mean	147 ± 149	141 ± 103
SEM, Feret	151 ± 56	141 ± 66
Size measurement on suspensions		
DLS, mean particle size	275 ± 4	253 ± 1
CLS (in distilled water)	193 ± 3	-
XRD (crystallite size)	41.5	33.8

Note: DLS and CLS results are reported with single standard deviations of repeated measurements; SEM and TEM results are reported with the width of the size distribution over the measured particles.

5.3 Discussion and conclusions

The results of Section 5.1 show that for spherical, monodisperse materials good agreement between laboratories and methods is achievable. However, even slight deviations from the assumptions implicitly used in the methods applied for ERM-FD304 lead to less agreement. For the zinc oxides, presented in Section 5.2, no agreement is reached between methods, for several reasons. A first reason is the much higher polydispersity of the ZnO materials as compared to the silica materials. Also, the ZnO materials, which are powders, need to be suspended first for some methods. In addition, the powders are highly aggregated and agglomerated, and the particles are not spherical.

The observed disagreement between methods is only partly due to a lack of maturity of the methods. To a large extent, the disagreements are inherent in particle characterisation. The same disagreements exist for sizing of larger particles, which has been standardised for decades. This is not because of lack of progress, but because of inherent differences of the properties targeted by the methods. This is not necessarily undesirable. Different methods assess different aspects of particle size, or assess size in different conditions. This opens the possibility for carefully selecting the method that best fits the nanomaterial, its intended application, and the intended use of the measurement results.

It also has become clear that currently laboratories do not usually report number-based mean or median diameters. This situation can change with a more common use of the *definition*. However, the larger scatter of volume-weighted results compared to intensity-weighted results reported for the silica reference materials also highlights the inherent problems that accompany the conversion of signals.

Finally, it is noted that the indications for measurement uncertainty given in the various examples vary significantly. The variations indicated as error bars in Figure 11 are much larger than some variations indicated in Table 4, which are only standard deviations of repeated measurements, often even only from repeated readings. It is therefore crucial to investigate critically which uncertainties are reported by laboratories to avoid misinterpretation of apparently very precise results.

6. Measurement of nanoparticles in final products

A separate issue is to check in final products whether ingredients have been properly labelled, if this is required by legislation, for example whether a product contains nanomaterials without being mentioned on the label. The question here is 'does this product contain nanomaterials' rather than 'is this a nanomaterial?' This question is significantly more complex than just deciding whether a material fulfils the *definition*, as it raises questions concerning sample preparation, change of particles during production of the final product, discrimination of added particles from naturally present particles and mixtures of several materials.

The methods described in Chapter 4 generally do not distinguish between particles of a different chemical composition. This means that other structures (for example, proteins) that are present in the product will also be recognised as nanoparticles. It is therefore necessary to separate the particles of interest from the rest of the final product. This process constitutes an additional part of sample preparation, as it prepares the analytical sample for the final determination.

Although sample preparation is crucial for a successful (and meaningful) measurement, it may also change the particles and therefore may lead to wrong results. The main sources of potential misinterpretation are agglomeration and dissolution.

- **Agglomeration and aggregation**: During sample preparation, initially dispersed particles may form aggregates which cannot be separated anymore, leading to the general agglomeration and aggregation problems discussed above.

- **Dissolution**: Constituent particles may (partly) dissolve, leading to changes in the particle size distributions. Dissolution might remove very small particles, thus shifting the number size distribution to larger diameters and hence potentially changing a nanomaterial into a non-nanomaterial. On the other hand, dissolution may also reduce the size of particles that initially were larger than 100 nm, thus changing a non-nanomaterial into a nanomaterial in line with the *definition*.

To avoid such changes, sample preparation methods will have to be tailored to the combination's particle-matrix in question. For instance, some particles such as TiO_2 are chemically very stable, so significant dissolution is unlikely. Organic particles, on the other hand, are chemically less stable and there is a significant chance that the sample preparation methods also affect the particles themselves. Moreover, the interactions between the particles and the surrounding 'matrix' of the product will also determine the choice of the sample preparation method.

Particles may also change from the time of the addition of the materials as an ingredient to the production process until the finalisation of the product. Relevant potential changes are the same as during sample preparation, namely aggregation and dissolution. There have also been reports of materials containing nanoparticles which were not added as a nanomaterial ingredient, but which were created in situ, either spontaneously or intentionally [47].

Extrapolating from their experience in analytical chemistry, the authors of this report regard it as most likely that a multitude of sample preparation methods must be developed and standardised to allow comparable quantification of a wide range of nanoparticles in a wide range of materials.

7. Conclusions

The intended regulatory use of the *definition* of a nanomaterial requires consideration of how to best implement it, relying on the possibility to verify through measurements whether or not a material meets the definition. Across the different available measurement methods, discussed in this report, the following critical issues are identified:

- Particles have several external dimensions that can be interpreted as *particle size*. Moreover, most measurement methods provide an *apparent* and test-condition–dependent value for an average external particle dimension. Therefore, different size measurement methods may provide significantly different size values.

- Many methods deliver size distributions that need to be mathematically converted to the number-based size distribution required in the *definition*. This conversion is based on a number of assumptions, and becomes increasingly prone to error, difficult or even impossible if the mass fraction of nanoscale particles is not sufficiently large.

- No method is available that can reliably distinguish whether a large particle is an aggregate or a single, polycrystalline particle, and at the same time measure the size of large numbers of individual constituent particles. Most size measurement methods necessitate a sample preparation procedure that breaks up particles into the constituent particles for which the size must be measured. Aggregates cannot be broken up and no methods are available to reliably determine the size of the constituent particles in aggregates.

- No single method alone can cover, in a single measurement, for all materials the complete size range from lower than 1 nm to well above 100 nm, as would be required for a universal assessment according to the *definition*. In particular, it is expected that working ranges will strongly depend on particle mass fractions.

Summarising the current technical limitations, none of the currently available methods can determine for all kinds of potential nanomaterials whether they fulfil the *definition* or not. Implementation of the *definition* via measurements poses significant difficulties for polydisperse materials and is currently usually not possible for aggregated materials if the size distribution of their constituent primary particles must be determined, unless the aggregates as particulate material themselves fulfil the nanomaterial definition.

The future improvement of measurement technology, development of analytical methods and standardised sample preparation protocols may partly resolve the mentioned limitations. If rapid implementation of the *definition* through measurements is needed, dedicated guidance documents will have to be provided for specific materials and sectors, with clear and justified indication of the relevant particle size measurement methods and test conditions. A combination of several methods, ideally supported by information on the manufacturing process of the material under investigation, will have to be employed for robust assessments. The reliability of each of the measurement methods used in such combined, tiered processes will need to be thoroughly checked in dedicated method validation and interlaboratory comparison studies. Such technical developments and experiences should be taken into account for a future revision of the *definition* stipulated by the Recommendation.

Acknowledgements

The authors wish to thank Frank Babick (TU Dresden, DE), Charles Clifford (NPL, UK), Peter Klobes (BAM, DE), Michael Krumrey (PTB, DE), Jan Mast (CODA-CERVA, BE), Germ Visser (DSM, NL) and Michael Stintz (TU Dresden, DE) for their critical review and comments to selected parts of this report.

References

[1] Resolution P6_TA(2009)0328 of the European Parliament on regulatory aspects of nanomaterials, 2009.

[2] Lövestam, G., Rauscher, H., Roebben, G., Sokull Klüttgen, B., Gibson, N., Putaud, J.-Ph., Stamm, H., 'Considerations on a definition of nanomaterial for regulatory purposes', *JRC Reference Report*, EUR 24403 EN, European Union, Luxembourg, 2010. ISBN 978-92-79-16014-1

[3] SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Opinion on the scientific basis for the definition of the term "nanomaterial", 8 December 2010.

[4] COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterial, 2011/696/EU, 2011.

[5] Regulation No 1223/2009 of the European Parliament and of the Council on cosmetic products, 2009.

[6] Regulation No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers, 2011.

[7] ISO/TS 27687:2008 Nanotechnologies - Terminology and definitions for nano-objects - Nanoparticle, nanofibre and nanoplate, International Organization for Standardization, Geneva, 2008.

[8] Van Doren, E.A.F., De Temmerman, P.-J.R.H., Francisco, M.A.D., Mast, J., 'Determination of the volume-specific surface area by using transmission electron tomography for characterization and definition of nanomaterials', *Journal of Nanobiotechnology*, Vol. 9:17, 2011. doi:10.1186/1477-3155-9-17

[9] Roebben, G., 'Viscous energy dissipation in silicon nitride at high temperatures', Dissertation K.U. Leuven, 1999. ISBN 90-5682-165-2

[10] ISO 13322-1:2004 Particle size analysis - Image analysis methods - Part 1: Static image analysis methods, International Organization for Standardization, Geneva, 2004.

[11] ISO 9276-2:2001 Representation of results of particle size analysis - Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions, International Organization for Standardization, Geneva, 2001.

[12] ISO/TS 80004-3:2010 Nanotechnologies - Vocabulary - Part 3: Carbon nano-objects, International Organization for Standardization, Geneva, 2010.

[13] ISO 14488:2007 Particulate materials – Sampling and sample splitting for the determination of particulate properties, International Organization for Standardization, Geneva, 2007.

[14] Roebben, G., Ramirez-Garcia, S., Hackley, V., Roesslein, M., Klaessig, F., Kestens, V., Lynch, I., Garner, M.C., Rawle, A., Elder, A., Colvin, V., Kreyling, W., Krug, H., Lewicka, Z., McNeil, S., Nel, A., Patri, A., Wick, P., Wiesner, M., Tian Xia, Oberdörster, G., Dawson, K., 'Interlaboratory Comparison of Size and Surface Charge Measurements on Nanoparticles prior to Biological Impact Assessment', *J. Nanoparticle Research*, Vol. 13, 2011, pp. 2675–2687. doi:10.1007/s11051-011-0423-y

[15] Taurozzi, J.S., Hackley, V.A., Wiesner, M.R., 'Ultrasonic dispersion of nanoparticles for environmental, health and safety assessment: issues and recommendations', *Nanotoxicology*, 5, 2011, pp. 711–729. doi:10.3109/17435390.2010.528846

[16] ISO 1382:2008 Rubber - Vocabulary, International Organization for Standardization, Geneva, 2008.

[17] ISO 8780-1:1990 Pigments and extenders - Methods of dispersion for assessment of dispersion characteristics - Part 1: Introduction, International Organization for Standardization, Geneva, 1990.

[18] ISO 4618:2006 Paints and varnishes - Terms and definitions, International Organization for Standardization, Geneva, 2006.

[19] Merkus, H.G., 'Particle size measurements: Fundamentals, practice, quality', *Particle Technology Series*, Vol. 17, Springer, Berlin, 2009.

[20] ISO/IEC Guide 99 International Vocabulary of metrology – Basic and general concepts and associated terms (VIM), International Organization for Standardization, Geneva, 2007.

[21] Giechaskiel, B., Bergmann, A., 'Validation of 14 used, re-calibrated and new TSI 3790 condensation particle counters according to the UN-ECE Regulation 83', *J. Aerosol Science*, Vol. 42, 2011, pp. 195–203.

[22] Hassellöv, M., Readman, J.W., Ranville, J.F., Tiede, K., 'Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles', *Ecotoxicology*, Vol. 17, 2008, pp. 344–361.

[23] ISO 9276-1 to -6 Representation of results of particle size analysis, International Organization for Standardization, Geneva, 1998-2008.

[24] Braun, A., Franks, K., Kestens, V., Roebben, G., Lamberty, A., Linsinger, T., 'Certification of equivalent spherical diameters of silica nanoparticles in water', ERM-FD100, *Report EUR 24620* EN, European Union, Luxembourg, 2011. ISBN 978-92-79-18676-9

[25] Singh, C., Friedrichs, S., Levin, M., Birkedal, R., Jensen, K.A., Pojana, G., Wohlleben, W., Schulte, S., Wiench, K., Turney, T., Koulaeva, O., Marshall, D., Hund-Rinke, K., Kördel, W., Van Doren, E., De Temmerman, P-J., Abi Daoud Francisco, M., Mast, J., Gibson, N., Koeber, R., Linsinger, T., Klein, C.L., 'NM-Series of Representative Manufactured Nanomaterials - Zinc Oxide NM-110, NM-111, NM-112, NM-113, Characterisation and Test Item Preparation', *EUR Report 25066* EN, European Union, Luxembourg, 2011. ISBN 978-92-79-22215-3

[26] ISO 29301:2010 Microbeam analysis - Analytical transmission electron microscopy -Methods for calibrating image magnification by using reference materials having periodic structures, International Organization for Standardization, Geneva, 2010.

[27] ISO 22412:2008 Dynamic light scattering, International Organization for Standardization, Geneva, 2008.

[28] Braun, A., Couteau, O., Franks, K., Kestens, V., Roebben, G., Lamberty, A., Linsinger, T.P.J., 'Validation of dynamic light scattering and centrifugal liquid sedimentation methods for nanoparticle characterisation', *Advanced Powder Technology*, Vol. 22, 2010, pp. 766–770.

[29] ISO 13318-1:2001 Determination of particle size distribution by centrifugal liquid sedimentation methods -- Part 1: General principles and guidelines, International Organization for Standardization, Geneva, 2001.

[30] ISO 13318-2:2007 Determination of particle size distribution by centrifugal liquid sedimentation methods - Part 2: Photocentrifuge method, International Organization for Standardization, Geneva, 2007.

[31] ISO 13318-3:2004 Determination of particle size distribution by centrifugal liquid sedimentation methods - Part 3: Centrifugal X-ray method, International Organization for Standardization, Geneva, 2004.

[32] ISO TS 13762:2001 Particle size analysis - Small angle X-ray scattering method, International Organization for Standardization, Geneva, 2001.

[33] Calzolai, L., Gilliland, D., Pascual Garcìa, C., Rossi, F., 'Separation and characterization of gold nanoparticle mixtures by flow-field-flow fractionation', *J. Chromatogr. A*, Vol. 1218, 2011, pp. 4234–4239.

[34] Songsilawat, K., Shiowatana, J., Siripinyanond, A., 'Flow field-flow fractionation with off-line electrothermal atomic absorption spectrometry for size characterization of silver nanoparticles', *J. Chromatogr. A*, Vol. 1218, 2011, pp. 4213–4218.

[35] Gallego-Urrea, J.A., Tuoriniemi, J., Hassellöv, M., 'Applications of particle-tracking analysis to the determination of size distributions and concentrations of nanoparticles in environmental, biological and food samples', *Trends in Analytical Chemistry*, Vol. 30, 2011, pp. 473–483.

[36] Couteau, O., Roebben, G., 'Measurement of the size of spherical nanoparticles by means of atomic force microscopy', *Measurement Science and Technology*, Vol. 22, 2011, art. no. 065101.

[37] Misumi, I., Gonda, S., Sato, O., Yasutake, M., Kokawa, R., Fujii, T., Kojima, N., Kitamura, S., Tamochi, R., Kitta, J.-I., Kurosawa, T., 'Round-robin measurements of 100- and 60-nm scales among a deep-ultraviolet laser diffractometer, a scanning electron microscope and various atomic force microscopes', *Meas. Sci. Technol.*, Vol. 18, 2007, pp. 803–812.

[38] ISO 11039:2012 Surface chemical analysis -- Scanning-probe microscopy -- Measurement of drift rate, International Organization for Standardization, Geneva, 2012.

[39] ISO 27911:2011 Surface chemical analysis -- Scanning-probe microscopy -- Definition and calibration of the lateral resolution of a near-field optical microscope, International Organization for Standardization, Geneva, 2011.

[40] ASTM E2530 - 06 Standard Practice for Calibrating the Z-Magnification of an Atomic Force Microscope at Subnanometer Displacement Levels Using Si(111) Monatomic Steps, 2006.

[41] ASTM E2382 - 04 Guide to Scanner and Tip Related Artefacts in Scanning Tunneling Microscopy and Atomic Force Microscopy, 2004.

[42] ISO 9277:2010 Determination of the specific surface area of solids by gas adsorption -- BET method, International Organization for Standardization, Geneva, 2010.

[43] 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, International Organization for Standardization, Geneva, 2003.

[44] ISO 18852:2012 Rubber compounding ingredients - Determination of multipoint nitrogen surface area (NSA) and statistical thickness surface area (STSA), International Organization for Standardization, Geneva, 2012.

[45] Franks, K., Braun, A., Charoud-Got, J., Couteau, O., Kestens, V., Lamberty, A., Linsinger, T.P.J., Roebben, G., 'Certification of the equivalent spherical diameters of silica nanoparticles in aqueous solution', ERM-FD304, *Report EUR 25018* EN, European Union, Luxembourg, 2012. ISBN 978-92-79-21867-5

[46] Lamberty, A., Franks, K., Roebben, G., Braun, A., Kestens, V., Linsinger, T.P.J., 'Interlaboratory comparison for the measurement of particle size and zeta potential of silica nanoparticles in an aqueous suspension', *Journal of Nanoparticle Research*, Vol. 13, 2011, pp. 7317–7329.

[47] Glover, R.D., Miller, J.M., Hutchison, J.E., 'Generation of metal nanoparticles from silver and copper objects: nanoparticle dynamics on surfaces and potential sources of nanoparticles in the environment', *ACS Nano*, Vol. 5, 2011, pp. 8950–8957.

European Commission
EUR 25404 – Joint Research Centre – Institute for Reference Materials and Measurements

Title: **Requirements on measurements for the implementation of the European Commission definition of the term “nanomaterial”**

Authors: Linsinger T., Roebben G., Gilliland D., Calzolai L., Rossi F., Gibson N., Klein C.

Luxembourg: Publications Office of the European Union

2012 – 52 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424 (online), ISSN 1018-5593 (print)

ISBN 978-92-79-25602-8 (PDF)
ISBN 978-92-79-25603-5 (print)

doi:10.2787/63490

Abstract

The European Commission recently published its recommendation on a common definition of the term “nanomaterial” for regulatory purposes. Proper implementation of this definition requires appropriate tools and methodologies for which measurement aspects are crucial. This report describes the requirements for particle size measurements of nanomaterials based on the definition. It discusses the related generic measurement issues and reviews the capabilities of the measurement methods currently available. Moreover, it illustrates with practical examples the measurement issues that remain to be solved.

This report does not cover other related issues, such as the implementation of the definition by means other than through measurements, or methods to detect specific nanomaterials (such as fullerenes and single-wall carbon nanotubes), measurements to assess exposure to or effects of nanomaterials, or the detection and measurement of nanomaterials in consumer products. These will be addressed in a follow-up report.

LA-NA-25404-EN-N

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new standards, methods and tools, and sharing and transferring its know-how to the Member States and international community.

Key policy areas include: environment and climate change; energy and transport; agriculture and food security; health and consumer protection; information society and digital agenda; safety and security including nuclear; all supported through a cross-cutting and multi-disciplinary approach.

ISBN 978-92-79-25602-8

Publications Office