



The EU FP7 NanoDefine Project

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

The NanoDefine Methods Manual

NanoDefine Technical Report D7.6

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The NanoDefine Consortium

2015

NanoDefine in a nutshell:

The EU FP7 NanoDefine project was launched in November 2013 and will run until October 2017. The project is dedicated to support the implementation of the EU Recommendation on the Definition of Nanomaterial by the provision of the required analytical tools and respective guidance. Main goal is to develop a novel tiered approach consisting of (i) rapid and cost-efficient screening methods and (ii) confirmatory measurement methods. The "NanoDefiner" eTool will guide potential end-users, such as concerned industries and regulatory bodies as well as enforcement and contract laboratories, to reliably classify if a material is nano, or not. To achieve this objective, a comprehensive inter-laboratory evaluation of the performance of current characterisation techniques, instruments and software is performed. Instruments, software and methods are further developed. Their capacity to reliably measure the size of particulates in the size range 1-100 nm and above (according to the EU definition) is validated. Technical reports on project results are published to reach out to relevant stakeholders, such as policy makers, regulators, industries and the wider scientific community, to present and discuss our goals and results, to ensure a continuous exchange of views, needs and experiences obtained from different fields of expertise and application, and to finally integrate the resulting feedback into our ongoing work on the size-related classification of nanomaterials.

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Legal Note

This document contains general recommendations supporting the user in the decision whether a material is a nanomaterial according to the *EC Recommendation on the Definition of Nanomaterial*. However, users are reminded that the texts of the appropriate EC legal acts are the only authentic legal reference and that the information in this document does not constitute legal advice. Usage of the information remains under the sole responsibility of the user. The NanoDefine Consortium Partners do not accept any liability with regard to the contents of this document.

Disclaimer

Certain commercial equipment, instrument brand names and materials are identified in this document as examples or to specify adequately an experimental procedure. In no case does such identification imply recommendation or endorsement by the NanoDefine Consortium, nor does it imply that the material or equipment is necessarily the best available for the purpose and for the material under consideration.

1 Glossary

This document does not have a glossary

2 Abbreviations and acronyms

AFM Atomic Force Microscopy
ALS Angular Light Scattering
AUC Analytical Ultra Centrifugation
BET Brunauer-Emmett-Teller
CLS Centrifugal Liquid Sedimentation
CM Characterisation Method
DMAS Differential Electrical Mobility Analysis
DMPS Differential Mobility Particle Sizer
DLS Dynamic Light Scattering
DUM Dynamic Ultramicroscopy
EM Electron Microscopy
ESZ Electrical sensing zone
FFF Field-Flow-Fractionation
LD Laser Diffraction
PTA Particle Tracking Analysis
SAXS Small-angle X-ray scattering
SEM Scanning Electron Microscopy
SFM Scanning Force Microscopy
SLS Static Light Scattering
SMPS Scanning Mobility Particle Sizer
SOP Standard Operating Procedure
sp-ICP-MS Single Particle ICP-MS
TEM Transmission Electron Microscopy
TRPS Tunable Resistive Pulse Sensing
USSp Ultrasonic Spectroscopy
VSSA Volume-Specific Specific Surface Area
WP Work Package

3 Summary

The NanoDefine Methods Manual is an outcome of task 7.2 of WP7 and should be considered as a preliminary version of the Vademecum for the NanoDefiner e-tool user. The main objective of this Manual is to provide guidance through the nanomaterial characterization process, on the used characterization methods as well as their application range and their limits. It assists the user in choosing the most appropriate measurement method(s) to identify/classify any substance or mixture for a specific purpose, according to the EC recommendation for a definition of nanomaterial.

This Manual will be publically available as standalone document as well as a web-based resource. The web-based resource will consist of individual modules which can be updated and expanded as methods are improved or newly developed.

This deliverable is strongly linked to the outcomes of other WPs and inputs provided by the partners, as e.g.: methods templates to describe the characteristics of screening and confirmatory methods selected for testing in WP 3 (D7.1, D7.2), material classification system (D 7.3) or techniques evaluation report (D3.1).

As all the information required for this manual are not yet available at project midterm, some sections will be accomplished and some will be up-dated in accordance to the final outcome of various WPs. The result will be D7.7 (Methods Manual update, M48) which should be considered as a final version of the NanoDefine Methods Manual.

This version of the manual consists of a short introduction to the methods manual in which basic information on the document, its use and employed terminology are provided to the user. The NanoDefine material classification system and the evaluation system of the characterization techniques are discussed in detail in sections 6 and 7. Section 8 of the document is dedicated to sampling and discusses preliminarily standard operating procedures for sample preparation. Basic principles of the characterization methods as well as their detailed performance evaluation are presented in Section 9. The document concludes with a presentation and discussion of the NanoDefine Decision Support Flow Scheme which aims to guide the user logically through a sequence of tasks, decision nodes and options in order to decide whether a material is a nanomaterial according to the EC Definition. This framework will also allow for expert judgement at every decision node.

4 Introduction (of the Deliverable)

The task 7.2 of WP7 consists of developing a NanoDefine Methods Manual document, which should be considered as a Vademecum for the user of the NanoDefiner e-tool to give clear guidance throughout the entire nanomaterial characterization process on the used characterization methods as well as their application range and their limits. The Manual will be available as standalone document as well as a web-based resource.

This manual is strongly linked to the other WPs as it depends on the inputs provided by the partners, as e.g.: methods templates to describe the characteristics of every tier 1 (screening) method selected for testing in WP 3, including those addressing the volume specific surface area, as well as every tier 2 (confirmatory) methods. Those templates allow describing method applicability in terms of materials, sample preparation requirements (with input from WP 2), performance and reference capability, in relation to the technical performance criteria (T 7.1) and the decision criteria developed in T 7.3, including economic criteria. The confirmatory (tier 2) methods encompass (a) combined and (b) imaging methods. Application of confirmatory methods was more time consuming and expensive and required a higher degree of expertise than those in tier 1. Hence, separate templates were developed for tier 1 and tier 2 methods. The templates were filled in and used in WP 2, 3, 4 and 5 to tag the methods according to their suitability for certain materials and the decision criteria and priorities developed in T 7.1 and T 7.3. They will be reviewed and updated at the final stage of the project to take into account results of WP 4 and 5.

In section 5, the introduction of the methods manual, the way to use it and the description of the information proposed to the user are available. Developed material classification system is described in the section 6 and section 7 describes the evaluation system of the characterization methods (from D7.1). Section 8 is dedicated to the sampling and discusses standard operating procedures for sample preparation (to be added in the 7.7 version after D7.2 delivery). Basic principles as well as detail evaluation of the characterisation method are presented in the Section 9. The document is finalised with the presentation and discussion of Decision Support Flow Scheme which aims to guide the user logically through a sequence of tasks, decision nodes and options in order to decide whether a material is a nanomaterial according to the EC Definition. This framework will also allow for expert judgement at every decision node.

As all the information required for this manual are not yet available, some sections can be completed only in the D7.7 (Methods Manual update M48).

5 Introduction to the Methods Manual

Nanotechnology is a key enabling technology. Still existing uncertainties concerning EHS need to be addressed to explore the full potential of this new technology. One challenge consists in the development of methods that reliably identify, characterize and quantify nanomaterials (NM) both as substance and in various products and matrices. The European Commission has recommended a definition of NM as reference to determine whether an unknown material can be considered as 'nanomaterial' (2011/696/EU). The NanoDefine project explicitly addresses this question.

A consortium of European top RTD performers, metrology institutes and nanomaterials and instrument manufacturers has been established to mobilize the critical mass of expertise required to support the implementation of the definition. Within framework of NanoDefine project, based on a comprehensive evaluation of existing methodologies and a rigorous intra-lab and inter-lab comparison, validated measurement methods and instruments are being developed that are robust, readily implementable, cost-effective and capable to reliably measure the size of particles in the range of 1–100 nm and above, with different shapes, coatings and for the widest possible range of materials, in various complex media and products. One major outcome of the project will be the establishment of an integrated tiered approach including validated rapid screening methods (tier 1) and validated in depth methods (tier 2), with a user manual to guide end-users, such as manufacturers, regulatory bodies and contract laboratories, to implement the developed methodology for nanomaterials characterization. Consequently software, the NanoDefiner e-tool, is being developed to guide user to the most reliable and cost-efficient measurement method to identify/classify any substance or mixture according to the EC recommendation for a nanomaterial definition. This is accompanied by the NanoDefine Methods Manual.

The aim of this document is to provide a user with basic information on the nanomaterials characterisation methods, which were chosen to be incorporated in NanoDefiner tool. This manual explains the logic of choice of the method along with method evaluation process and the course of material classification applied for its assessment. Moreover this document shortly discusses and recommends dispersion, sampling and sample preparation techniques for the material characterisation. Additional detailed information on standard operating procedure (SOP) applicable to the characterization methods are included in annexes (*All annexes are scheduled to be added in the deliverable D7.7*)

In the chapter 6, the material classification scheme is detailed in order to provide explanations on how the classification was structured (mono-type particles material, multi-type particles material and product/article).

Each method described in this manual was firstly evaluated against criteria depending on the substance to analyse (the applicability to different groups of substances) and depending on the technical factors. The criteria and some definition used to assess these methods are described in the chapter 7.

Chapter 8 tackles very important issues of sample preparation; sampling and appropriate dispersion protocols (sample preparation without dispersion, sample preparation by generalized dispersion methods). It discusses sample preparation following different pathways and presents different type of protocols like standardized protocols for high priority materials groups, dedicated to microscopy methods, for product sample preparation methods or for sample carrier preparation and deposition of samples (Electron Microscopy) (*This section will be completed in the D7.7*)

Chapter 9 introduces the reader with basic information on the type of methods incorporated in the NanoDefiner tool. It discusses limitation of presented techniques and it includes the results of method evaluation, which are shown in the dedicated tables described in the first section of this manual. This chapter describes briefly following methods: The Particle tracking analysis (PTA) / Dynamic ultramicroscopy (DUM), the Single particle ICP-MS (sp ICP-MS), the Differential electrical mobility analysis (DMAS) or SMPS, the Analytical Centrifugation (AC), the Tunable Resistive Pulse Sensing (TRPS) / Electrical sensing zone (ESZ) / nano Coulter counter, the Ultrasonic spectroscopy (USSp), the Dynamic Light Scattering (DLS), the Small-angle X-ray scattering (SAXS), the BET for determination of volume-specific surface area (VSSA), and the porosimetry techniques for determination of the outer volume-specific surface area. (*SOPs will be described in details in annexes which are foreseen to be added in D7.7*).

Finally, this chapter also describes methods like e.g. Electronic Microscopy (EM), Atomic Force Microscopy (AFM (or SFM)), Field Flow Fractionation (FFF) (simplified low-cost system, coupled to counting

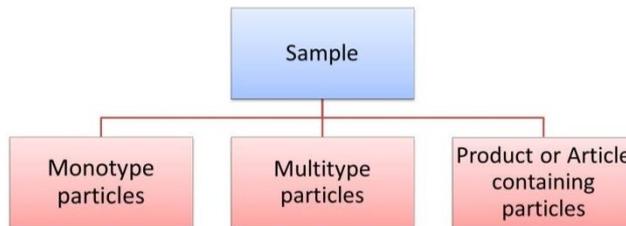
techniques, and conventional FFF with separation-multidetector analysis on standard and complex samples) which are recommended in the e-tool.

In the chapter the Decision Support Flow Scheme is presented and discussed, which aims to guide the user logically through a sequence of tasks, decision nodes and options in order to decide whether a material is a nanomaterial according to the EC Definition. This framework will also allow for expert judgement at every decision node.

6 Material Classification System

The aim of this section of the document is to give a user a general overview of the material classification system developed in NanoDefine project and applied in NanoDefiner e-tool. Each of the classification criteria is discussed in details and the logical pathway for nanomaterials sorting is shown on the examples. Thus considering the performance characteristics of specific methods, i.e. what kind of material they actually can characterise in a reliable way, we propose three main classes of materials as main drivers of this classification scheme (see figures below):

- **Monotype particles:** all particles have essentially the same chemical and structural composition. An ensemble of monotype particles may constitute a 'nanoform' as defined by the European Chemicals Agency¹ and a 'discrete form' of substance in nanoscale as defined by US EPA². A non-nanoform under specific conditions.
- **Multitype particles:** a material that consists of particles of different chemical or structural compositions.
- **Articles and consumer products that contain mono- and/or multitype particles.**



| Monotype particles | | Multitype particles | | Product or article containing particles | |
|--------------------|---------------------|---------------------|--|---|--|
| Particles | Composite particles | | | Different types of particles | |
| | | | | | |

If the particles are all of the same type, a characterisation method needs to be suitable for that type only, whereas if a material consists of different particle types, the chosen technique should be applicable to all particle types present.

An article Error! Bookmark not defined..Error! Bookmark not defined. according to REACH and relevant guidance provided by ECHA is an object which, during production, is given a special shape, surface or design which determines its function to a greater degree than does its chemical composition. Such an article could contain different chemical particles. This category includes also **consumer products**,² which is defined according to CEN as items intended for consumers or likely to be used by consumers, even if not intended for them.

As nanomaterials have specific structural properties additional definition of **composite** and **matrix** was developed within the framework of NanoDefine project and used in the e-tool.

Composite is a solid substance that consists of two or more phase-separated constituents

Depending on the internal structure, **particles of the composite** can be divided into three types:

| | |
|--|---|
| Core-shell particles: are particles which have a core of one constituent embedded in another constituent. |  |
| Particles with multiple coatings: are particles that consist of successive layers of various constituents. |  |
| Particles composed by a mix of two or more different constituents: are particles that consist of two or more constituents homogeneously or not present in the structure. |  |

In NanoDefine project a **matrix** was defined as: a non-particulate constituent or component of a material.

The developed NanoDefiner materials classification scheme is summed up on the Figure 1.

One should keep in mind that the presented Materials Classification Scheme was created to enable the appropriate choice of the nanomaterial method characterisation. Consequently in the scheme much additional information appears which are not required in the EC recommendation for nanomaterial definition like e.g. chemical composition, but are considered to be essential for the correct application of the NanoDefiner tool.

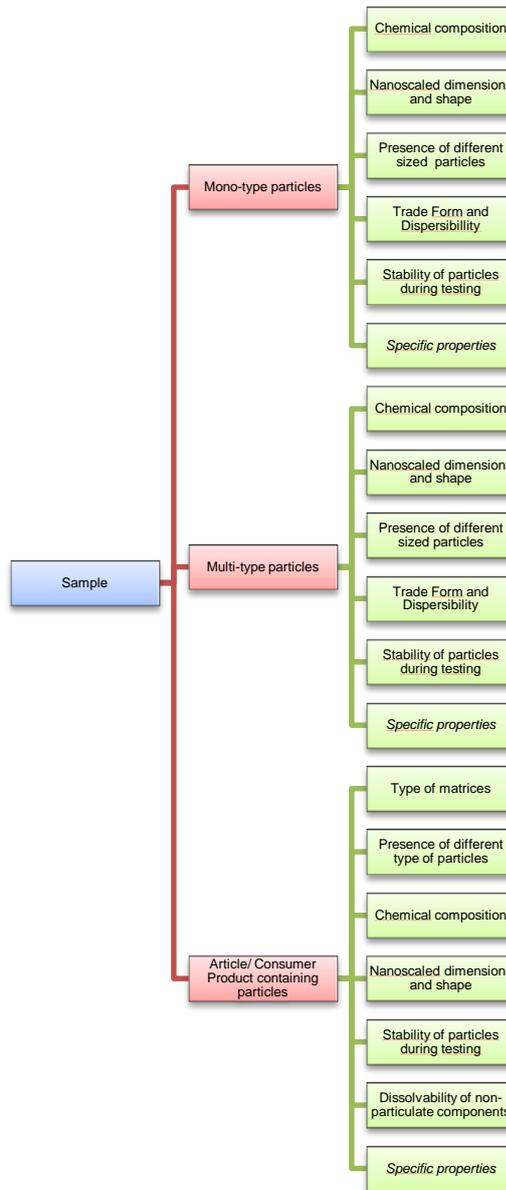


Figure 1: Overview of the material classification system

To classify a material the crucial information is if the material to analyse is a mono-type, multi-type or an article a commercial product (embedded in a solid or liquid/gel matrix). This information determines which other criteria have to be taken into account in the classification process.

Optional parameters for classification are written in italic style. In each of the following sections (part 6.1, 6.2 and 6.3), the decision framework for material classification is provided in detail. Moreover, a figure summarizing the classification is given at the beginning of each section.

Section **6.1** discusses details of the classification if the material to characterise is a **mono-type particles material**. In section **6.2**, guidance is proposed and explained case of **multi-type particles material**. Finally, section **6.3** provides details for the decision framework if an **article/ consumer product** has to be characterised.

6.1 Mono-type substance

As it was already explained a mono-type particles all particles which have essentially the same chemical and structural composition It has to be underlined that mono-type substance still may consist of

many constituents, unintentionally present impurities or intentionally added additives to stabilise the substance. Presence of all these substances may have a great influence on the final results generated by NanoDefiner tool and therefore should be considered for reporting while applying materials classification scheme. It is also important to notice that if the composition of the substance is not known or doubtful the user should continue the analysis in the "multi-type" particles scheme.

Figure 2 below shows in detail the classification scheme for the mono-type particles. Each endpoint considered is further described in detail below.

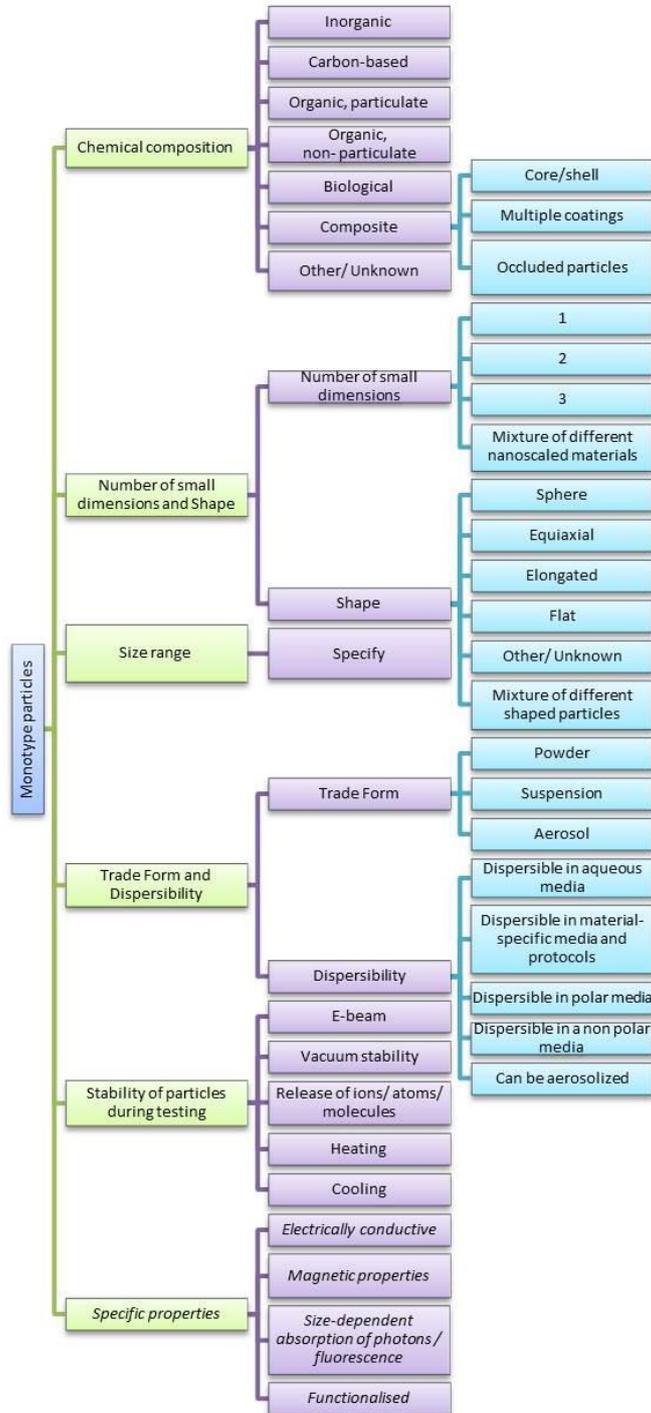


Figure 2: Overview of the material classification system for mono-type particles

6.1.1 Chemical Composition

Though not explicitly required by the EC recommendation of definition for nanomaterial, due to the method characterisation futures, knowledge of the chemical identity of the material is important for the selection of the appropriate sizing method or if certain materials need specific attention.

- Inorganic materials such as metals, ceramics, salts, oxides (significant content of inorganic elements homogeneously incorporated in all constituent particles)
- Carbon-based (CNT, nanodiamond, carbon black...)
- Organic, particulate (polymers, dyes, etc..)
- Organic, non-particulate (dendrimers, liposomes, supramolecular assemblies...)
- Biologic (nucleic acid, peptide, protein)
- Composite
- Other/ Unknown

6.1.1.1 Composite structure

Composite is a solid substance that consists of two or more phase-separated constituents. Depending on the internal structure, **particles of the composite** can be divided into three types:

| | |
|--|---|
| Core-shell particles: are particles which have a core of one constituent embedded in another constituent. |  |
| Particles with multiple coatings: are particles that consist of successive layers of various constituents. |  |
| Particles composed by a mix of two or more different constituents: are particles that consist of two or more constituents homogeneously or not present in the structure. |  |

6.1.2 Dimensions and shape

The particles shape³ and the number of small dimensions of the substance to be analysed have also to be known and be integrated in the material classification.

The number of small dimensions is:

- 1
- 2
- 3
- Mixture of different small particles

The shape of nanomaterials is classified as below:

- Sphere or irregular globular
- Equiaxial (Prismatic, Cubic, Tetrahedral)

- Elongated (Tubes, fibres, rods)
- Flat (irregular flakes and discs)
- Mixture of different shaped nanoparticles
- Other / Unknown

6.1.3 Presence of particles outside the nanoscale

This category should be applied if the user deals with a material in which a great part of particles is bigger or smaller than nanoparticles or if the type of the nanoparticle in the material is unknown. The substance to analyse is classified as below:

- Significant presence of particles smaller than nanoparticles ($d < 1\text{ nm}$ in all dimensions)
- Significant presence of particles bigger than nanoparticles ($d > 100\text{ nm}$ in all dimensions)
- The user does not know which kind of particles is mixed with the nanoparticles to analyse

6.1.4 Trade Form and Dispersibility

6.1.4.1 Trade Form (including samples used for R&D purposes)

This criterion concerns the trade form of the material. Is it

- A powder?
- A suspension?
- An aerosol?

6.1.4.2 Dispersibility

It is essential to determine if the substance to analyse can be dispersed by standardized protocols that specify both a dispersion medium and a dispersion protocol in order to determine which characterisation method could be applied for the analysis.

- Dispersible in aqueous media (by generalized protocols)
- Dispersible in material-specific media and protocols
- Can be aerosolized

6.1.5 Stability of particles during testing

The stability of the substance during testing is also important in order to decide which characterisation method could be applied.

Does the user know if the substance is stable when applying:

- Electron beam?
- Vacuum?
- Heating? If yes, specify above which temperature in °C

- Cooling? If yes, specify below which temperature in °C
- Does the material release ions/ atoms/molecules in its environment under certain conditions (heating, solutions etc.)?

6.1.6 Specific properties

Some specific properties of the material such as conductivity or surface functionalisation could also be important for the classification of the material, thus additional information on the specific properties of the analysed material are needed.

Does the user know if the substance to analyse

- is electrically conductive? If it is known, specify if the material is conductive, semi conductive or insulating.
- has some magnetic properties? If yes, specify which ones.
- exhibits size-dependent absorption of photons/fluorescence? If yes, specify which technique could be used for characterisation. (UV, fluorescence spectrometry)
- is functionalised or not? If yes, specify the composition of functionalization if it is known.
- does the outer part of particles have some special characteristics (hydrophobic, hydrophilic, reactive groups)? If yes, specify which ones.

6.1.7 Example

In this part, an example is given in order to illustrate how the classification for a mono-type particles could be used.

6.1.7.1 Example: SWCNT

In figure 3 below, the substance to analyse is a sample of single walled carbon nanotubes (SWCNTs). Those CNTs could be dispersed in DMF, exhibit semi-conductive properties and are not functionalised. As the user knows that Raman spectroscopy could be used to calculate the diameter for this type of CNTs, the size dependent spectroscopy box is filled in. The user makes the hypothesis that there are no other nanoparticles so he does not fill the corresponding box.

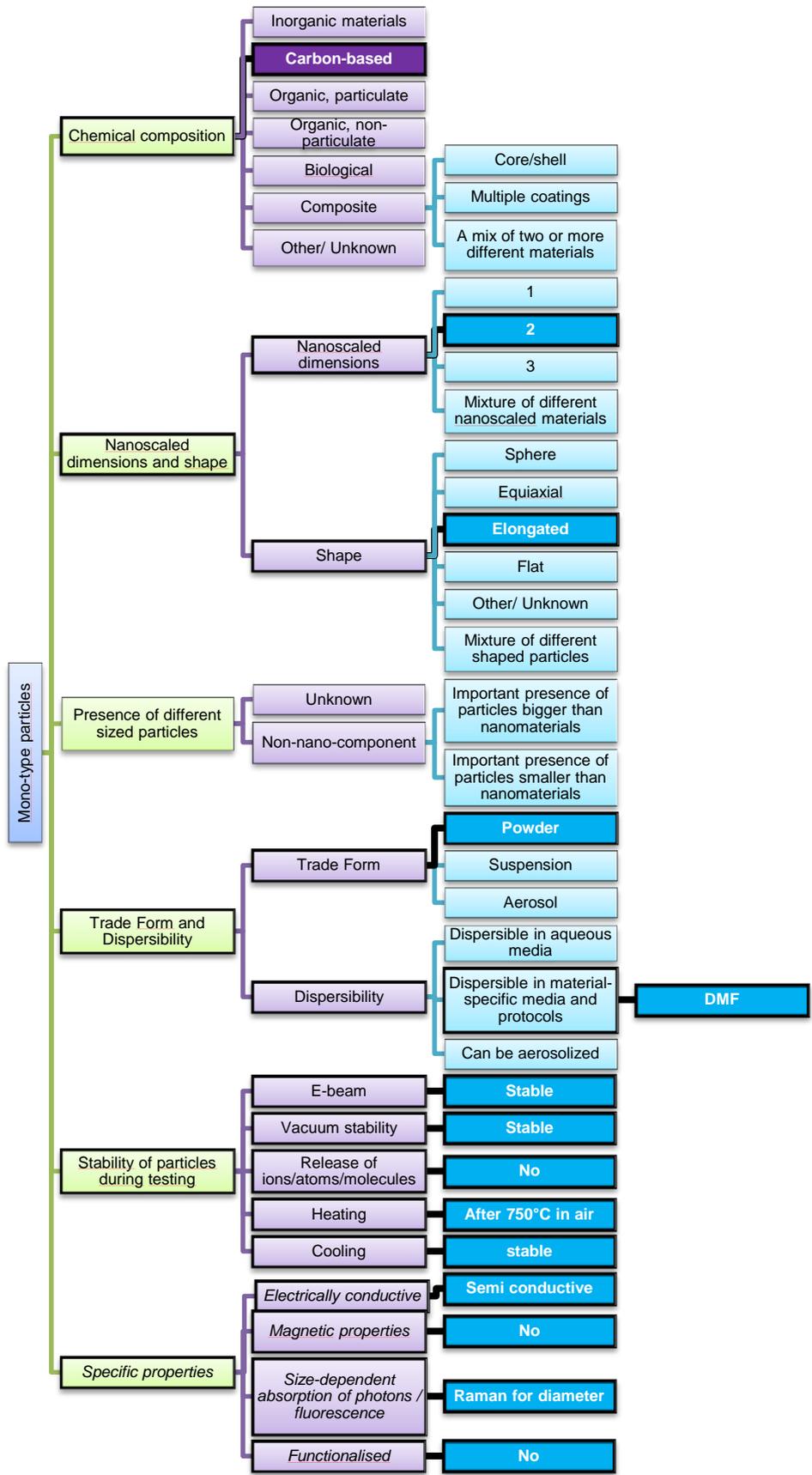


Figure 3: Example of the use of the material classification system (CNTs)

6.1.7.2 Example: silica nanospheres

In the figure 4 below an example of the classification of silica nanospheres is shown. The trade form of this material is powder. These nanoparticles could be dispersed in water with specific surfactant and need a control of pH. The user knows that there is a large amount of bigger particles present in the material. He knows also that those nanoparticles are sensitive to heating above 600 °C. He chooses not to fill in the specific properties chart box as it is not required.

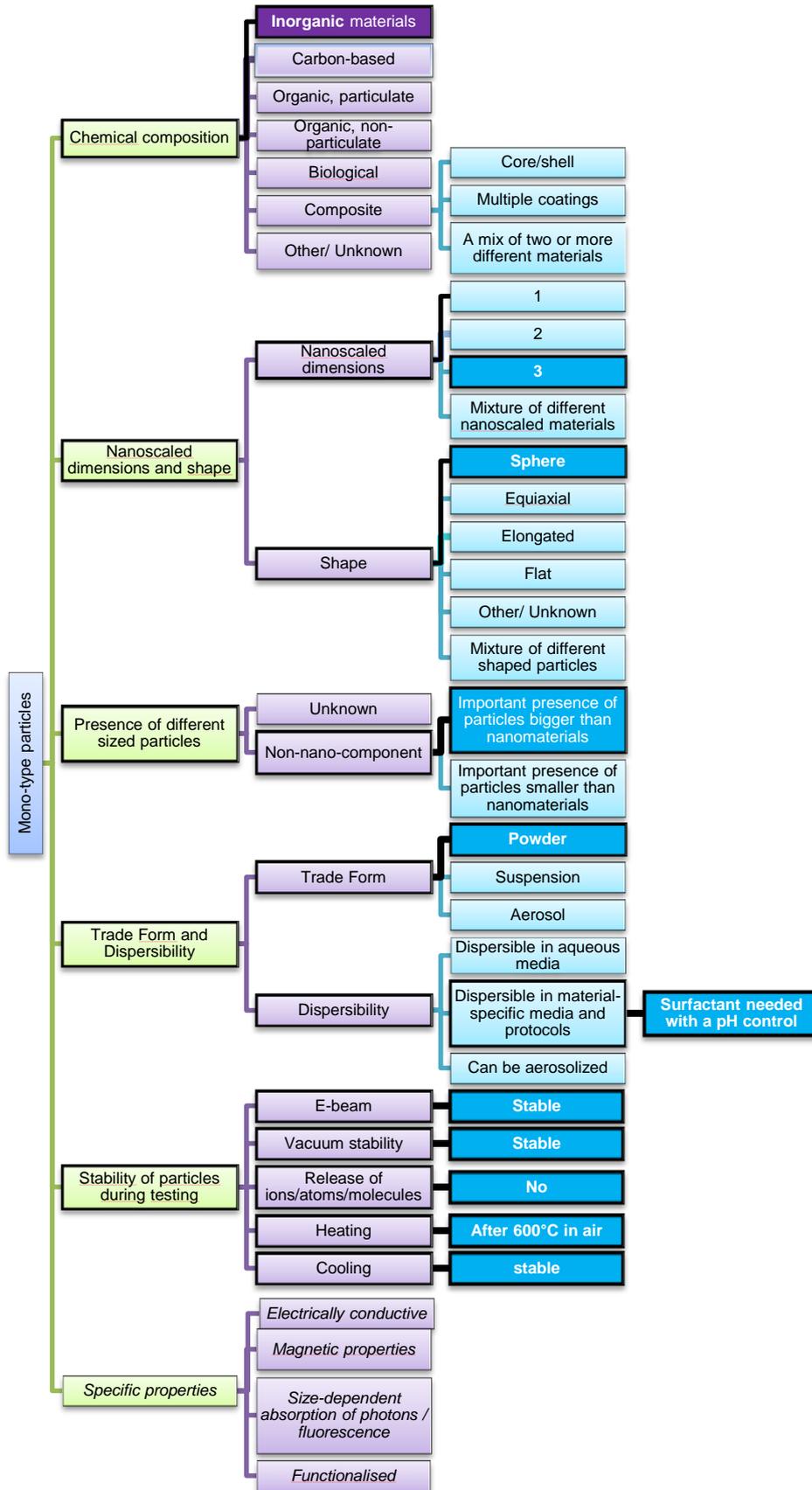


Figure 4: Example of the use of the material classification system (Silica nanospheres)

6.1.7.3 Example: a core/shell composite (nanoparticles of gold coated with silver)

In the figure 5 below, an example of classification scheme for the substance which consists of gold and silver nanospheres is shown. The composite structure of this material consists of golden core and a silver shell. These particles are suspended in colloidal suspensions. The user doesn't know if there are bigger or smaller particles so he fills the "unknown" box. These nanoparticles could be suspended in water with CTAB or SDS surfactants. As the user knows that Surface Plasmon Resonance technique could be used to calculate the diameter for this type of particles, the size dependent spectroscopy chart box is filled in.

6.2 Multi-type particles

As previously explained Multi-type particles means a material that consists of particles of different chemical or structural compositions.

Materials being a result of both or chemical reaction or physical blending falls into this category if they consist of different type of particles.

Figure 6 explains in detail the classification of the material which should be characterised as multi-type particles material. Optional parameters for classification are written in italic style.

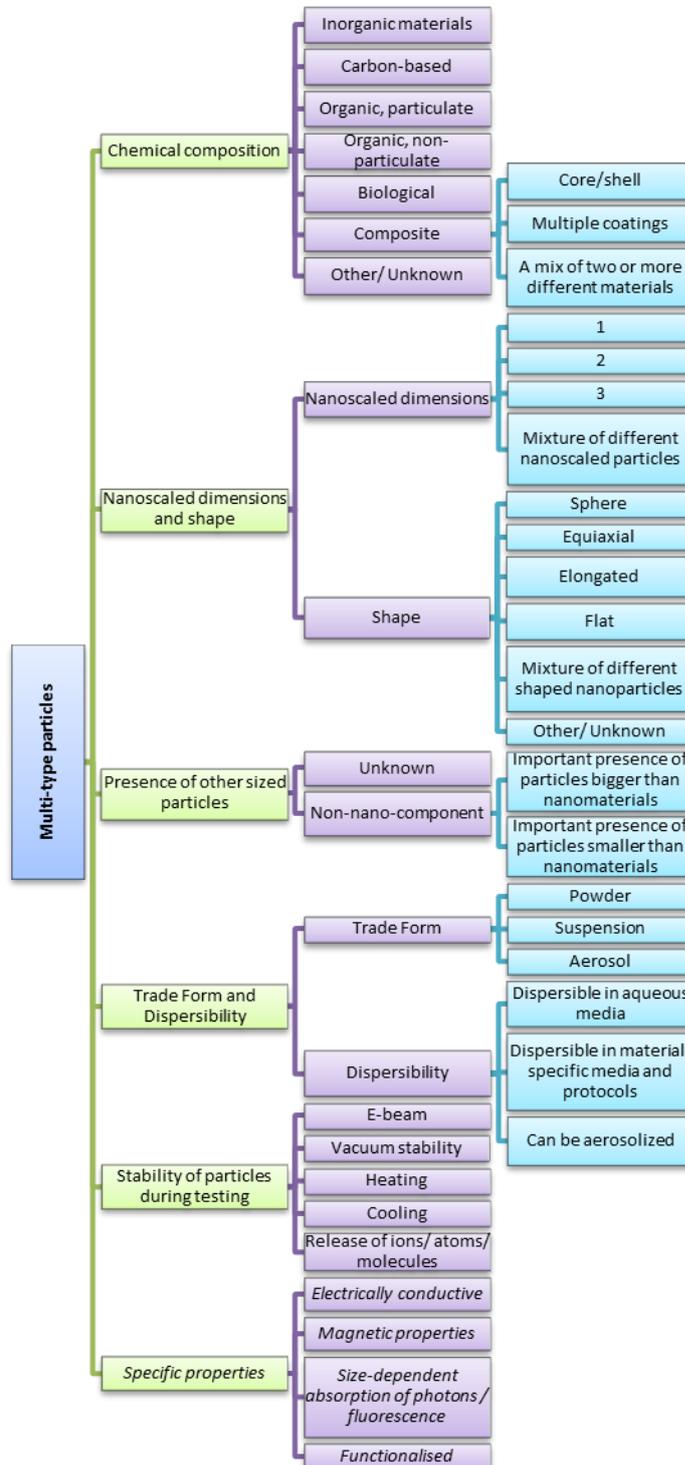


Figure 6: Overview of the material classification system for mono-type particles

6.2.1 Chemical composition

The chemical nature of the mixture is important for the application of specific analytical methods.

- Inorganic materials such as metals, ceramics, salts, oxides (significant content of inorganic elements homogeneously incorporated in all constituent particles)
- Carbon-based (CNT, nanodiamond, carbon black...)
- Organic, particulate (polymers, dyes, etc.)
- Organic, non-particulate (dendrimers, liposomes, supramolecular assemblies...)
- Biological (nucleic acid, peptide, protein)
- Composite
- Other/ Unknown

6.2.1.1 Composite structure

Composite is a solid substance that consists of two or more phase-separated constituents

Depending on the internal structure, **particles of the composite** can be divided into three types:

| | |
|--|---|
| Core-shell particles: are particles which have a core of one constituent embedded in another constituent. |  |
| Particles with multiple coatings: are particles that consist of successive layers of various constituents. |  |
| Particles composed by a mix of two or more different constituents: are particles that consist of two or more constituents homogeneously or not present in the structure. |  |

6.2.2 Small dimensions and shape

The shape⁵ and their small dimensions of the mixture to analyse have to be also known and integrated in the material classifications.

The number of small dimensions is:

- 1
- 2
- 3
- Mixture of different small particles (for example nanotubes and nanospheres)

The shape of particles is classified as below:

- Sphere or irregular globular
- Equiaxial (Prismatic, Cubic, Tetrahedral)
- Elongated (Tubes, fibres, rods)
- Flat (irregular flakes and discs)
- Mixture of different shaped nanoparticles
- Other / Unknown

6.2.3 Presence of other sized nanoparticles

It is relevant to know if the mixture is made only with nanoparticles or if it is a mixture of bigger and smaller particles.

The mixture to analyse can be classified as below:

- Significant presence of particles smaller than nanoparticles ($d < 1$ nm in all dimensions)
- Significant presence of particles bigger than nanoparticles ($d > 100$ nm in all dimensions)
- The user does not know which kind of particles is mixed with the nanoparticles to analyse

6.2.4 Trade form and Dispersibility

6.2.4.1 Trade Form (including samples used for R&D purposes)

This criterion concerns the trade form of the material. Is it

- A powder?
- A suspension?
- An aerosol?

6.2.4.2 Dispersibility

It is essential to know if the mixture to analyse can be dispersed by standardized protocols that specify both a dispersion medium and a dispersion protocol.

- Dispersible in aqueous media (by generalized protocols)
- Dispersible in material-specific media and protocols
- Can be aerosolized

6.2.5 Stability during testing

The stability of the mixture to analyse is also important to know which characterisation method could be suitable.

Does the user know if the substance is stable when applying:

- Electron beam?
- Vacuum?
- Heating? If yes, specify above which temperature in °C
- Cooling? If yes, specify below which temperature in °C • Does the material release ions/ atoms/molecules in its environment under certain circumstances (like e.g. heating, solution etc.)?

6.2.6 Specific properties

Some specific properties of the material such as conductivity or surface functionalisation could also be important while choosing appropriate characterisation method. Thus additional information on the specific properties of the analysed material is needed.

Does the user know if the substance to analyse

- is electrically conductive? If it is known, specify if the material is conductive, semi conductive or insulating.
- has some magnetic properties? If yes, specify which ones.
- exhibits size-dependent absorption of photons/fluorescence? If yes, specify which technique could be used for characterisation. (UV, fluorescence spectrometry)
- is functionalised or not? If yes, specify if the nanoparticles are functionalised or not. If known, specify the composition of the functionalisation
- Does the surface of particles have some special characteristics (hydrophobic, hydrophilic, reactive groups)? If yes, specify which ones

6.2.7 Examples

Here an example is given in order to illustrate how this scheme of classification could be applied.

6.2.7.1 Example of a multi-type particles material: physical blending

In the figure 7 below an example of mixture composed of silica and polystyrene spherical particles is presented. The supplier who gives this mixture to the user declares that the material is made with nanoparticles. The user does not know if there are some bigger or smaller particles present in the material. This mixture could be dispersed according to protocols with specific surfactant and needs a control of pH. As the user knows this mixture is not sensitive to e-beam but ignores in which temperature nanoparticles could be degraded, he writes in the box that he does not know those parameters. The user fills also optional parameters such as: particles are not functionalised, not conductive and do not exhibit magnetic properties.

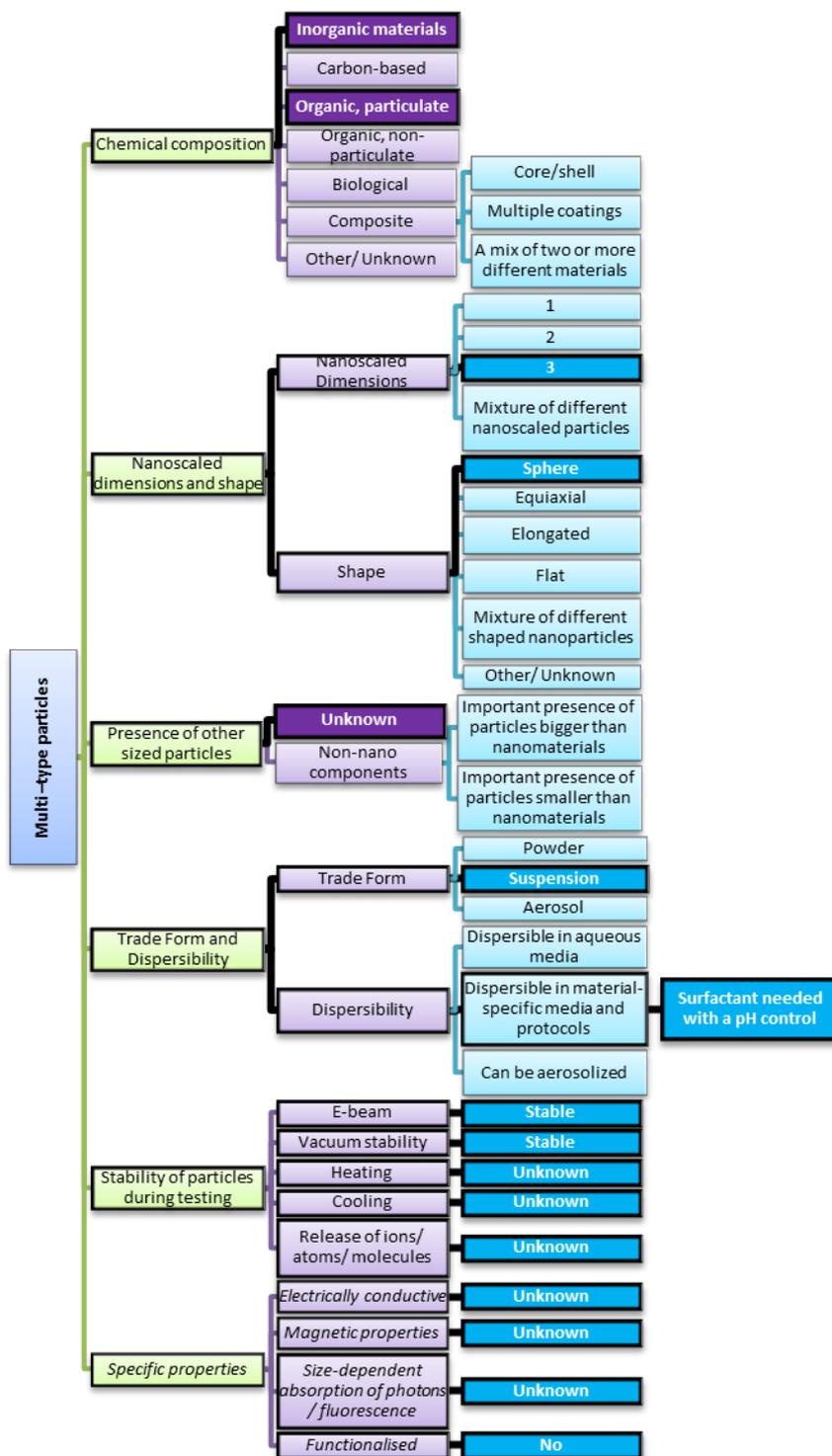
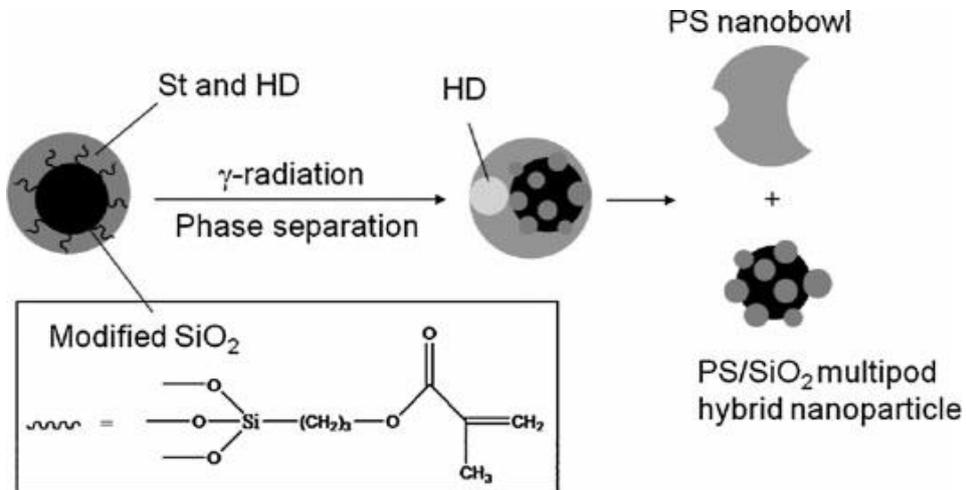


Figure 7: Example of the use of the material classification system (mixture of polystyrene and silica spheres).

6.2.7.2 Example of a multi-type particles material

This example illustrates how to apply classification scheme to the material which is a product of the re-

action described in by Ge X. ^[4] and shown on the figure below. In this reaction two types of nanoparticles are produced: polystyrene nanoparticles and polystyrene and silica composite nanoparticles. The user knows that the material under consideration consists of particles which are nanometric in three dimensions and that this material contains particles with different shapes and a large amount of bigger nanoparticles. This material is available as a colloid and it can be suspended in water by employing surfactant such as SDS.



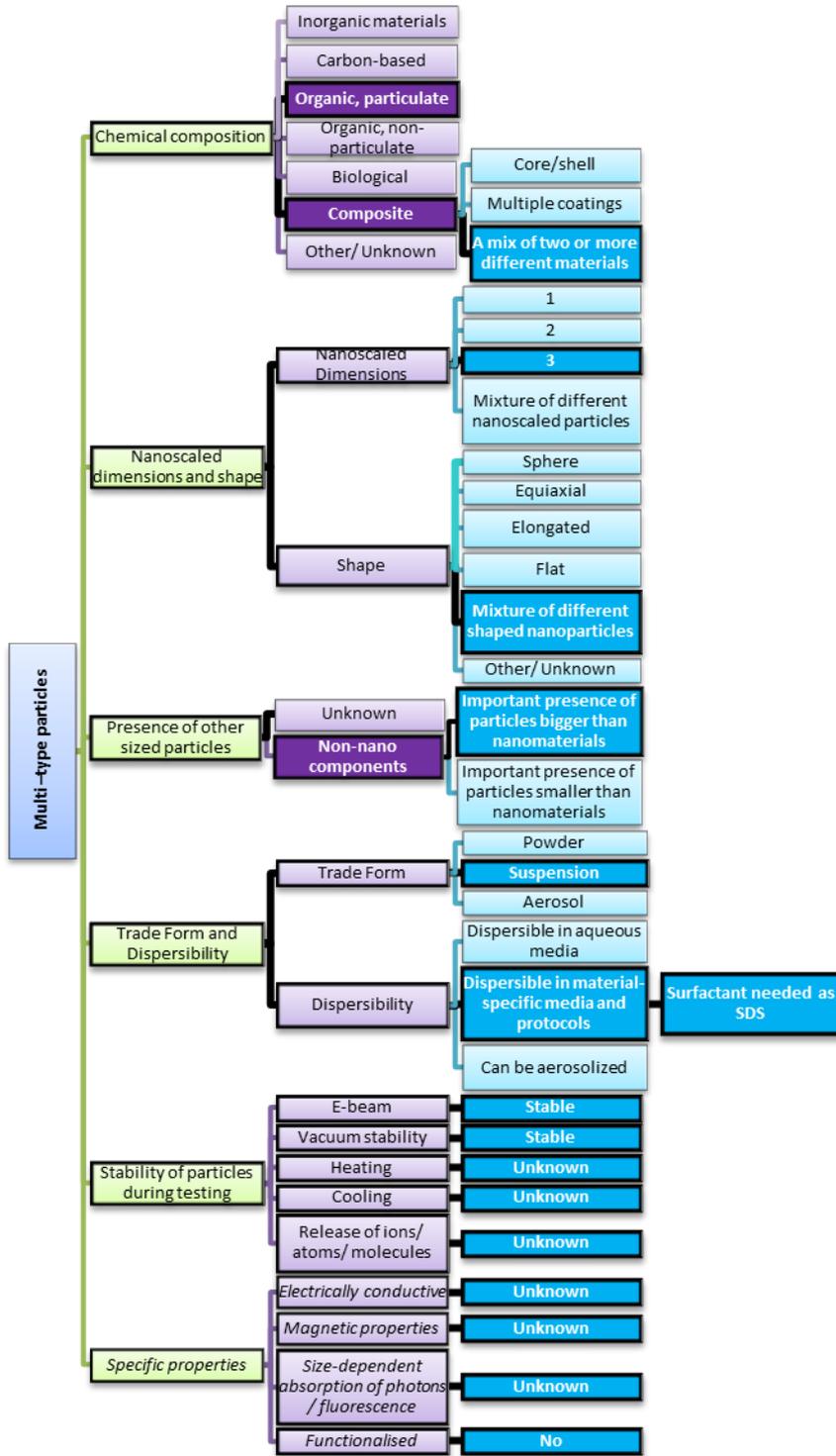


Figure 8: Example of the use of the material classification system (multi-type material of polystyrene and polystyrene/silica nanoparticles)

6.3 Article or Product

The figure 9 below details the classification system in case an article or a consumer product has to be characterised. Optional parameters for classification are written in *italic style*.

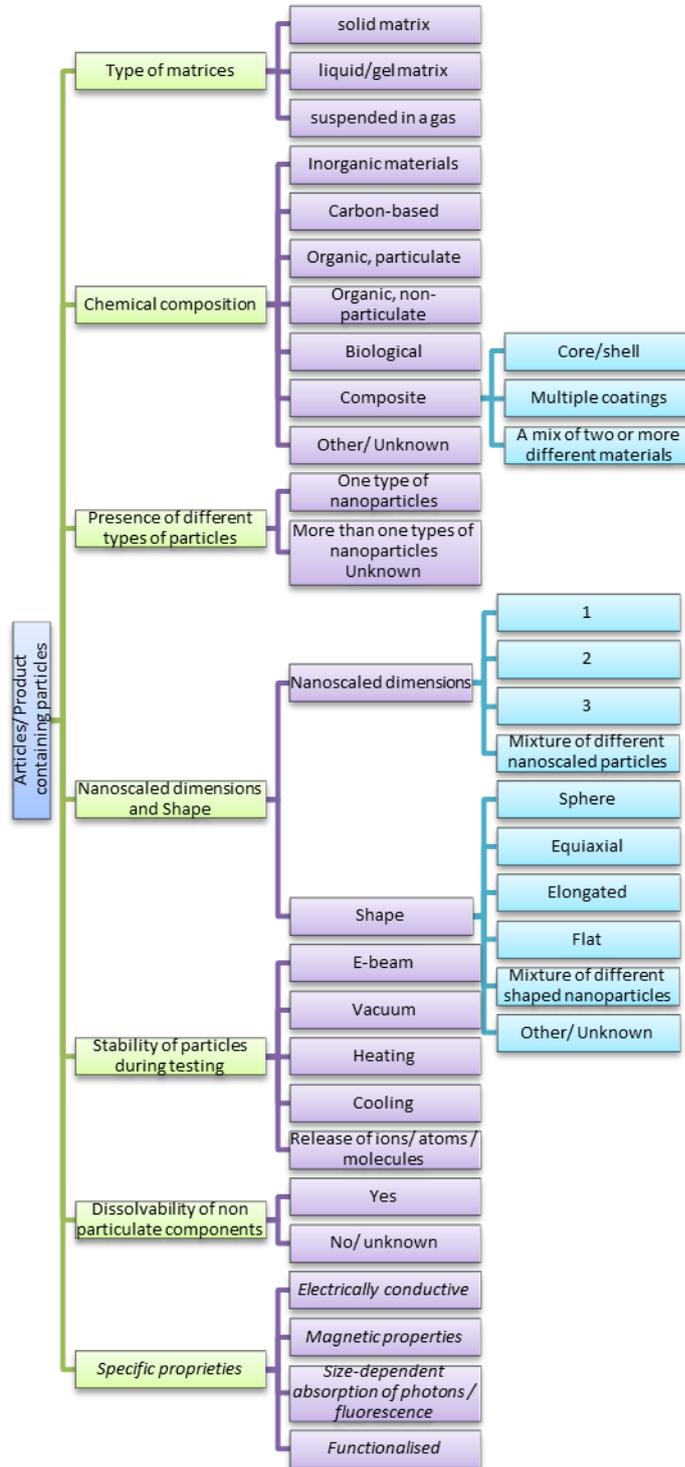


Figure 9: Overview of the material classification system in case of an article or a product

6.3.1 Type of matrix

A matrix is a non-particulate constituent or component of a material.

The type of matrices in which particles are embedded or suspended is an important criterion which must be taken into account. The product to analyse can be classified as below:

- embedded in a solid matrix
- embedded in a liquid/gel matrix
- suspended in a gas (aerosol...)

6.3.2 Chemical composition

The chemical nature of particles is important for the application of specific analytical methods.

- Inorganic materials such as metals, ceramics, salts, oxides (significant content of inorganic elements homogeneously incorporated in all constituent particles)
- Carbon-based (CNT, nanodiamond, carbon black...)
- Organic, particulate (polymers, dyes, etc.,)
- Organic, non-particulate (dendrimers, liposomes, supramolecular assemblies...)
- Biological (nucleic acid, peptide, protein)
- Composite
- Other/ Unknown

6.3.2.1 Composite structure

If the particles have a composite structure the definition below should be employed for the classification of the material.

Composite is a solid substance that consists of two or more phase-separated constituents

Depending on the internal structure, **particles of the composite** can be divided into three types:

| | |
|--|---|
| Core-shell particles: are particles which have a core of one constituent embedded in another constituent. |  |
| Particles with multiple coatings: are particles that consist of successive layers of various constituents. |  |
| Particles composed by a mix of two or more different constituents: are particles that consist of two or more constituents homogeneously or not present in the structure. |  |

6.3.3 Presence of different types of particles

It can be relevant to know if the article/consumer product contains of chemically different types of particles. Two cases are possible:

- One type of particles
- More than one type of particles/ unknown

If the user does not know, he should select the second category.

6.3.4 Small dimensions and shape

The shape⁵ and the small dimensions of the particles present in the product are also integrated in the material classification.

The number of small dimensions is:

- 1
- 2
- 3
- Mixture of different small particles (for example nanotubes and nanospheres)

The shape of nanomaterials is classified as below:

- Sphere or irregular globular
- Equiaxial (Prismatic, Cubic, Tetrahedral)
- Elongated (Tubes, fibres, rods)
- Flat (irregular flakes and discs)
- Mixture of different shaped nanoparticles
- Other / Unknown

6.3.5 Stability of particles during testing

The stability of the product to analyse is also important to know in order to apply an appropriate characterisation method.

Does the user know if the substance is stable against

- Electron beam?
- Vacuum?
- Heating? If yes, specify above which temperature in °C
- Cooling? If yes, specify below which temperature in °C
- Does the material release ions/ atoms/molecules in its environment?

6.3.6 Dissolvability of non-particulate components

The ability of the matrix to be dissolved or not is an important criteria to determine which characterisation method would be appropriate. The material could be classified as below:

- non particulate components of the matrix can be dissolved. If yes, give details under which conditions.
- non particulate components of the matrix cannot be dissolved/ unknown

Note: Further information will be given in a later stage of the project (decision framework manual)

6.3.7 Specific properties

Some specific properties of the material such as conductivity or surface functionalisation could also be important thus additional information on the specific properties of the analysed article/consumer product are needed.

Does the user know if the substance to analyse

- is electrically conductive? If it is known, specify if the material is conductive, semi conductive or insulating.
- has some magnetic properties? If yes, specify which ones.
- exhibits size-dependent absorption of photons/fluorescence? If yes, specify which technique could be used for characterisation. (UV, fluorescence spectrometry)
- is functionalised or not? If yes, specify if the nanoparticles are functionalised or not. If known, specify the composition of functionalisation.
- Does the surface of particles have some special characteristics (hydrophobic, hydrophilic, reactive groups)? If yes, specify which ones.

6.3.8 Example

Here, an example is given in order to illustrate how the classification scheme could be used.

In figure 10 below, the product to analyse is a sunscreen with titanium dioxide nanoparticles. Nanoparticles have irregular shape. The cream can be studied with electron microscopy after dilution but the user does not know if the product is sensitive to heating and cooling. The matrix can be dissolved easily. The user does not know specific properties of the product so he does not fill the corresponding boxes.

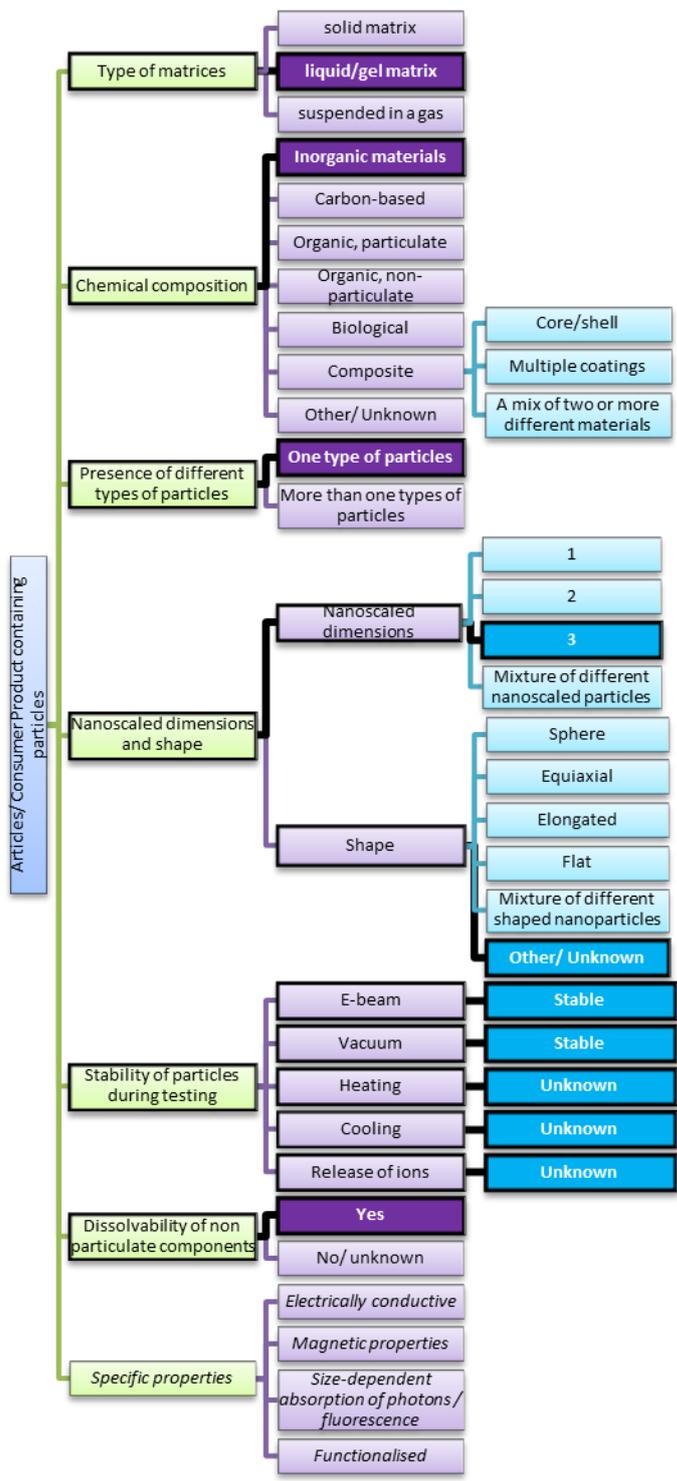


Figure 10: Example of the use of the material classification system (sunscreen with titanium dioxide particles)

7 Performance criteria

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

Each characterisation method was evaluated against criteria depending on the substance to analyse (if the characterisation method is suitable for this type of substance) and on the technical factors. The applied criteria, discussed below are not listed according to their priority.

7.1 Applicability to different groups of substances

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

Certain characterisation methods are only applicable to nanoparticles dispersed in a liquid phase; others for aerosols or for powders and granulates. For the characterization of nanomaterials e.g. in articles or consumer products it is necessary to know if a method can characterise nanoparticles when they are embedded in a matrix). One should also take into consideration that some nanoparticles may change significantly during sample preparation, hence excluding certain techniques. As the physical state of the sample has a major impact on the choice of the characterization method the primary grouping was done based on the type of the sample physical state:

- Dispersed in liquids (pure or not)
- Solid particulate form (powder...)
- Dispersed or embedded in different types of matrices (paste, resin, elastomer...)

7.1.2 Dispersibility according to dispersion protocols

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

Size distribution can be affected by sample preparation protocol, such as the choice of the dispersion media and the particle concentration but also by aggregation and agglomeration if the dispersant is not appropriately chosen. It should be underlined that nanoparticles surface chemistry strongly affects the dispersibility properties of nanomaterials hence dividing them in five major groups:

- Dispersible in aqueous media (by generalized protocols)
- Dispersible in non-polar liquids (by generalized protocols)
- Dispersible in polar liquids (by generalized protocols)
- Dispersible in material-specific media (by specific protocols)
- Can be aerosolized

7.1.3 Substance Nature

One of the most important criterions is the nature of the substance itself considering that the characterisation method has to be chosen and/or adapted accordingly. For instance, a UV-Vis spectrometry can only serve as size measurement technique if the material exhibits Surface Plasmon Resonances, e.g. silver or gold nanoparticles.⁵

Consequently materials can be grouped as follows:

- Inorganic materials such as metals, ceramics, salts, oxides (significant content of inorganic elements homogeneously incorporated in all constituent particles)
- Particles which exhibit size-dependent absorption of photons / fluorescence (metals, quantum dots⁶...)
- Carbon-based (CNT, Nanodiamond, Carbon Black...)
- Organic, particular (Polymers, dyes, etc., nanonized, precipitated)

- Organic, non-particular (Dendrimers, Liposomes, Supramolecular assemblies...)
- Biological (nucleic acid, peptide, protein)
- Composite
- Other

7.1.3.1 Composite structure

Composite is a solid substance that consists of two or more phase-separated constituents. Depending on the internal structure, **particles of the composite** can be divided into three types:

| | |
|--|---|
| Core-shell particles: are particles which have a core of one constituent embedded in another constituent. |  |
| Particles with multiple coatings: are particles that consist of successive layers of various constituents. |  |
| Particles composed by a mix of two or more different constituents: are particles that consist of two or more constituents homogeneously or not present in the structure. |  |

If the substance to be analysed is a composite it is important to know more about its structure in order to apply a suitable characterisation method including the correct data evaluation. The structure of a composite material to be analysed can lead to different results depending on a characterization method employed. Hence, it is necessary to know if a specific technique is able to determine the particle size without interference caused by the composite structure

7.1.4 Particle shape and number of small dimensions

Some methods may be inappropriate for characterization of particle with certain shape. In this context, knowledge of the number of small dimensions of the particle is very important factor influencing the choice of the characterization method.

The number of small dimensions is:

- 1
- 2
- 3

Mixture of different small particles (for example nanotubes and nanospheres)

The shape of the particles can be divided in five general groups:

- Sphere
- Equiaxial (Prismatic, Cubic, Tetrahedral)
- Elongated (Tubes, fibres, rods)
- Flat (Flakes and discs)
- Other

7.1.5 Thermal degradation sensitivity

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

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

7.1.6 Cooling degradation sensitivity

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

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

7.1.7 E-beam sensitivity

Some substances are sensitive to electron beam, and therefore cannot be characterised reliably with EM, or require more sophisticated EM techniques, such as cryo-EM or low-dose techniques. This criterion enables to know if it would be relevant to analyse those substances with the respective method.

- Sensitive to e-beam
- Not sensitive to e-beam

7.1.8 Sample dispersity and modality

Almost all samples are polydisperse to a certain degree. Therefore it is important to recognize if a specific characterisation method can be used to analyse polydispersed samples without obtaining false results. Hence dispersity and modality of the sample has to be considered as an important factor in the performance of the method.

- Monodisperse sample
- Polydisperse sample
- Multimodal sample
- Monomodal sample

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

7.1.9 Optional criteria

7.1.9.1 Electrical conductivity

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

- Conductive

- Semiconductive
- Insulator

7.1.9.2 Magnetic properties

Magnetic particles may be characterised with some specific measurement techniques such as magnetic force microscopy (MFM)⁷ or magnetic particle spectrometer.^{8,9} Thermomagnetic,¹⁰ dc magnetization¹¹ and ac susceptibility¹² measurements enable also to determine the size distribution of magnetic particles. In this regard, nanoparticles could be classified as:

- Magnetic
- Non magnetic

7.1.9.3 Functionalisation / no functionalization on the surface

One important criterion is to know if the nanoparticles are functionalised on the surface or not. Characterisation methods have to be adapted according to this feature. More details about functionalization are shown in the chapter 7 Material classification system.

- Functionalised
- Not functionalised

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

7.1.9.4 Agglomeration/ aggregation state

In some substances nanoparticles are aggregated or have a tendency to agglomerate. This specific feature of the material needs to be accounted for in the performed data analysis. To this end nanoparticles can be classified as:

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

7.2 Method performance parameters and criteria

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

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

7.2.2 Working range

Working range is a range in which the method provides reliable results (may be dependent also on the material and its preparation and on instrument type).

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

7.2.3 Trueness

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

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

7.2.4 Robustness

Robustness is defined as a Influence of slight variations in the test protocol on the outcome of the test.

7.2.5 Precision

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

7.2.6 Resolution

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

7.2.7 Size distribution

The following criterion was considered: Does the method provide a size distribution¹³ or a certain average value?

7.2.8 Selectivity

Selectivity is how well can nanoparticles according to the EC recommendation for the nanomaterial definition be distinguished from

- non-nanoparticles of the same composition
- non-nanoparticles of another composition (matrix particles, mixtures of powders)
- nanoparticles of another composition (mixtures of powders)

Moreover, if the substance to analyse is not pure, it is essential to know if and how the results are affected by impurities, including dissolved ionic species from the same substance.

7.2.9 Capability to measure aggregation

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

7.2.10 Capability to measure single particles

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

7.2.11 Counting constituent particles in aggregates

Is the method able to count constituent particles in aggregates?

7.2.12 Composition

Does the method provide information on the chemical composition?

7.2.13 Specification of the measurand (diameter)

Size measurement is method dependent.¹⁴ Different methods address different measurands (equivalent diameters) which need to be specified (hydrodynamic diameter, Stokes diameter, projected area diameter, etc.).

7.2.14 Non-destructive / destructive

This criterion indicates whether the method is destructive or not.

7.3 Final Table

The outcomes of the ranking of each characterisation method are presented in the dedicated table. For the clarity each table was divided into two sections: first one (blue rows) related to the type of substance to analyse: where the evaluation of the, method suitability to be used for the characterisation of certain types of materials is presented and the second part (yellow rows), related to the technique itself: where characteristics of each characterisation method according to the criteria are specified.

| Criteria | Criteria | Characterization |
|---|--|------------------|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | |
| | Solid particulate form | |
| | Dispersed or embedded in different kinds of matrices | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | |
| | Dispersible in non-polar liquids | |
| | Dispersible in polar liquids | |
| | Dispersible in material-specific media | |
| | Can be aerosolized | |
| Substance Nature | Inorganic | |
| | Size-dependent absorption / fluorescence | |
| | Carbon based | |
| | Organic, particulate | |
| | Organic, non-particulate | |
| | Biological | |
| | Composite | |
| | Other | |
| Composite (particles) | Core/shell | |
| | Multiple coatings | |
| | A mix of two or more different materials | |
| Number of nanoscaled dimensions | 1 | |
| | 2 | |
| | 3 | |
| Shape of nanoparticles | Sphere or assimilated | |
| | Equiaxial | |
| | Tubes, fibres, rods | |

| | | |
|--|----------------------------------|--|
| | Flakes and discs | |
| | Other | |
| Thermal degradation sensitivity | Above 0°C | |
| | Sensitivity above 25°C | |
| | Sensitivity above 37°C | |
| | Sensitivity above 50°C | |
| | Sensitivity above 100°C | |
| | Sensitivity above 150°C | |
| | Sensitivity above 500°C | |
| | Sensitivity above 1000°C | |
| | | |
| Cooling degradation sensitivity | Sensitive below 25 °C | |
| | Sensitive below 0 °C | |
| | Sensitive below -18 °C | |
| | Sensitive below -35 °C | |
| | Sensitive below -78 °C | |
| | Sensitive below -195 °C | |
| E- beam sensitivity | e- beam sensitive | |
| | Not e-beam sensitive | |
| Sample dispersity and modality | Monodisperse sample | |
| | Polydisperse sample | |
| | Monomodal sample | |
| | Multimodal sample | |
| Conductivity properties | Conductive | |
| | Semiconductive | |
| | Insulator | |
| Magnetic properties | Magnetic | |
| | Non magnetic | |
| Functionalization / no functionalisation | Functionalised | |
| | Not functionalised | |
| Agglomeration/ aggregation state | Aggregated nanoparticles | |
| | Nanoparticles are not aggregated | |
| | Nanoparticles agglomerate | |
| | Nanoparticles do not ag- | |

| | glomerate | |
|---|---|--|
| counting, separative or ensemble techniques | Single particle counting | |
| | Calculate number or concentration from ensemble methods | |
| | Method combination (hyphenated methods) | |
| Working range | Size range | |
| | Concentration range | |
| | Minimum needed sample | |
| | Linearity/proportionality | |
| | Limits of detection/quantification | |
| | Sensitivity (Counting efficiency) as a function of size | |
| Limits of detection/quantification | What is the lower limit to detect | |
| Trueness | Indicate the trueness of this CM | |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | |
| Robustness | Specify the robustness of this CM. | |
| Precision | Specify the precision of the CM | |
| Resolution | Specify the resolution of this CM | |
| Size distribution | Is it possible to measure size distribution? | |
| Selectivity | discrimination from non-nanoparticles of the same composition | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | |
| | discrimination from nanoparticles of another composition | |
| | Impurities | |

| | | |
|--|--|--|
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | |
| Measures individual particles | Does this CM measure individual particles? | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | |
| Composition | Does this CM analyse composition? | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | |
| Destructive method or not | Is it a destructive method? | |
| Other Specificity | | |

Table 1: Table of method assessment

8 Sampling and Sample Preparation

As the deliverables discussing sample preparation and dispersion protocols are scheduled on 24th and 36th month of the project this chapter is going to up-dated in the 7.7 version of the NanoDefine Methods Manual. Already available SOPs are included in Annexes.

8.1 Sampling

8.2 Sample preparation without dispersion

8.3 Sample preparation by generalized Dispersion Methods

8.4 Standardised protocols for high priority materials groups

8.4.1 Summary of preliminary dispersion protocols for priority materials

Text to be up-dated in the deliverable 7.7

Table 2 List of the priority materials and status of dispersion protocols

| No. | Material | <u>Lead partic- ipants</u> | <u>Status of protocol/Comments</u> |
|-----------|--|--------------------------------|---|
| <u>1</u> | IRMM-382 – MWCNT | CODA- CERVA | Adequate for general use Use of generic NanoGenoTox proto- col- TEM verification of effectiveness required |
| <u>2</u> | IRMM-380 – Organic pigment yellow 83 (nano grade) | JRC | Procedure considered as finalised |
| <u>3</u> | IRMM-386 – Organic pigment yellow 83 (coarse grade) | JRC | Procedure considered as finalised |
| <u>4</u> | IRMM-384 – CaCO ₃ (fine grade) | JRC | Adequate for general use Unresolved question regarding possi- ble dissolution of small particles |
| <u>5</u> | IRMM-383 – Nano steel | CODA- CERVA | Not yet developed- platelet form make it unsuitable for use with CLS/DLS obliging the evaluation by TEM |
| <u>6</u> | IRMM-387 – BaSO ₄ (ultrafine grade) | DTU | Adequate for general use |
| <u>7</u> | IRMM-381 – BaSO ₄ (fine grade) | DTU | Adequate for general use |
| <u>8</u> | IRMM-385 – Kaolin | CODA- CERVA | Preliminary method from JRC based on CLS measurements - in depth veri- fication by TEM necessary as platelet shape poorly suited to CLS. There is possible risk of fragmentation by US which will be studied by CODA- CERVA |
| <u>9</u> | BAM-11 – Zeolite powder | DTU | Not yet available |
| <u>10</u> | IRMM-389 – Basic methacry- late copolymer particles (BMC) | TUD | Not yet available |
| <u>11</u> | Coated TiO ₂ | JRC | Procedure considered as finalised |

Table 3:List of the priority materials and mean size after dispersion protocols

| No. | Material | Size after dispersion protocol | Expected mean size * |
|-----------|---|--|---|
| <u>1</u> | IRMM-382 – MWCNT | Not available | N/A |
| <u>2</u> | IRMM-380 – Organic pigment yellow 83 (nano grade) | 40nm (CLS wt .basis) | 50nm from TEM |
| <u>3</u> | IRMM-386 – Organic pigment yellow 83 (coarse grade) | 240nm (CLS wt .basis) | 182nm from TEM |
| <u>4</u> | IRMM-384 – CaCO ₃ (fine grade) | 392nm (CLS wt .basis) | 168nm from TEM 390nm from manufacturer |
| <u>5</u> | IRMM-383 – Nano steel | Not available | 75nm from TEM |
| <u>6</u> | IRMM-387 – BaSO ₄ (ultrafine grade) | 124nm (DLS Intensity weighted) | 40.3nm from TEM |
| <u>7</u> | IRMM-381 – BaSO ₄ (fine grade) | 377nm (DLS Intensity weighted) | 234nm from TEM |
| <u>8</u> | IRMM-385 – Kaolin | 184nm CLS weight basis Data not to be considered reliable as platelet form unsuitable for CLS measurement | 181nm from TEM |
| <u>9</u> | BAM-11 – Zeolite powder | Not available | 165nm from TEM |
| <u>10</u> | IRMM-389 – Basic methacrylate copolymer particles (BMC) | Not available | 2322nm from TEM |
| <u>11</u> | Coated TiO ₂ | 281nm (CLS wt. basis) | 213nm from TEM |

* Where indicated "from TEM" the data reported is based on number size distributions derived from the TEM analysis. It should be noted that the CLS and DLS sizes reported are not number based values but weight based for CLS and intensity based for the DLS. As such, these values would normally be expected to be greater than the equivalent mean size if expressed on a particle number basis.

8.5 Protocols or preparation of methods for microscopy methods

8.6 Protocols for product sample preparation methods

8.7 Protocols for sample carrier preparation and deposition of samples (Electron Microscopy)

9 Measurement Methods

This chapter discusses available characterisation methods for nanomaterials. In the introduction general information on different types of the methods that allow for the determination of size and size distributions are explained and an overview of techniques is presented in the form of user-friendly tables. Such tables provide the user with a possibility for quick selection of the method which may be appropriate for the characterisation of given material. Yet it is highly recommended to use detail performance table, which are describe below, for final selection of the method to be employed.

This chapter is divided into four main sections in which counting, fractioning, ensemble and integral methods are introduces to the reader. Basic principles of each evaluated method are explained and general performance information is presented in the dedicated table in which main advantages and disadvantages of the technique are also highlighted.

Furthermore, additional very detail information on the performance and suitability of each method are presented in a form of table which allows to the user quick and simple identification of the question and clear answer. Any additional information that should be considered while choosing the most appropriate method is highlighted with the yellow warning sign  to drawn attention of the user. It is also strongly suggested to pay attention to any additional information included in the column "notes" as it may carry great weight for the assessed case.

9.1 General introduction to the methods

There are several means and physical phenomena that allow for the determination of size and size distributions (e. g. imaging, sedimentation, extinction). A more general distinction of particle sizing techniques is based on how the weights of the individual size fractions are determined (cf. Stintz 2005, Stintz et al. 2010):

- counting techniques (measuring particle properties at individual particles)
- fractionating techniques (measuring the amount or concentration of size/property classes after fractionating the particle system)
- ensemble techniques (measuring the spectral or parametric response of a representative particle ensemble of the total particle system)
- integral methods.

9.1.1 Counting methods

In the counting methods individual particles are measured and counts of similar size particles are place into a "size bins" to construct a size distribution.

Counting methods inherently yield particle number weighted distributions (Q_0) of a certain particle property or of a physical quantity that is related to a certain particle property (e. g. particle size, or the average displacement as a measure of the diffusion coefficient). They rely on the individualisation of the particle sample, which can be either achieved by analysing microscopy images (e. g. from electron microscopes) or by sufficient sample dilution or by reduction of sample or measurement volume. The probed particle property may be either geometric (in particular for image analysis), optical (e. g. scattering cross section), or related to mobility (diffusion coefficient).

9.1.2 Fractionating methods

In the Fractioning methods an outside force/process is used too separate particles according to the size and subsequently the quantities of the separated different sizes are determined to construct a size distribution.

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

rays is mass proportional – Q_3).

9.1.3 Ensemble methods

In the ensemble methods all particles in the sample are measured in the same time and the size distribution is extracted from a combined signal for all particles.

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

9.1.4 Integral methods

Additional to those methods that resolve the distribution of particle size there are a few methods which solely measure an integral (effective/mean) property of the particle systems such as the specific surface area (S_V or S_m) or the turbidity of a suspension. These properties can be directly converted into mean values of PSD (e. g. $S_V \rightarrow$ harmonic mean of the volume weighted PSD). Note that ensemble methods – in principle – also yield such integral properties (e. g. the mean decay of signal fluctuation in DLS which gives x_{cum} , i.e. the harmonic mean of the intensity weighted size distribution). The measurement of integral properties can be conducted with relatively high accuracy. Hence, it is widely used to detect changes in size distribution even though it does not provide any piece of information on the distribution width.

9.1.5 General recommendations for metrics conversion

This chapter will be up-dated in the 7.7 version of the NanoDefine Methods Manual. The results reported in the deliverables of WP3 are going to be included and discussed here.

9.1.6 Evaluation table

General overview of the recommended characterisation methods described in this chapter is shown in the Tables Table 4 to Table 7. The criteria selected are of two representative natures: direct relation to the EC recommendation for a definition of nanomaterial and analytical one. For the clarity only the scores fair, good and very good were highlighted in the tables. It has to be underline that these tables aim to give only a general overview of the recommended methods and for an appropriate selection the detail performance tables should be used.

Table 4: Evaluation of the methods: Size range

| Type of method | Method | | Size range | | | | | |
|----------------|--------|-----------|------------|-----------|-----------|-----------|-----------|-----------|
| | | | nm | | | µm | | |
| | | | 1-10 | 10-30 | 30-100 | 0.1-1 | 1-10 | >10 |
| Counting | EM | SEM | | Good | Very good | Very good | Very good | Very good |
| | | TSEM | Good | Good | Very good | Very good | Very good | Very good |
| | | TEM | Very good | Very good | Very good | Very good | Very good | Good |
| | | SFM/AFM | Good | Very good | Very good | Good | Fair | |
| | | PTA | | Fair | Good | Very good | | |
| | | TRPS | | Fair | Fair | Good | Very good | Good |
| | | sp ICP-MS | | Fair | Good | Very good | | |
| Fractionating | | FFF | Good | Very good | Very good | Very good | Fair | |
| | | AC | Fair | Good | Very good | Very good | Good | |
| | | DMAS | Good | Very good | Very good | | | |
| | | SEC | Fair | Good | Very good | Fair | Fair | |
| Ensemble | | DLS | Good | Very good | Very good | Very good | Fair | |
| | | SAXS | Good | Good | Very good | | | |
| | | USSp | | Good | Very good | Good | Fair | Fair |
| | | XRD | Good | Good | Very good | | | |
| | | ALS | | Fair | Good | Very good | Very good | Very good |
| | | OS | Good | Good | Very good | Fair | Fair | |
| Integral | | BET | Good | Good | Very good | Very good | Very good | Fair |
| Legend | | | Fair | Good | Very good | | | |

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

Table 5: Evaluation of the methods: Material

| Type of method | Method | | Sample | | | Type of material | | | | | | | | | Shape | | | |
|----------------|--------|------|--------|--|--|------------------|--------------|----------------------|-------------------------|------------|-----------|------------|-------------------|-----|--------|-----------|--------------------|--------------|
| | | | | | | Inorganic | Carbon based | Organic. Particulate | Organic Non particulate | Biological | Composite | Core/Shell | Multiple coatings | Mix | Sphere | Equiaxial | Tubes, fibres rods | Flakes discs |
| Counting | EM | SEM | | | | | | | | | | | | | | | | |
| | | TSEM | | | | | | | | | | | | | | | | |
| | | TEM | | | | | | | | | | | | | | | | |
| | SFM | | | | | | | | | | | | | | | | | |
| | PTA | | | | | | | | | | | | | | | | | |
| | TRPS | | | | | | | | | | | | | | | | | |
| | ICP-MS | | | | | | | | | | | | | | | | | |
| Fractionating | FFF | | | | | | | | | | | | | | | | | |
| | AC | | | | | | | | | | | | | | | | | |
| | DMA S | | | | | | | | | | | | | | | | | |
| | SEC | | | | | | | | | | | | | | | | | |
| Ensemble | DLS | | | | | | | | | | | | | | | | | |
| | SAXS | | | | | | | | | | | | | | | | | |
| | USSp | | | | | | | | | | | | | | | | | |
| | XRD | | | | | | | | | | | | | | | | | |
| | ALS | | | | | | | | | | | | | | | | | |
| Integral | OS | | | | | | | | | | | | | | | | | |
| | BET | | | | | | | | | | | | | | | | | |
| Legend | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | |

Table 6: Evaluation of the methods: Particles, aggregates and agglomerates.

| Type of method | Method | | Size distribution | Measures aggregates /agglomerates | Measures individual particles | Counting const. particles in aggregations | Measures primary particles in aggregated samples | Measures primary particles in not aggregated samples | Measures primary particles in agglomerated samples | Measures primary particles in not agglomerated samples |
|----------------|--------|--------|-------------------|-----------------------------------|-------------------------------|---|--|--|--|--|
| Counting | EM | SEM | Very good | Fair | Very good | Good | Very good | Very good | Very good | Very good |
| | | TSEM | Very good | Fair | Very good | Good | Very good | Very good | Very good | Very good |
| | | TEM | Very good | Fair | Very good | Good | Very good | Very good | Very good | Very good |
| | | SFM | Good | Fair | | Fair | | Very good | | Very good |
| | | PTA | Very good | Good | Very good | | | Very good | | Very good |
| | | TRPS | Very good | | Very good | | | Very good | | Very good |
| | | ICP-MS | Very good | | Very good | | | Very good | | Very good |
| Fractionating | FFF | | Very good | | | | Very good | | Very good | Very good |
| | AC | | Very good | | | | Very good | | Very good | Very good |
| | DMAS | | Very good | | Very good | | Very good | | Very good | Very good |
| | SEC | | | | | | | | | |
| Ensemble | DLS | | Very good | Good | | | Very good | | Very good | Very good |
| | SAXS | | Very good | Good | | Very good | Very good | | Very good | Very good |
| | USSp | | Very good | | | Very good | Very good | Very good | Very good | Very good |
| | XRD | | | | | | | | | |
| | ALS | | | | | | | | | |
| | OS | | | | | | | | | |
| Integral | BET | | | | Good | | Very good | Very good | Very good | |
| Legend | | | Fair | | | Good | | Very good | | |

Table 7: Evaluation of the methods: Additional information

| Type of method | Method | Direct counting CM | Convertibility to number weighted PSD / Quantitative accuracy | Access to the smallest dimension of each particle | Measurement of the material as it is | ISO standards available | Size Accuracy | Chemical selectivity | Access to primary particles? |
|----------------|-----------|--------------------|---|---|--------------------------------------|-------------------------|---------------|----------------------|------------------------------|
| Counting | EM | SEM | Very good | Very good | | Fair | Very good | (+EDX) | Very good |
| | | TSEM | Very good | Very good | | Very good | Very good | (+EDX) | Very good |
| | | TEM | Very good | Very good | | Very good | Very good | (+EDX) | Very good |
| | SFM | Very good | | Very good | | Very good | Very good | | Fair |
| | PTA | Very good | | | | Very good | Very good | | Fair |
| | TRPS | Very good | | | | Very good | Very good | | |
| | sp ICP-MS | Very good | | | | Fair | Very good | Very good | |
| Fractionating | FFF | | | | | | Very good | (+Detector) | Very good |
| | AC | | Fair | | | Very good | Very good | | |
| | DMAS | Very good | | | | Very good | Very good | | |
| | SEC | | Fair | | | | Very good | | |
| Ensemble | DLS | | | | | Very good | Very good | | |
| | SAXS | | | Fair | Very good | Very good | Very good | Fair | Very good |
| | USSp | | | | | Very good | Very good | | |
| | XRD | | | | Very good | Very good | Fair | Very good | Very good |
| | ALS | | | | | Very good | Very good | | |
| | OS | | | | | | Very good | | |
| Integral | BET | | | | Fair | Very good | | | Fair |
| Legend | | Fair | Good | Very good | | | | | |

9.2 Counting methods

9.2.1 Particle tracking analysis (PTA), Dynamic ultramicroscopy (DUM)

9.2.1.1 Measuring principle

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

In contrast to the majority of sizing techniques for NP suspensions PTA is in principle capable of measuring the particle number concentration. However, the reliability of such a measurement depends very much on material properties and distribution width. Taking the current state-of-the-art the concentration measurement is not very reliable in the general case and needs further investigations¹⁵.

9.2.1.2 Performance – general remarks

The application limits of ultramicroscopy result from the requirements that the particle distances should be much larger than the optical resolution limit of the microscope and that the scattered light of all individual particles is sufficiently strong for detection. While the former can be achieved by appropriate dilution, the latter requirement is missed for particles below a material-specific size limit.

Additionally, there are principal difficulties in detecting weak scatters in the presence of strong scatters. That means, though providing number weighted PSD (similar to ordinary microscopy); the dynamic ultramicroscopy has a bias to strongly scattering particles (similar to dynamic light scattering¹⁶). This concerns very broad size distributions as well as multi-component particle systems.

9.2.1.3 General performance table. Particle tracking analysis (PTA) / Dynamic ultramicroscopy (DUM)

| Main features | |
|--|---|
| Type of samples | Suspended particles |
| Type of sizing | counting technique (by identifying individual objects in video images) |
| Particle property measured | translational hydrodynamic diameter |
| Type of quantity | particle number |
| Size range | 10 nm ... 1 µm (depending on the scattering properties of the material) |
| Concentration range | << 1 vol.-% |
| Information content | good, yet not perfect resolution (widening of PSD due to stochastic nature of Brownian motion, statistic uncertainty for rare particle sizes, insensitivity for very fine particles). |
| Main advantages | |
| <ul style="list-style-type: none"> • yields number weighted size distributions Q_0. | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • measures aggregate size rather than size of the constituent particles. • poor sensitivity in the fine particle range • lower size limit depends on scattering properties of particles • limited dynamic range | |

Table 8: Performance table PTA/DUM

9.2.1.4 Detailed performance table. Particle tracking analysis (PTA) / Dynamic ultramicroscopy (DUM)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/ No) | Notes |
|---|--|----------------------------------|--------------------------------------|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes | |
| | Dispersible in polar liquids | Yes | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes ⚠ | If scattering is sufficiently strong |
| | Organic, non-particulate | Yes ⚠ | If scattering is sufficiently strong |
| | Biological | Yes ⚠ | Only stationary |
| | Composite | No | If matrix phase is solid |
| | Other | | |
| Composite (particles) | Core/shell | Yes ⚠ | Only outer size |
| | Multiple coatings | Yes ⚠ | Only outer size |
| | A mix of two or more different materials | Yes ⚠ | Only outer size |
| Number of nanoscaled dimensions | 1 | No | Does not resolve shape |
| | 2 | No | Does not resolve shape |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods | No | |
| | Flakes and discs | No | |

| | | | |
|--|------------------------------------|-------|---|
| | Other | | |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes ⚠ | Insensitive to fine NPs for very broad PSDs |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes ⚠ | Insensitive to fine NPs for very broad PSDs |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | No | |
| | Nanoparticles are not agglomerated | Yes | |
| counting, separative | Single particle counting | Yes | |

| | | | |
|--|---|--------------|--|
| or ensemble techniques | Calculate number or concentration from ensemble methods | No | |
| | Method combination (hyphenated methods) | No | |
| Working range | Size range | 10 nm – 1 µm | Lower limit is material dependent |
| | Concentration range | << 1 vol.-% | |
| | Minimum needed sample amount | 10 mL | |
| | Linearity/proportionality | Yes ⚠ No | yes with regard to diffusion velocity, i.e. size Not really, with regard to concentration |
| | Limits of detection/quantification | N/A | Depend on optical contrast and size |
| | Sensitivity (Counting efficiency) as a function of size | good ⚠ | Poor for very fine NPs in the presence of large particles |
| Limits of detection/quantification | What is the lower limit to detect | N/A | See size range |
| Trueness | Indicate the trueness of this CM | N/A | Size: “falseness” if wrong calibration of microscope and wrong model parameters (e. g. viscosity) |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | N/A | Number/frequency: falseness if inappropriate illumination and image analysis or if too a high particle concentration |
| Robustness | Specify the robustness of this CM | poor | |
| Precision | Specify the precision of the CM | N/A | PSD parameters to be defined (PSD means, width, etc.) |
| Resolution | Specify the resolution of this CM | good | |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | No | unless similar optical properties |
| | discrimination from nanoparticles of another composition | No | |
| | Impurities | N/A | Yes, dissolved impurities are ignored. ⚠ |

| | | | |
|--|--|---------------------------------------|---|
| | | | No, particulate impurities (in the right size range and with sufficient contrast) are detected |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | Yes ⚠ | Size vs. time However, no identification of agglomerates/ aggregates below 1 µm, and for larger agglomerates/ aggregates no meaningful data analysis |
| Measures individual particles | Does this CM measure individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | No | |
| Composition | Does this CM analyse composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | "translational hydrodynamic diameter" | (long time self-)diffusion coefficient of particles |
| Destructive method or not | Is it a destructive method? | Yes | Usually, because of dilution |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 9: Template for characterisation of PTA/DUM

9.2.2 Single particle ICP-MS (sp ICP-MS)

9.2.2.1 Measuring principle

Single particle ICP-MS is based on the measurement of highly diluted nanoparticle dispersions by ICP-MS operated in time resolved mode for a pre-selected mass-to-charge ratio (m/z) value^{17,18,19,20,21}. Ideally, individual particles enter the ion source and are atomised and ionised in the plasma torch to produce a plume of element ions that is transferred to the mass spectrometric detector. The discrete measurement intervals (dwell times) of the MS are set to a value (≤ 10 ms) that allows the registration of the signal of the ion plume from only one particle. A prerequisite to operate in the single particle mode is (besides the short dwell times) that the concentration of particles is small enough to avoid simultaneous ionisation of more than one particle or the generation of overlapping ion plumes per dwell time. When these requirements are met the signal intensity is proportional to the mass of the respective element in the particle. The diameter of spherical particles can then be calculated from the measured mass based on the known or assumed stoichiometry and density of the target analyte. The number concentration of the particles in the measured dispersion can be inferred from the number of signals, the infusion rate, nebulisation efficiency and the acquisition time.

9.2.2.2 Performance – general remarks

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

Current application limits include:

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

9.2.2.3 General performance table. Single particle ICP-MS (sp-ICP-MS)

| Main features | |
|--|---|
| Type of samples | Suspended particles |
| Type of sizing | Calculated from mass |
| Particle property measured | Mass |
| Type of quantity | particle number concentration, mass concentration |
| Size range | depending on target element, e.g.: <ul style="list-style-type: none"> • Au 15 – 1000 nm, • Ag 20 - 1000 nm, • TiO₂ 50 – 1000 nm, • SiO₂ 200 – 1000 nm |
| Concentration range | depending on element, particle size, instrument (e.g. Ag 60 nm: 5 – 500 ng/L) |
| Information content | good (chemical composition, particle size, particle number concentration, mass concentration) |
| Main advantages | |
| <ul style="list-style-type: none"> • measures individual particles, • chemically specific, • rapid, • cost-efficient | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • particle size limits do not go down to 1 nm, • accurate size determination limited to spherical particles, • does not resolve particles in aggregates and agglomerates (if not deagglomerated in sample preparation) | |

Table 10: Performance table sp-ICP-MS

9.2.2.4 Detailed performance table. Single particle ICP-MS (sp-ICP-MS)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/ No) | Notes |
|---|--|---|---|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes  | only aqueous liquids |
| | Solid particulate form | Yes | after dispersion in water |
| | Dispersed or embedded in different kinds of matrices | Yes | after appropriate sample preparation (digestion, extraction, clean-up etc.) |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | No | |
| | Dispersible in polar liquids other than water | No | |
| | Dispersible in material-specific media |  No | only aqueous media (which can be modified with e.g. dispersants, buffers, low percentage of organic solvents) |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes  | sensitivity and interferences depending on element |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | No | |
| | Organic, particulate | No | |
| | Organic, non-particulate | No | |
| | Biological | No | |
| | Composite | Yes  | if particle contains detectable elements (of inorganic nature) |
| | Other | | |
| Composite (particles) | Core/shell | Yes  | if particle contains detectable elements |
| | Multiple coatings | Yes  | if particle contains detectable elements |
| | A mix of two or more different materials | Yes  | if particle contains detectable elements |
| Number of nanoscaled dimensions | 1 | No | if overall particle mass does not exceed linear range of method; |

| | | | |
|---------------------------------|--------------------------|-------|---|
| | | | theoretical max. 10 µm in one dimension (droplet size; needs practical verification) |
| | 2 | No | if overall particle mass does not exceed linear range of method; theoretical max. 10 µm in one dimension (droplet size; needs practical verification) |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes ⚠ | method can measure objects of different shapes, but does not provide shape information |
| | Equiaxial | Yes ⚠ | method can measure objects of different shapes, but does not provide shape information |
| | Tubes, fibres, rods | No | method can measure objects of different shapes, but does not provide shape information |
| | Flakes and discs | No | method can measure objects of different shapes, but does not provide shape information |
| | Other | No | method can measure objects of different shapes, but does not provide shape information |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | |
| | Monomodal sample | Yes | |

| | | | |
|--|---|------------------------------|---|
| | Multimodal sample | Yes | |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | gives no information on type of functionalisation |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | No | |
| | Nanoparticles are not agglomerated | Yes | |
| counting, separative or ensemble techniques | Single particle counting | Yes | |
| | Calculate number or concentration from ensemble methods | No | |
| | Method combination (hyphenated methods) | No | |
| Working range | Size range | N/A | depending on element (e.g. Ag: ca. 20 – 1000 nm) |
| | Concentration range | N/A | depending on element, particle size, instrument (e.g. Ag 60 nm: 5 – 500 ng/L) |
| | Minimum needed sample amount | 5 mL of injection dispersion | usually not an issue due to the high dilution factors (1000 – 100000) of the original sample |
| | Linearity/proportionality | N/A | linear range depending on element, particle size, instrument |
| | Limits of detection/quantification | N/A | depending on element (e.g. Ag: ca. 20 nm) |
| | Sensitivity (Counting efficiency) as a function of size | N/A | see LoD |
| Limits of detection/quantification | What is the lower limit to detect | | depending on element (e.g. Ag: ca. 20 nm) |
| Trueness | Indicate the trueness of this CM | good | depending on analyte, matrix, laboratory (e.g. own results for Ag in meat trueness for size, number concentration and mass concentration > 90%) |

| | | | |
|--|---|------|---|
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | good | starting from 2 x LoD |
| Robustness | Specify the robustness of this CM | good | |
| Precision | Specify the precision of the CM | good | good precision can be achieved, but in first interlab study (including less experienced labs) some deviations were observed |
| Resolution | Specify the resolution of this CM | good | |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | Yes | |
| | discrimination from nanoparticles of another composition | Yes | |
| | Impurities | N/A | |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | No | |
| Measures individual particles | Does this CM measure individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | No | |
| Composition | Does this CM analyse composition? | Yes | at the moment only one m/z |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | mass | can be used to calculate diameter for spherical, non-porous particles |
| Destructive method or not | Is it a destructive method? | Yes | |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 11: Template for characterisation of sp-ICP-MS

9.2.3 Tunable Resistive Pulse Sensing (TRPS)² / Electrical sensing zone (ESZ) / nano Coulter counter

9.2.3.1 Measuring principle

A Coulter counter is an apparatus for counting and sizing particles suspended in electrolytes²²; the measuring technique is also called electrical sensing zone. It is used for cells, bacteria, prokaryotic cells and virus particles and more recently for fine particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. As fluid containing particles or cells is drawn through each micro channel, each particle causes a brief change to the electrical resistance of the liquid. The counter detects these changes in electrical resistance. The Coulter principle relies on the fact that particles moving in an electric field cause measurable disturbances in that field. The magnitudes of these disturbances are proportional to the volume of the particles in the field^{23, 24, 25}. First, the particles should be suspended in a conducting liquid. Second, the electrical field should be physically constricted so that the movement of particles in the field causes detectable changes in the current. Finally, the particles should be dilute enough so that only one at a time passes through the physical constriction, preventing an artefact known as coincidence.

9.2.3.2 Performance – general remarks

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

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

9.2.3.3 General performance table. Tunable Resistive Pulse Sensing (TRPS)

| Main features | |
|-----------------------------------|---|
| Type of samples | Suspended particles |
| Type of sizing | single particle counting and sizing |
| Particle property measured | particle volume, equivalent sphere diameter |
| Type of quantity | particle number |
| size range | <ul style="list-style-type: none"> • minimum size >70 nm • maximum is in the range 1-10 µm |
| concentration range | 10 ⁵ - 10 ¹² particles / mL |

² TRPS (tunable resistive pulse sensing) is the preferred designation of the manufacturer (ison) for their newly introduced instrument (qnano): particles dispersed in water with dissolved salt move through the single pore of an elastic separator (hence the 'tunable' detection interval) which separates two electrodes that detect the ion current. Whenever a single particle blocks the pore, the current reduces, and the duration and depth of this 'pulse' provide information on size. The sequential detection of blockade events constitutes a size distribution in number metrics without further conversion.

This detection principle is related, but not identical to the conventional ESZ (electrical sensing zone), and hence the designation 'Nano Coulter counter' for TRPS is not preferred.

| | |
|--|---|
| information content | Good as it gives a particle number based PSD based on direct counting. Particle number concentration can also be determined. Information on particle charge is also possible in some instruments. |
| Main advantages | |
| <ul style="list-style-type: none"> • very sensitive, • true single particle counting | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • cannot distinguish single particles from agglomerate/aggregates, • lower size limit (70 nm) does not allow access to the whole range necessary for the definition • non-spherical particle shape or particle aggregates will introduce errors • limited dynamic range because of clogging | |

Table 12: Performance table TRPS / ESZ / nano Coulter counter

9.2.3.4 Detailed performance table. Tunable Resistive Pulse Sensing (TRPS)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|---------------------------|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | No | |
| | Dispersible in polar liquids | No | |
| | Dispersible in material-specific media | Yes | Needs to be aqueous based |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | No | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | No | |
| | Biological | Yes | |
| | Composite | Yes ⚠ | only outer size |

| | | | |
|---------------------------------|--|-------|---|
| | Other | | |
| Composite (particles) | Core/shell | Yes ⚠ | only outer size |
| | Multiple coatings | Yes ⚠ | only outer size |
| | A mix of two or more different materials | Yes | Possible in certain cases |
| Number of nanoscaled dimensions | 1 | No | |
| | 2 | No | Volume correct, if $L/D < 3$ |
| | 3 | Yes | Measures particle volume, replacing liquid volume |
| Shape of nanoparticles | Sphere or similar | Yes ⚠ | Does not resolve shape |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods | No | Volume correct, if $L/D < 3$ |
| | Flakes and discs | No | |
| | Other | No | |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |

| | | | |
|---|---|---------------------------------|---|
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes ⚠ | But lower size limit depends on sensing pore size, which may be blocked |
| | Nanoparticles are agglomerated | No | |
| | Nanoparticles are not agglomerated | Yes ⚠ | But lower size limit depends on sensing pore size, which may be blocked |
| counting, separate or ensemble tech- niques | Single particle counting | Yes | |
| | Calculate number or concentration from ensemble methods | No | |
| | Method combination (hyphenated methods) | No | |
| Working range | Size range | 50 nm - 10 µm | But lower size limit depends on sensing pore size, which may be blocked |
| | Concentration range | 10^5 - 10^{12} particles/mL | |
| | Minimum needed sample amount | 500 µL | |
| | Linearity/proportionality | Yes | Pulse amplitude linear to displaced electrolyte volume |
| | Limits of detection/quantification | 10^5 particles/mL | |
| | Sensitivity (Counting efficiency) as a function of size | Yes | |
| Limits of detection/quantification | What is the lower limit to detect | 10^5 particles/mL | |
| Trueness | Indicate the trueness of this CM | N/A | |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | N/A | |
| Robustness | Specify the robustness of this CM | N/A | |
| Precision | Specify the precision of the CM | N/A | |
| Resolution | Specify the resolution of this CM | N/A | |

| | | | |
|--|---|---------------------------------|--|
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | No | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | No | |
| | discrimination from nanoparticles of another composition | Yes | Limited discrimination reported to be possible |
| | Impurities | N/A | Yes, dissolved impurities are ignored. No, particulate impurities (in the right size range and with sufficient contrast) are detected |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | No | |
| Measures individual particles | Does this CM measure individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | No | |
| Composition | Does this CM analyse composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | volume (or equivalent diameter) | |
| Destructive method or not | Is it a destructive method? | Yes | |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 13: Template for characterisation of TRPS.

9.2.4 Electron Microscopy

9.2.4.1 Measuring principle

One analytical method widely used for sample visualization down to the nm scale is Electron Microscopy.

py (EM). This technic uses electron beam to visualise nanomaterials.

In the Scanning Electron Microscopy (SEM), images are constructed based on electrons coming from the sample surface.²⁶ In case of Transmission Electron Microscopy (TEM), images are constructed based on electron passing through the samples. Both SEM and TEM give 2-dimensional projections of 3-dimensional particles. EM is a counting method and can be used to build the required number based size distributions²⁶. Most of EM measurements are performed in high vacuum chambers. Therefore, the use of EM is limited to the analysis of particles which are not sensitive by the combined action of high vacuum and electron beams. EM enables the visualization of the morphology of particles by the jape of their projections. But the analysis of flattened particles (e.g. disks, flakes) could be problematic because the smallest axis of the particle could be hidden, and it could give an overestimated measured size comparatively to the size relevant for the definition.

In SEM, the sample to be examined is bombarded with a finely focused electron beam which is scanned over a defined field. Low-energy secondary electrons (SE) are released after inelastic collisions with the atoms in the specimen as well as high-energy backscattered electrons (BSE) after elastic collisions. Depending on their kinetic energy the information range carried by the released electrons varies from the micrometre range (typically for BSE) down to the nanometre range (typically for SE). Hence, the SE are suited for high-resolution morphological characterization of the specimen surface at nanometre scale and even individual nanoparticles may be visualized and lateral dimensions measured in an SEM. Depending on the instrument used but also strongly on the challenging sample preparation onto typical sample substrates/holders accurate size characterization of nanoparticles with sizes down to several tens of nm is possible.²⁷

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

This CM is able to count individual particles. Depending on the number of NPs acquired in an image, mostly several images are necessary to reach a good counting statistics. The automation tools such as motorized stage and sequential image acquisition should be available in order to speed up the whole measurement process. Also automatic image processing of batch images speeds up the determination of the size distribution.

Whereas in the case of an SEM typical beam voltages up to 30 kV may be applied and SE/BSE are collected by various detectors in the case of TEM the beam voltage is up to 300 kV. The samples to be analyzed must be in the range of the electron transparency so that the electrons transmitted through the thin sample are collected. The highly energetic electron beam is even finer than in the SEM case (more sophisticated aberration correctors being typically also available), so that spatial resolution well below 1 nm can be attained. Similar requirements as in SEM for NPs regarding sample preparation, suitable substrate (typically the so-called TEM grids), vacuum suitability,²⁸ calibration of the pixel size and automation of both acquisition and image processing hold true for TEM, too. By combining TEM imaging and semi-automatic image analysis, are valid for TEM, which allows characterizing the size, shape, and surface topology of colloidal nanomaterials,²⁹ aggregated nanomaterials^{30,31}; and primary particles in aggregates (or, in terms of the EC definition, constituent particles)).³² A characterization methodology which includes a systematic selection procedure for unbiased random image collection, semi-automatic image analysis and data processing has been validated for size, shape and surface topology measurements of silica nanoparticles. The expanded uncertainty of size measurements of two colloidal silica certified reference materials was estimated to be about 3 %.

TEM can be operated in the scanning mode, i.e. STEM, when the electron beam is focused into a narrow spot which is scanned over the sample. According to the range of angles by which they are scattered in the sample the transmitted electrons can be differentiated: bright-field electrons are those elec-

trons slightly or not at all scattered and dark-field electrons are those collected concentrically to the optical microscope axis. The so-called HAADF-STEM (High-Angle Annular Dark-Field STEM) imaging mode results when only the strongly scattered electrons are collected with an annular dark-field detector. The contrast of this type of imaging with atomic resolution is directly related to the atomic number (Z-contrast image).

One hybrid type of electron microscopy is constituted by the SEM able to work in the transmission mode, i. e. TSEM, T-SEM or STEM in SEM etc. This means that by using TEM grids as supports for NPs the transmitted electrons (of lower energies than in the TEM case) are more or less absorbed by the NPs and a so called STEM detector placed under the sample holder detects them. The alternative is to use a special mount, i.e. single-unit transmission setup, which enables to perform TSEM with the available SE/BSE detector and not having necessary an additional STEM detector. It was recently demonstrated that both types are well suited for metrological measurement of NP size and size distribution down to a size of about 10 nm^{33, 34;35}.

9.2.4.2 Performance – general remarks

Current limitations in application include:

- Strong dependency from sample preparation (incl. suitable substrate)
- Possibility to use vacuum with certain types of material (organic, coated etc.)
- SEM is not able to measure accurately the size of NPs below several tens of nm (depending on instrument)
- Automation (batch image acquisition and batch image processing)
- Time-consumption that depends on the complexity of sample preparation and degree of automation
- High cost, but still in the same order of magnitude as ICP-MS
- Accuracy in allocation of the NPs in the image processing software

Analytical figures of merit to be considered:

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

Systematic results of metrological measurement of NP size and size distribution by SEM, TEM and TSEM were carried out recently in the frame of various, specifically dedicated round robin tests.³⁶ According to reported results, EM provides traceable results which are also consistent, i.e. comparable, with those obtained by AFM, SAXS and SMPS, but not with DLS.

9.2.4.3 General performance table. Electron Microscopy (EM)

| Main features | |
|-----------------|--|
| Type of samples | particles properly deposited onto substrates |
| Type of sizing | counting technique (by identifying individual objects in images) |

| | |
|---|--|
| Particle property measured | Feret diameter, Equivalent circle diameter (ECD), wide range of 1D and 2D size, shape and surface measurands |
| Type of quantity | particle number |
| Size range | <ul style="list-style-type: none"> • SEM: 30 nm - 100 μm • TEM (incl. HAADF-STEM): <1 nm - 10 μm • TSEM: 10 nm - 10 μm |
| Concentration range | "0" (individual particles) ... monolayer (immobilised particles) |
| Information content | <ul style="list-style-type: none"> • Good in x-y direction (parallel to the substrate); |
| Main advantages | |
| <ul style="list-style-type: none"> • yields well-resolved number weighted size distributions from measurement of individual particles. • size, shape and surface measurands can be measured on 2D images • sub-nm resolution for TEM, nm resolution for SEM • able to distinguish and measure aggregates and isolated particles. Classification between single and agglomerated particles is possible • constituent particles in certain aggregates can be detected and their size can be measured (depending on the size of the constituent particles) • access to smallest dimension of particles in X-Y plane (TEM) • capable of chemical specificity of single particles by the attached EDX • crystallographic information is available by electron diffraction • significant instrumental developments (spatial resolution, automation, EDX detector sensitivity, table-top instruments, etc.) | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • strongly dependent on sample preparation • needs vacuum and expensive instrumentation • automation in image processing: in progress • limited dynamic range (highest size/lowest size < 40) based on one image only | |

9.2.4.4 Detailed performance table. Electron Microscopy (EM)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|---|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | No | Possible if a cryo-stage is available |
| | Solid particulate form | Yes | |
| | Dispersed or embedded in different kinds of matrices | Yes ⚠ | Only at the surface, in thin films or in ultramicrotomed sections |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | No | Yes ⚠ after successful deposition on substrates |
| | Dispersible in non-polar liquids | No | Yes ⚠ after successful deposition on substrates |

| | | | |
|---------------------------------|--|-----|--|
| | Dispersible in polar liquids | No | Yes ⚠ after successful deposition on substrates |
| | Dispersible in material-specific media | No | Yes ⚠ after successful deposition on substrates |
| | Can be aerosolized | No | Yes ⚠ after successful deposition on substrates |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite | Yes | |
| Composite (particles) | Other | Yes | |
| | Core/shell | Yes | |
| | Multiple coatings | Yes | Difficult |
| Number of nanoscaled dimensions | A mix of two or more different materials | Yes | |
| | 1 | No | Sometimes, the thickness can be measured when tilting the sample |
| | 2 | Yes | |
| Shape of nanoparticles | 3 | Yes | |
| | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods | Yes | |
| | Flakes and discs | No | |
| Thermal degradation sensitivity | Other | Yes | |
| | Above 0°C | No | ⚠ Yes, with cryo stage |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| Sensitivity above 1000°C | Yes | | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |

| | | | |
|---|---|--------------|--|
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | No | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | With altered image quality |
| | Insulator | Yes | SEM: low-voltage option or conductive high resolution sputter-coating needed |
| Magnetic properties | Magnetic | Yes | Depending on strength of magnetic field and worsening the CM figures of merit |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | Yes | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes | |
| | Nanoparticles are not agglomerated | Yes | |
| counting, separate or ensemble techniques | Single particle counting | Yes | |
| | Calculate number or concentration from ensemble methods | No | |
| | Method combination (hyphenated methods) | Yes | |
| Working range | Size range | > 1 nm | Lower range varies in dependence on instrument type, sample type and preparation |
| | Concentration range | "mono-layer" | EM measures accurately only "dry" particles deposited on a substrate. Droplet of 0.1-1 µL at 0.1%-vol. conc. typically sufficient. |
| | Minimum needed sample amount | 0.1 µL | Minimum 500 NPs for a monodisperse/ monomodal sample |

| | | | |
|--|---|---------------|---|
| | | | (Motzkus et al., 2013) |
| | Linearity/proportionality | Yes | |
| | Limits of detection/quantification | 1 nm to 10 nm | Depending on instrument, sample type and preparation, etc. |
| | Sensitivity (Counting efficiency) as a function of size | good | |
| Limits of detection/quantification | What is the lower limit to detect | 1 nm to 10 nm | Depending on instrument, sample type and preparation, etc. |
| Trueness | Indicate the trueness of this CM | good | Based on traceability of reference materials; critical for NPs below 10-20 nm with SEM |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | average | To be evaluated for specific cases |
| Robustness | Specify the robustness of this CM | average | Strong dependency on sample preparation To be evaluated for specific cases (WP5) |
| Precision | Specify the precision of the CM | 1 nm to 10 nm | Depending on many parameters, mainly preparation; better for TEM and poorer for SEM. To be evaluated for specific cases (WP5) |
| Resolution | Specify the resolution of this CM | 1 nm to 10 nm | Depending on many parameters, mainly preparation; better for TEM and poorer for SEM. To be evaluated for specific cases (WP5) |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | Yes ⚠ | In cases when image contrast is high enough |
| | discrimination from nanoparticles of another composition | Yes ⚠ | In cases when image contrast is high enough |
| | Impurities | Yes ⚠ | TEM: Very large particles, in the μm range and above cannot be measured accurately SEM: Very small particles (nm range) may not be detectable |

| | | | |
|--|--|---------------------------------|---|
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | Yes ⚠ | Difficult and possible only after sampling on substrates and in vacuum |
| Measures individual particles | Does this CM measure individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | Yes ⚠ | Depending on contrast and size of aggregate |
| Composition | Does this CM analyse composition? | No | However, Z contrast sometimes possible and EDX with poorer spatial resolution is almost always available at a SEM |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | "Diameter", "Feret diameter" | |
| Destructive method or not | Is it a destructive method? | Yes | Sample must be prepared on substrates or as thin films, etc. |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | Yes | |
| Sample support | Does this CM need preparation on suited supports? | Yes | |

Table 14: Template for characterisation of EM

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

9.2.5.1 Measuring principle

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

Particles need to be fixed to the surfaces in order to be characterised and to be moved by the tip. AFM is an imaging method and can be used to measure the size of polydisperse and polyshaped particles. Organic particles can also be analysed with this technique.

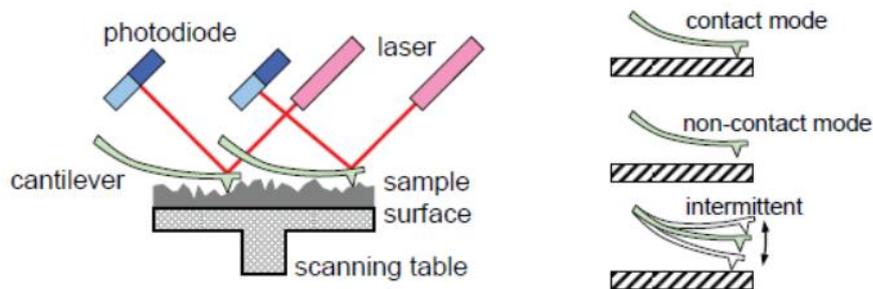


Figure 11: Measuring principle of SFM

This technique able to image colloidal particles or powders was developed by Binnig and his co-workers in the 1980s.³⁷ AFM (or SFM) is the most used type of scanning probe microscopy (SPM). The SFM and its related techniques are based on the interaction between a very fine probe tip with the atoms or molecules at the surface of the sample (cf. Figure 23).^{38, 39} This can be used to resolve surface morphologies or particles on a substrate with vertical/out-of-plane resolution of 0.1 nm to 10 nm (lateral/in-plane resolution: 10 nm). The AFM is usually employed for the characterisation of films and surfaces (e. g. roughness), whereas the morphological characterisation of particles is a less frequent application. Its real strength is the sensitivity to the forces between probe and sample, which allows an evaluation of surface chemistry (e. g. functional groups, hydrophobicity) and the quantification of particle interactions, or interactions between particles and surfaces (e. g. adhesion, friction^{40, 41}). Depending on the situation, forces that are measured in AFM include mechanical contact force, van der Waals forces, capillary forces, chemical bonding, electrostatic forces, magnetic forces, etc. As well as force, additional quantities may simultaneously be measured through the use of specialised types of probe. Samples in air or in liquid can be analysed, but the sample must adhere to a substrate and be rigid and well dispersed on it. The roughness of the substrate must be less than the size of the nanoparticles being measured. The AFM can be operated in several modes. In general, imaging modes are divided into static (also called contact) modes and a variety of dynamic (or non-contact) modes where the cantilever is vibrated (Figure 11). The use of the AFM in biology, biochemistry and bionanotechnology, also for the characterization of nanomaterials (size, shape), are reviewed in an article by Kada et al.⁴²

The results of imaging methods are (mainly) particle number weighted particle size distributions (PSDs). It means that the sample size (number of probed particles) should be sufficiently high for ensuring low uncertainty in class frequencies. Moreover, the sample size required to achieve a certain confidence level increases with polydispersity. The accuracy of the measured particle properties depends on a variety of factors (e. g. magnification or spatial resolution of the scanning mode, or image processing). Most crucial, however, is the representativeness of the imaged particles for the whole particle system. That requires that the particle deposition on the substrate is neither size-selective nor inhomogeneous.⁴³ In general, sample preparation is a key issue for imaging methods.

9.2.5.2 General performance table. Scanning force microscopy (SFM), or atomic force microscopy (AFM)

| Main features | |
|----------------------------|--|
| Type of samples | Particles of (almost) any material, in vacuum, air or liquid, but they must be immobilized on a substrate |
| Type of sizing | Counting technique |
| Particle property measured | Particle height above the level of a substrate |
| Type of quantity | Particle number, size of individual particles |
| Size range | <ul style="list-style-type: none"> • 1 nm ... >> 1 μm (z-size, "height", most reliable), lateral size not recommended, depends on tip geometry) |

| | |
|---|---|
| Concentration range | "0" (individual particles) ... monolayer (immobilised particles) |
| Information content | <ul style="list-style-type: none"> • Good in z-direction ("height"); • not reliable in x-y direction (laterally, parallel to the substrate) |
| Main advantages | |
| <ul style="list-style-type: none"> • measures individual particles, no conversion necessary • access to the minimum dimension of a particle • measures a wide range of materials • instruments are widely available and not expensive | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • needs immobilization of particles on suitable substrates • lateral size measurements are not reliable, depend on tip conditions • low sample throughput, slow • limited dynamic range | |

9.2.5.3 Detailed performance table. Scanning force microscopy (SFM), or atomic force microscopy (AFM).

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

| | | | |
|---------------------------------|--|---|---|
| | | | zation on substrates |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite | Yes | |
| | Other | Yes | |
| Composite (particles) | Core/shell | Yes  | Only external size |
| | Multiple coatings | Yes  | Only external size |
| | A mix of two or more different materials | Yes  | If the difference in size is not too big and if the maximum size remains lower than the scanner range; other limitations, such as differences in hardness, might apply; additional info is required for identification of the different materials |
| Number of nanoscaled dimensions | 1 | Yes | |
| | 2 | Yes | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods | Yes | |
| | Flakes and discs | Yes | |
| | Other | Yes | |
| Thermal degradation sensitivity | Above 0°C | No |  Yes, with cryo stage |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |

| | | | |
|---|---|--|--|
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | Yes ⚠ | Only aggregate size, with limitations (size and roughness) |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Only agglomerate size, with limitations (size, roughness and hardness) |
| | Nanoparticles are not agglomerated | Yes | |
| counting, separative or ensemble techniques | Single particle counting | Yes | |
| | Calculate number or concentration from ensemble methods | No | |
| | Method combination (hyphenated methods) | Yes | Example: collecting fractions from FFF and re-dispersing them on adequate substrates for AFM |
| Working range | Size range | > about 1 nm for height; < 100 µm for lateral size | Range varies in dependence on instrument type, sample type and preparation |
| | Concentration range | “mono-layer” | Droplet of 10 µL at 0.1% vol. conc. typically sufficient |
| | Minimum needed sample amount | 0.1 µL for liquid | Droplet of 10 µL at 0.1% vol. conc. typically sufficient |

| | | | |
|--|---|-------------------------------|--|
| | | suspension 1 mg for powder | |
| | Linearity/proportionality | Yes | |
| | Limits of detection/quantification | 1 nm / several tens of nm | About 1 nm for height and several tens of nm for lateral dimensions (depending on tip convolution) |
| | Sensitivity (Counting efficiency) as a function of size | good | Low throughput |
| Limits of detection/quantification | What is the lower limit to detect | 1 nm / several tens of nm | Individual particles can be detected (but statistics!) For individual particles: About 1 nm for height and a few tens of nm for lateral dimensions (depending on tip convolution) |
| Trueness | Indicate the trueness of this CM | good | only for height, as convolution with the tip geometry leads to a bias in lateral dimensions |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | average | Depending on polydispersity and sample preparation; to be evaluated for specific cases |
| Robustness | Specify the robustness of this CM | average | Strong dependency on sample preparation; to be evaluated for specific cases |
| Precision | Specify the precision of the CM | < 1 nm for height | Depending on many parameters; precision on lateral size depends, among others, on tip convolution; to be evaluated for specific cases (WP5). Depends also on the type of material. |
| Resolution | Specify the resolution of this CM | 1 nm to a few nm | Depending on dimension (height versus lateral), instrument type and imaging conditions and material type |
| Size distribution | Is it possible to measure size distribution? | Yes ⚠ | if the size difference is not too big |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes ⚠ | if the size difference is not too big |
| | discrimination from non-nanoparticles of another composition (matrix particles) | Yes ⚠ | In cases when image contrast is high enough |

| | | | |
|--|--|-----------------------|---|
| | discrimination from nanoparticles of another composition | average | In cases when hardness properties are significantly different (in intermittent contact mode); modes based on other properties (electrical, magnetic, thermal...) could be helpful, but need to be evaluated |
| | Impurities | N/A | |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | average | Only small aggregates if well separated on the substrate; agglomerates are expected to be more difficult to image due to their bigger size and poor mechanical properties |
| Measures individual particles | Does this CM measure individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | No | |
| Composition | Does this CM analyse composition? | No | Although coupling with Raman or FTIR does exist, but remains limited (in resolution, among others) and not widespread |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | height / lateral size | Bias on lateral size due to tip convolution. |
| Destructive method or not | Is it a destructive method? | Yes | Sample must be immobilised on substrates or as thin films, etc. |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | Also possible, but not required. |
| Sample support | Does this CM need preparation on suited supports? | Yes | |

Table 15: Template for characterisation of AFM

9.3 Fractionating methods

9.3.1 Differential electrical mobility analysis (DMAS)

9.3.1.1 Measuring principle

There are different terminologies for aerosol particle size spectrometers based on electrical mobility: MPPS for mobility particle size spectrometer, SMPS for scanning mobility particle sizer, DMPS for differential mobility particle sizer and DMAS for differential mobility analysing system.

DMAS combines a particle classifier (Differential Mobility Analyser DMA or DEMC for differential electrical mobility classifier) that transmits particles within a narrow interval of sizes from an initially polydisperse aerosol, and a detector (for example, a Condensation Particle Counter CPC) that counts the particles within that differential size interval. First, the aerosol passes through an inertial impactor to avoid that particles larger than 1 micrometre enter the DMA column, then the aerosol enters a particle charge conditioner like a charge neutraliser to be conditioned, so, particles that carry several charges lose their charge excess. Once the aerosol is well conditioned particles are selected using electrical classification inside DMA column: an electric field is created and the airborne particles drift along the DMA according to their electrical mobility Z_d . It is related to the particle diameter d_p via the expression:

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

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

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

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

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

Figure 12 presents the fundamental components of DMAS (ISO 15900: 2009). The pre-conditioner indicated in this figure serves generally two goals: removing the large particles with impactor the most used and, if necessary, reducing the sample humidity using a dryer. Concerning the aerosol detector, there are two types of detector: a CPC and an aerosol electrometer. Concerning the particle charge conditioner, a bipolar diffusion particle charger (also called an aerosol neutralizer) is often used in SMPS. This is often done using a radioactive source like ^{85}Kr or a bipolar ion generator. These chargers establish the equilibrium charge distribution on the aerosol particles. Unipolar Corona chargers may also be used in a DMAS. The DMAS is operated by software controlling the sheath air flow, reading the aerosol flow, reading other system parameters such as T , p , setting the voltage, and reading the CPC output.

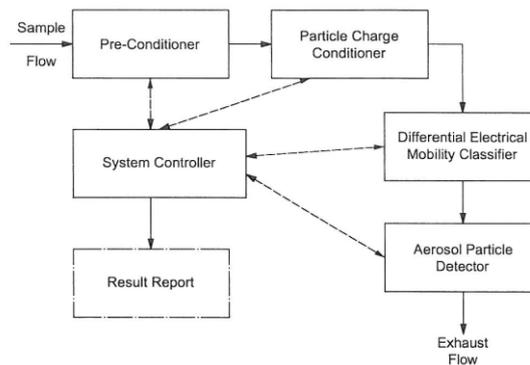


Figure 12: Fundamental components of the differential mobility analysing system (DMAS) (ISO 15900: 2009)

9.3.1.2 Performance – general remarks

Current limitations of this method include:

- Stability of the aerosol:

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

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

Data analysis, resolution of particle size distribution etc

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

The performances of four SMPS were evaluated by Fissan et al.⁴⁴ under the same conditions for flow rates, flow ratio, input monodisperse aerosols, and transport-line lengths in the 6–50 nm size range. Their results provide a quantitative comparison of the mobility resolution and diffusion loss of the nanometre aerosols in such systems. Moreover, the performance assessment of Fast MPS (FMPS) and Ultrafine Water-based Condensation Particle Counter (UWCPC) equipped SMPS was performed by Jeong and Evans⁴⁵ under various conditions on urban ambient particles, urban indoor particles, rural ambient particles, and laboratory-generated particles. Asbach et al.⁴⁶ tested four different mobility particle sizers on NaCl and diesel soot particles measurements. Recently the paper of Wiedensohler et al.⁴⁷ talks about harmonization of measurement procedures to facilitate high quality long-term observations of atmospheric particle size number distributions obtained by SMPS. Some results of metrological measurement of NP size and size distribution by SMPS have been carried out recently in the frame of various interlaboratory comparisons specially dedicated to this purpose⁴⁸. Electro-spray-differential mobility analysis (ES-DMA), a technique that exerts electrical and drag forces on clusters, can be used to determine the size and packing of colloidal small clusters (and aggregates more generally) of nanoparticles⁴⁹.

9.3.1.3 General performance table. Differential electrical mobility analysis (DMAS)

| Main features | |
|-----------------------------------|--|
| Type of samples | airborne submicrometer particles |
| Type of sizing | Fractionation ensemble technique |
| Particle property measured | number size distribution (or Count size distribution) |
| Type of quantity | an equivalent diameter called electrical mobility diameter |
| Size range | 2.5 nm to 1 µm (Range varies in dependence on instrument type like DMA and CPC and the parameter used (flow rate,...)) |

| | |
|--|--|
| Concentration range | 1 to 10 ⁸ particles/cm ³ (Maximum concentration of CPC is 10 ⁷ part/cm ³) |
| Information content | 2 modes of measurement: <ul style="list-style-type: none"> • Scanning mode: SMPS (scanning mobility particle sizer); often described for a continuous voltage scan. Up scan: 20 to 300 seconds • Stepping mode: DMPS (differential mobility particle sizer); often described for a stepwise voltage scan |
| Main advantages | |
| <ul style="list-style-type: none"> • well-resolved number size distributions • adapted to measure airborne submicrometre particles • quick measurement (a few min) • applicable to polydisperse population between 3 nm to 1 µm • non-destructive method • physical separation of particles with the electrical mobility | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • non-spherical particle shape or particle aggregates will introduce errors • primary particles in aggregates/agglomerates cannot be resolved | |

Table 16: Performance table DMAS

9.3.1.4 Detailed performance table. Differential electrical mobility analysis (DMA)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|-------------------------------------|-------------------------------------|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | No | measures only airborne NPs |
| | Solid particulate form | No | measures only airborne NPs |
| | Dispersed or embedded in different kinds of matrices | No | measures only airborne NPs |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | No | |
| | Dispersible in non-polar liquids | No | |
| | Dispersible in polar liquids | No | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | Yes | measures only particles aerosolized |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |

| | | | |
|---------------------------------|--|-------|---|
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite | Yes | |
| | Other | Yes | |
| Composite (particles) | Core/shell | Yes ⚠ | Only outer size |
| | Multiple coatings | Yes ⚠ | Only outer size |
| | A mix of two or more different materials | Yes ⚠ | Only outer size |
| Number of nanoscaled dimensions | 1 | No | measures an equivalent diameter called electrical mobility diameter |
| | 2 | No | measures an equivalent diameter called electrical mobility diameter |
| | 3 | Yes ⚠ | measures an equivalent diameter called electrical mobility diameter |
| Shape of nanoparticles | Sphere or similar | Yes | The diameter obtained can be compared with other technique if the particle shape is spherical |
| | Equiaxial | Yes ⚠ | does not resolve shape |
| | Tubes, fibres, rods | No | does not resolve shape |
| | Flakes and discs | No | does not resolve shape |
| | Other | | |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |

| | | | |
|--|---|------------------------------|---|
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | |
| | | | |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | No | |
| | Nanoparticles are not agglomerated | Yes | |
| counting, separative or ensemble techniques | Single particle counting | Yes | measures the number concentration in aerosol phase |
| | Calculate number or concentration from ensemble methods | No | Is not an ensemble method |
| | Method combination (hyphenated methods) | No | |
| Working range | Size range | 2 nm to 1 μ m | Range varies in dependence on instrument type like DMA and CPC and the parameter used (flow rate,...) |
| | Concentration range | $<10^6$ part/cm ³ | For CPC |
| | Minimum needed sample amount | 10^4 part/cm ³ | |
| | Linearity/proportionality | Yes No | With regard to concentration With regard to particle size (for low particle diameters the fraction of charged particles is very low) |
| | Limits of detection/quantification | > 2 nm | depends on the instrument type (3-5 nm) |
| | Sensitivity (Counting efficiency) as a function of size | good | There is a strong impact of the size particle < 10 nm for the |

| | | | |
|--|---|------------------|--|
| | | | counting efficiency (CPC) |
| Limits of detection/quantification | What is the lower limit to detect | 2 nm to 10 nm | Dependent on instrument type, and parameters used (flow rate,...) |
| Trueness | Indicate the trueness of this CM | good | |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | good | |
| Robustness | Specify the robustness of this CM | good | |
| Precision | Specify the precision of the CM | 2 channels | depends on width of channel used, particle diameter and instrument type |
| Resolution | Specify the resolution of this CM | 0.5 nm to 3.6 nm | for $d_p < 100$ nm Resolution depends on the size of particle and instrument type |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | Yes | |
| | discrimination from nanoparticles of another composition | No | |
| | Impurities | N/A | "impurities" only refer to residual particles (salt, polymers) after aerosolisation from suspension; this entry is irrelevant for aerosol characterisation |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | No | SMPS measures all size of particles (primary, agglomerate and aggregate) without knowing the state of agglomeration. It is necessary to use other method like SEM, TEM,... |
| Measures individual particles | Does this CM measure individual particles? | Yes | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | No | in rare cases it is possible to identify doublets, triplets and quartets |
| Composition | Does this CM analyse composition? | No | |

| | | | |
|--|---|--------------------------------|------------------------|
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | “electrical mobility diameter” | an equivalent diameter |
| Destructive method or not | Is it a destructive method? | Yes | |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 17: Template for characterisation of DMA

9.3.2 Field-Flow-Fractionation (FFF)

9.3.2.1 Measuring principle

FFF is a fractionation technique which separates particles according to their hydrodynamic size. The sample is pumped through a channel in a laminar flow (in the centre of the channel, the fluid moves faster than at the edges of the channel).⁵⁰ Perpendicularly to this flow, a field is applied. In most of the cases, it is a second flow but this flow can be electric, magnetic, centrifugal. Some particles would be then pushed to the edge of the channel where they move faster. Quantification of the amount of particles depends on the sort of detector used and its calibration. FFF is performed in liquids and is limited to the analysis of the particles which are able to be dispersed in liquids.⁵⁰

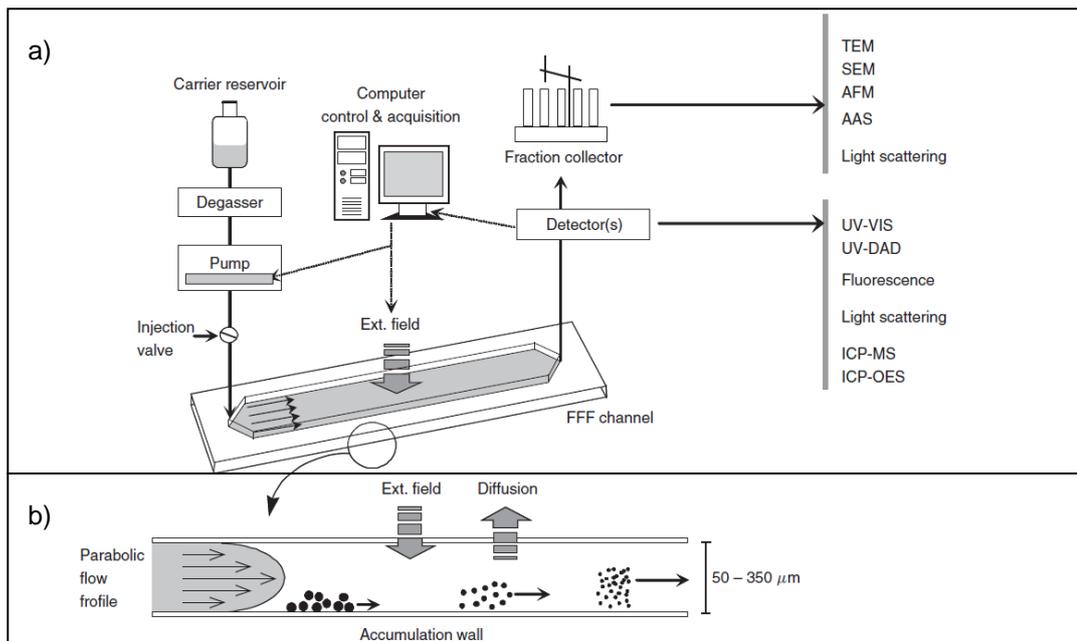


Figure 13 : a) schematic diagram of a FFF-System including commonly used detection systems; b) cross-section of the channel illustrating the separation mechanism (adapted from v. d. Kammer 2005)⁵⁰

FFF is a continuous analytical separation technique for macromolecules and colloidal suspensions. Until now several FFF techniques have been developed. They are based on various separation principles

such as particle diffusion (e.g. asymmetric flow field-flow-fractionation, AF4) or buoyant mass (e.g. sedimentation field-flow-fractionation, SedFFF). In combination with a suitable detection system FFF techniques enable us to derive particle size or molecular mass from sample specific properties, such as particle diffusion. The separation concept of all FFF techniques is similar. Particles are transported by laminar flow profile (flow field) through a channel. Separation is achieved by interactions of the particles with a concurrent second field force. The field force can be e.g. a perpendicular flow field (AF4) or centrifugal force (SedFFF). This interaction results in a heterogeneous distribution of the sample in the channel. For example during AF4 separation a forces drives the sample (i.e. dissolved and particulate components) towards the accumulation wall which is covered by an ultrafiltration membrane which is permeable for components smaller than the cut-off of the membrane. Components which are retained in the channel will be distributed in the channel profile according to their diffusional properties. Depending on the diffusion properties (i.e. size) particles will experience different laminar flows resulting in a separation according to their hydrodynamic size. The separated sample is detected online by a suitable detector.

9.3.2.2 Performance – general remarks

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

Current limits of application include:

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

9.3.2.3 General performance table. Field-Flow-Fractionation (FFF)

| Main features | |
|----------------------------|--|
| Type of samples | Suspended particles |
| Type of sizing | derived from calibration of the elution time with known size standards or by application of FFF theory or by coupled particle sizer. |
| Particle property measured | FlowFFF: particle diffusion coefficient (derived hydrodynamic diameter) |
| Type of quantity | detector dependent: UV/VIS: particle mass concentration if particles absorb light in the operation range of the detector, fluorescence: particle mass concentration if particle is fluorescing, MALLS → intensity of scattered light; ICPMS → element mass concentration (element must be constituent of the particle and stoichiometry known); if counting detectors are used, particle number concentrations can be determined (e.g. sp ICP-MS) |

| | |
|--|--|
| Size range | in general 1-1000 nm. Instrumental settings need to be tuned for the size range of interest, dynamic range (ratio of upper to lower measurable diameter) is typically 20 – 40 |
| Concentration range | adjustable, case-specific, the minimum and maximum acceptable concentration depends strongly on the sample characteristics, the size range and applied detector |
| Information content | <ul style="list-style-type: none"> • for AF4: diffusion coefficient, hydrodynamic radius; • for Sedimentation FFF: volumetric radius • further information depending on coupled detector e.g. <ul style="list-style-type: none"> • light scattering (SLS): rms-radius and geometric radius, intensity weighted size distribution • ICP-MS: chemical composition, mass concentration, mass based size distribution) • UV/DAD: indicator for chemical composition (element specific wave length absorption and surface plasmon resonance) |
| Main advantages | |
| <ul style="list-style-type: none"> • physical separation of particles: can operate on complex mixtures and matrices • element specific: can operate on complex mixtures and matrices • the technique provides mass-based size distributions even for particles composed of multiple elements. • coupling to single particle counting techniques possible in the course of NanoDefine | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • number-based size distribution cannot directly be measured. • it does not determined particles in aggregates and agglomerates. • limited dynamic range (different elution regimes for different particle sizes) | |

9.3.2.4 Detailed performance table. Field-Flow-Fractionation (FFF)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|-------------------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes ⚠ | Channels for organic liquids available. Not routinely used |
| | Dispersible in polar liquids | Yes | |
| | Dispersible in material-specific me- | Yes ⚠ | Case by case decision |

| | | | |
|--|--|------------|---|
| | dia | | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | Macromolecules and similar are possible |
| | Biological | Yes | |
| | Composite | Yes | |
| | Other | | |
| | Composite (particles) | Core/shell | Yes |
| Multiple coatings | | Yes | |
| A mix of two or more different materials | | Yes | |
| Number of nanoscaled dimensions | 1 | No | |
| | 2 | No | ⚠ Yes for small aspect ratios |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods | No | ⚠ Yes in certain cases. Separation can be achieved; sizing would require sample-similar standards or external sizing methods. |
| | Flakes and discs | No | ⚠ Yes in certain cases. Separation can be achieved; sizing would require sample-similar standards or external sizing methods. |
| | Other | | |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |

| | | | |
|--|---|--------------------|--|
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | No | |
| | Nanoparticles are not agglomerated | Yes | |
| counting, fractioning or ensemble techniques | Single particle counting | N/A | ⚠ Depends on detection system. If detector counts particles, particle number based distribution can be achieved. |
| | Calculate number or concentration from ensemble methods | Yes | |
| | Method combination (hyphenated methods) | Yes | |
| Working range | Size range | 1 - 1000 nm | |
| | Concentration range | 20 µg/L – 500 mg/L | Detector dependent |
| | Minimum needed sample amount | 10 µL | |

| | | | |
|--|---|-------------------------------------|---|
| | Linearity/proportionality | Yes | Detector dependent |
| | Limits of detection/quantification | > 1 nm | Detector dependent |
| | Sensitivity (Counting efficiency) as a function of size | good | Detector dependent |
| Limits of detection/quantification | What is the lower limit to detect | 1 nm to 10 nm | Membrane and detector dependent |
| Trueness | Indicate the trueness of this CM | good | If reference materials available |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | good | Mass quantification if performed by mass specific detector |
| Robustness | Specify the robustness of this CM | average | Important parameter is the membrane quality |
| Precision | Specify the precision of the CM | 1 nm to 10 nm | Can be tuned to needs |
| Resolution | Specify the resolution of this CM | 1 nm to 10 nm | determined by size standards |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | No | ⚠ Pre-treatment of the sample is necessary. |
| | discrimination from non-nanoparticles of another composition (matrix particles) | No | ⚠ Pre-treatment of the sample is necessary. Depends on detection technique. |
| | discrimination from nanoparticles of another composition | Yes | Depends on detection technique. |
| | Impurities | N/A | |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | No | |
| Measures individual particles | Does this CM measure individual particles? | No | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | No | |
| Composition | Does this CM analyse composition? | Yes | Depending on constituents and applied detection technique. |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | ⁴¹ Hydrodynamic diameter | Diffusion coefficient (FlowFFF) hydrodynamic diameter can be derived, volumetric diameter |

| | | | |
|---------------------------|---|-----|---|
| | | ter | (SedFFF or CFFF). In few cases when MALS is applicable also rms and geometrical diameter, respectively. |
| Destructive method or not | Is it a destructive method? | No | Fractions can be collected; sample will be diluted. |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 18: Template for characterisation of FFF

9.3.3 Analytical centrifugation (AC)

9.3.3.1 Measuring principle

Analytical centrifugation operates on the principle of separating particles by size using centrifugal sedimentation in a liquid medium. This leads to variations in the local particle concentration when measured along the settling path and/or over time. Settling distance and settling time correspond to the terminal settling velocity, which in the case of isolated particles solely depends on their individual size, shape, and density. Hence, concentration profiles or time curves reflect the size distribution of the particle system.

This basic concept can be varied with respect to the particle detection (i.e. concentration measurement) or the mode of operation. Nowadays, two fundamental types of centrifuges are distinguished: disc centrifuges and cuvette centrifuges:

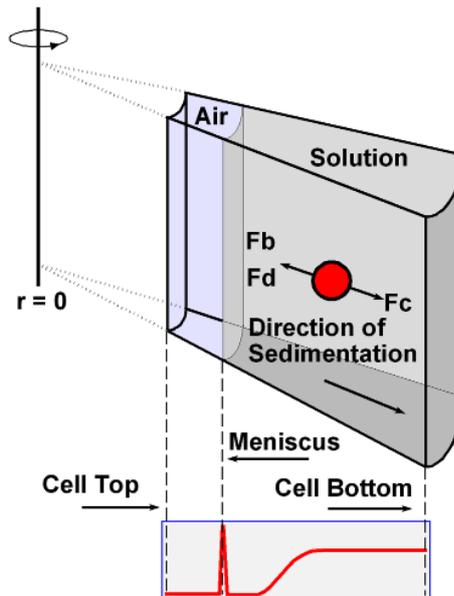


Figure 14: Schematic diagram of an AC cuvette centrifuge system

Disc centrifuges consist of a hollow disc which contains the suspension medium. Upon rotation, the liquid forms a stagnant layer on which a thin layer of the particle system is injected (line-start technique). The particles migrate according to their settling velocity to the outer diameter of the disc. All particles of

a certain size (or settling velocity) move in a narrow band with growing distance from the initial position. In the case of multidisperse particle systems, one can observe several of such bands in analogy to chromatographic techniques. The radial concentration profile is, hence, a distorted projection of the density function of the size distribution ($q(x_{\text{Stokes}})$). The line start technique requires a density gradient in the suspension medium (e. g. by sugar) before the particles are injected; otherwise there was a convective transport of particles within strands of the (heavy) suspension layer. The density gradient sets some practical limits to the measurement (e. g. duration) and has to be calibrated before conducting size measurements.

A different set-up and even mode of operation is found in cuvette centrifuges, where the particle sedimentation is observed in small cuvettes that are fixed on a rotating table. In this case, the particles are homogeneously suspended in the continuous phase before the centrifugation starts (homogeneous technique which is schematically presented on the Figure 14). During the centrifugation, all particles migrate towards the bottom of the cuvette, which results in the formation of a sediment, in a steady decline of local particle concentrations above the sediment, and in a monotone decrease of particle concentration in the direction from the sediment to the meniscus. The two types of variation in particle concentration, the temporal evolution, and the radial profile, can be considered as distorted projections of the cumulative function of the particle size distribution ($Q(x_{\text{Stokes}})$). The cuvette centrifuge was introduced by Svedberg and co-workers^{51, 52}. They called the instrument "ultra-centrifuge". Nowadays the term "Analytical Ultracentrifugation" (AUC) is only used for centrifugal accelerations above $100,000 \times g$.

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

- optical extinction (photocentrifuge), which yields volume weighted size distributions (q_3) for light-absorbing nanoparticles and intensity weighted size distribution for non-absorbing nanoparticles (q_6);
- X-ray absorption (X-Ray centrifuge), which always yields volume weighted size distributions (q_3);
- refractive index determination by interferometry, which (approximately) yields volume weighted size distributions for nanoparticles.

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

9.3.3.2 General Performance table. Analytical Centrifugation (AC)

| Main features | |
|----------------------------|---|
| Type of samples | Suspended particles |
| Type of sizing | fractionating ensemble technique |
| Particle property measured | <ul style="list-style-type: none"> • Stokes diameter determined from settling velocity and mass density, or • hydrodynamic diameter from settling velocity and the effective particle density (used for compact aggregates) |
| Type of quantity | <ul style="list-style-type: none"> • solid volume by X-ray, refractive index or turbidity measurement of light-absorbing and –scattering nano-particles (q_3) • squared solid volume by turbidity measurement non-absorbing nanoparticles (q_6) • for non-nanoparticles the physical quantity should be stated (e.g. extinction) |
| Size range | < 5 nm - 10 μm with minimum depending on particle density |
| Concentration range | $\leq 0.1 \text{ wt.}\%$ |
| Information content | rel. high for monodispersed and polydispersed non-aggregated materials |

| |
|---|
| Main advantages |
| <ul style="list-style-type: none"> • rapid screening • high resolution in size • and applicable to polydisperse materials |
| Main disadvantages |
| <ul style="list-style-type: none"> • cannot distinguish single particles from agglomerate/aggregates, • accuracy of size measurement is influenced by any uncertainty in the assumed values of materials density, • non-spherical particle geometry will introduce deviations to other equivalent diameters • limited dynamic range - needs a balance between sedimentation rate (depending on particle size), density of gradient and centrifugal speed. |

Table 19: Performance table AC

9.3.3.3 Detailed performance table. Analytical centrifugation / Centrifugation analysis - including Centrifugal Liquid Sedimentation (CLS) and Analytical Ultra Centrifugation (AUC)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|---|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes ⚠ | Depending on liquid compatibility of instrument |
| | Dispersible in polar liquids | Yes ⚠ | Depending on liquid compatibility of instrument |
| | Dispersible in material-specific media | Yes ⚠ | Depending on liquid compatibility of instrument |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes ⚠ | Size-dependent scattering relevant to algorithm converting detector signal to mass value. |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | No | No for CLS, but yes for AUC |

| | | | |
|---------------------------------|--|-------|---|
| | Biological | Yes ⚠ | Need to know material density- not always possible in biological samples |
| | Composite | No | |
| | Other | | |
| Composite (particles) | Core/shell | No | Yes if density values are known and one of either core or shell thickness are known ⚠ |
| | Multiple coatings | No | Theoretical possible but in practice very complex ⚠ |
| | A mix of two or more different materials | No | Not possible with CLS instrument (single-detector operation), but possible by combination of different optical (turbidity, RI, UV) and X-ray detectors. ⚠ |
| Number of nanoscaled dimensions | 1 | No | |
| | 2 | No | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes ⚠ | Approximations may be possible by use of aspect-ratio coefficients in software |
| | Tubes, fibres, rods | No | Approximations may be possible by use of aspect-ratio coefficients in software ⚠ |
| | Flakes and discs | No | If the lateral extension is known, the thickness distribution can be measured from below 1nm to above 100nm, and vice-versa. ⚠ |
| | Other | | |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |

| | | | |
|---|---|-----------------|---|
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | N/A | |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | No | |
| | Nanoparticles are not agglomerated | Yes | |
| counting, separate or ensemble tech- niques | Single particle counting | No | |
| | Calculate number or concentration from ensemble methods | No | |
| | Method combination (hyphenated methods) | Yes | Not with CLS (single detector). With AUC, multiple detectors acquire differently weighted size distributions during the same fractionation. |
| Working range | Size range | 5 nm-70 μ m | Range depends on instrument type, sample type and preparation. Maximum and minimum may not be possible in single run. From 0.5nm for AUC. |
| | Concentration range | >50 ppm | |
| | Minimum needed sample amount | 100 ng | Sample volume is typically around 100 μ l |
| | Linearity/proportionality | No | Non-linear |

| | | | |
|--|---|--------------------|---|
| | Limits of detection/quantification | 10 ppm / 50 ppm | Highly dependent on material, size and detector type |
| | Sensitivity (Counting efficiency) as a function of size | Yes | Decreasing sensitivity with size for optical detection. |
| Limits of detection/quantification | What is the lower limit to detect | 10 ppm / 50 ppm | Highly dependent on material, size and detector type |
| Trueness | Indicate the trueness of this CM | good | |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | N/A | Depends on material and the algorithms used to convert measured signal to weight% and then to number% |
| Robustness | Specify the robustness of this CM | good | Particle density must be reliably known |
| Precision | Specify the precision of the CM | good | Depends on the accuracy of the calibration standards used |
| Resolution | Specify the resolution of this CM | 2% of nominal size | For non-aggregated materials |
| Size distribution | Is it possible to measure size distribution? | Yes | Produces mass based size distribution |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | No | |
| | discrimination from nanoparticles of another composition | No | |
| | Impurities | N/A | |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | No | |
| Measures individual particles | Does this CM measure individual particles? | No | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | No | |
| Composition | Does this CM analyse composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | "Stokes diameter" | |

| | | | |
|---------------------------|---|-----|--|
| Destructive method or not | Is it a destructive method? | Yes | |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 20: Template for characterisation of Analytical centrifugation / Centrifugation analysis - including Centrifugal Liquid Sedimentation (CLS) and Analytical Ultra Centrifugation (AUC)

9.4 Ensemble methods

9.4.1 Dynamic light scattering (DLS)

9.4.1.1 Measuring principle

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

The principle in DLS is measurement of fluctuations in laser light scattered by vibrating particles suspended in a liquid as function of time (Figure 15). The vibration is due to Brownian motion caused by collision with solvent molecules of the liquid. The Brownian motion varies as a function of particle size and causes variation in the intensity of scattered light as function of time. A correlator compares the signal measured at a time t_0 with different very short time delays dt (autocorrelation). As the particles move, the correlation between t_0 and subsequent dt signals decreases with time, from a perfect correlation (1) at t_0 , to a complete decorrelation (0) at infinite time (order of milliseconds). In the case of big particles, the signal changes slowly and the correlation persists for a long time, whereas small particles have high Brownian movement causing rapid decorrelation.

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

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

k : Boltzmann's constant

D : translational diffusion coefficient

T : absolute temperature

η : viscosity

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

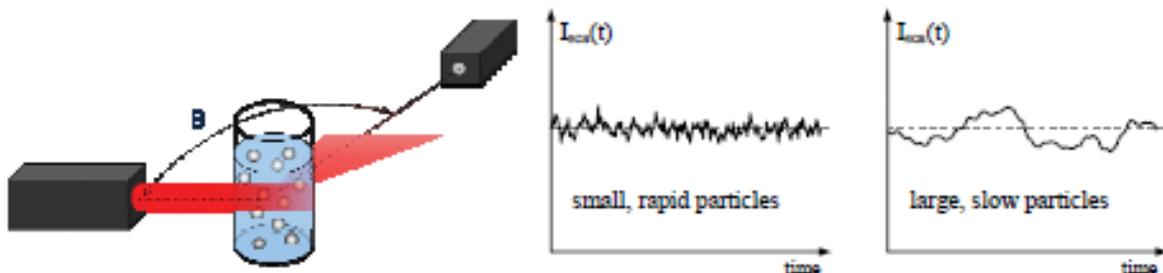


Figure 15: Measuring principle of DLS

The intensity fluctuations of DLS experiments can be analysed in terms of (ISO 22412, Xu 2000):
 a frequency spectrum (frequency analysis – FA), or
 a time correlation function (photon correlation spectroscopy – PCS).

PCS requires a different hardware than FA, but it can be shown that the results of both techniques are equivalent^{53, 54}. Today, a large variety of commercial or “self-made” DLS instruments are used. Apart from data processing (FA or PCS), they can be distinguished with regard to laser optics and signal modulation.

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

9.4.1.2 Performance – general remarks

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

Apart from upper concentration limits, there is a further one at the lower edge, where the intensity fluctuations start to become affected by the statistic variation of particle number in the measurement zone⁵⁵, a minimum of 100 particles should stay in the measurement zone. This is fulfilled for most colloidal suspensions; problems may arise for micrometre particles (i.e. $\geq 1 \mu\text{m}$).

Sedimentation sets a limit to the detection of coarse particles because the particle displacement of micrometre particles is governed by sedimentation rather than by diffusion. This is of particular importance for polydisperse particle systems, where diffusion and sedimentation are coupled⁵⁶. Moreover, sedimentation may affect the size distribution in the measurement zone. However, in the colloidal size range ($x \leq 1 \mu\text{m}$) there is virtually no impact of sedimentation on DLS results⁵⁷. A lower size limit exists only as much as the scattering intensity of the particles should considerably exceed that of the fluid molecules. Xu⁵⁸ proposes a minimum factor of 2.5; yet, for highly reliable DLS this value should be multiplied by 10.

During the last two decades, dynamic light scattering has evolved into a major characterisation technique for colloidal suspensions. A recent interlaboratory study into the characterisation of a monomodal colloidal silica⁵⁹ showed that state-of-the-art DLS instrumentation facilitate a highly reproducible and very reliable acquisition of correlation function and corresponding mean particle size x_{cum} . The study involved 17 participants from EU and USA, which provided 19 independent data sets from 6 different commercial instruments covering sideward scattering (90°) and backscattering. An earlier study with a different test material already indicated the high interlaboratory comparability⁶⁰.

9.4.1.3 General performance table. Dynamic Light Scattering (DLS).

| Main features | |
|----------------------------|---|
| Type of samples | Suspended particles |
| Type of sizing | spectroscopic ensemble technique |
| Particle property measured | (apparent = translation affected by rotation) hydrodynamic diameter |

| | |
|--|---|
| Type of quantity | intensity of scattered light; for NPs: $I_{sca} \propto x^6$ |
| Size range | 1 nm ... 1 μ m |
| Concentration range | ≤ 1 vol.-% (depends on the material) |
| Information content | relatively low, i.e. weak ability to accurately resolve PSD details |
| Main advantages | |
| <ul style="list-style-type: none"> • when DLS finds NPs then there are NPs, • rapid screening • a minimum amount of information about the sample is needed to run the analysis • testing is non-destructive (sample may be recovered) • small amount of sample is needed to run a test | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • in polydisperse or multi-component particle systems DLS will severely underestimate the amount of weakly scattering NPs • low resolution • assumption of spherical shape | |

Table 21: Performance table DLS

9.4.1.4 Detailed performance table. Dynamic light scattering (DLS)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/ No) | Notes |
|---|--|----------------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes | |
| | Dispersible in polar liquids | Yes | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes ⚠ | see size criteria |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | Yes | |
| | Composite | No | because of solid matrix |
| | Other | No | |
| Composite (particles) | Core/shell | Yes ⚠ | Outer particle size |
| | Multiple coatings | Yes ⚠ | Outer particle size |
| | A mix of two or more different materials | Yes ⚠ | Outer particle size |
| Number of nanoscaled dimensions | 1 | No | |
| | 2 | No | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes ⚠ | Conventional DLS measurement and analysis do not resolve shape |
| | Tubes, fibres, rods | No | Conventional DLS measurement and analysis do not resolve shape |

| | | | |
|--|------------------------------------|-----------------------|--|
| | Flakes and discs | No | Conventional DLS measurement and analysis do not resolve shape ⚠ |
| | Other | | |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| | Cooling degradation sensitivity | Sensitive below 25 °C | Yes |
| Sensitive below 0 °C | | Yes | |
| Sensitive below -18 °C | | Yes | |
| Sensitive below -35 °C | | Yes | |
| Sensitive below -78 °C | | Yes | |
| Sensitive below -195 °C | | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | Insensitive to fine NPs for very broad PSDs |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | Insensitive to fine NPs for very broad PSDs |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ aggregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | No | |
| | Nanoparticles are not agglomerated | Yes | |

| | | | |
|---|---|---|---|
| counting, separative or ensemble techniques | Single particle counting | No | |
| | Calculate number or concentration from ensemble methods | Yes | only estimates |
| | Method combination (hyphenated methods) | No | |
| Working range | Size range | 1 nm – 1 μ m | |
| | Concentration range | <0.1 vol.-% | |
| | Minimum needed sample amount | 2 mL | |
| | Linearity/proportionality | No Yes | No, with regard to size (decay of act or spectral shift) Yes with regard to concentration (total intensity) for diluted systems (where linear dependence on concentration) |
| | Limits of detection/quantification | N/A | Depends on optical contrast and size |
| | Sensitivity (Counting efficiency) as a function of size | N/A | Insensitive to very fine particles (weak scattering signal) and very coarse particles (do not contribute to signal fluctuation and disappear from measurement zone) |
| Limits of detection/quantification | What is the lower limit to detect | N/A | |
| Trueness | Indicate the trueness of this CM | N/A | Size: “falseness” if wrong model parameters (e.g. viscosity, wavelength) or if too a high concentration (multiple scattering, hydrodyn. interaction) |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | N/A | Intensity weights: “falseness” if multiple scattering or if too high laser intensities |
| Robustness | Specify the robustness of this CM | good | |
| Precision | Specify the precision of the CM | N/A | PSD average (mode, median, (power) means): high PSD width: satisfactory |
| Resolution | Specify the resolution of this CM | poor | |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes  | If NP dominate |
| | discrimination from non-nanoparticles of another composition (matrix particles) | No | Yes, only if NP dominate and have higher refractive index  |

| | | | |
|--|--|---|--|
| | discrimination from nanoparticles of another composition | No | |
| | Impurities | N/A | Yes, dissolved impurities are ignored. No, particulate impurities (in the right size range and with sufficient contrast) are detected |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | Yes  | (size vs time) However, no identification of aggregates/ agglomerates |
| Measures individual particles | Does this CM measure individual particles? | No | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | No | |
| Composition | Does this CM analyse composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | "Effective hydrodynamic diameter" | Short time self-diffusion: translation affected by rotation for non-spherical objects |
| Destructive method or not | Is it a destructive method? | Yes | If dilution is required; otherwise not |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 22: Template for characterisation of DLS

9.4.2 FUltrasonic spectroscopy (USSp)

9.4.2.1 Measuring principle

Ultrasonic spectroscopy is the generic term for all particle sizing techniques that are based on the frequency dependent measurement of sound velocity or attenuation in the ultrasonic domain (mostly within 100 kHz to 200 MHz). While velocity spectroscopy is mainly used for the study of inter- and intramolecular processes, attenuation spectroscopy has found its major application in particle sizing^{61,62}. The

most promising feature of acoustic characterisation techniques is their applicability to highly concentrated particle systems (up to 70 vol.-%) under non-equilibrium conditions⁶³. That means it offers the opportunity to monitor the state of dispersion of dense product streams, to control the de-agglomeration of suspensions or the homogenisation of emulsions, and to study polymerisation or crystallisation processes.

As mention before the attenuation spectroscopy has found application in particle sizing⁶². The principle of this technique is that plane sound waves moving through a particle suspension are attenuated in a predictable manner according to size and concentration of the particles in suspension.

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

9.4.2.2 Performance – general remarks

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

A major difficulty for the particle sizing by USSp is that the acoustic behaviour depends on a variety of material parameters. This is of particular relevance for emulsions (14 properties), whereas for aqueous suspensions only the viscosity and sound speed of liquid and the density contrast have to be known⁶⁴.

In colloidal suspensions, the sound propagation is typically governed by the acoustophoretic motion of particles. For monodisperse spheroids that do not deviate too much from a spherical shape (aspect ratio < 10/1) the attenuation spectrum essentially reflects the volume specific surface area of the particles⁶⁵. Similar results would probably be obtained for any convex particle shape. For particle aggregates, the inner structure is decisive. Regarding the type of quantity, acoustically measured size distributions are ideally volume weighted distributions.

It could be shown that the results of ultrasonic spectroscopy agree fairly well with those of other characterization methods and are hardly affected by the extent of sample dilution⁶⁶. Interlaboratory comparisons of ultrasonic spectroscopy measurements on suspensions of inorganic particles also showed good agreement⁶⁷. That is why ultrasonic spectroscopy is considered as a powerful tool for monitoring colloidal processes. However, the method does not allow for a very sharp resolution of size distributions in the colloidal size range⁶⁸.

9.4.2.3 General Performance table. Ultrasonic spectroscopy (USSp)

| Main features | |
|----------------------------|----------------------------------|
| Type of samples | Suspended particles |
| Type of sizing | spectroscopic ensemble technique |
| Particle property measured | acoustophoretic diameter |
| Type of quantity | particle volume |
| Size range | 10 nm - 100 µm |

| | |
|--|---|
| Concentration range | > 1 vol.-% |
| Information content | <ul style="list-style-type: none"> • relatively low for $x \leq 1 \mu\text{m}$, • relatively high for $x \geq 10 \mu\text{m}$ |
| Main advantages | |
| <ul style="list-style-type: none"> • for aggregates of NPs USSP detects the internal aggregate structure rather than the outer proportions, • does not require dilution for dense suspensions (which may affect the state of dispersion) • technique relatively easy to implement • able to measure turbid suspensions | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • does not yield particle number-weighted distributions • does not allow the characterisation of dilute suspensions, i.e. requires a lot of substance • has a low resolution • needs intense data evaluation based on mathematical modelling | |

Table 23: Performance table USSp

9.4.2.4 Detailed performance table. Ultrasonic spectroscopy (USSp)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/ No) | Notes |
|---|--|----------------------------------|---|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | No | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes | |
| | Dispersible in non-polar liquids | Yes ⚠ | If not too viscous |
| | Dispersible in polar liquids | Yes ⚠ | If not too viscous |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes ⚠ | But not always |
| | Organic, non-particulate | No | |
| | Biological | No | |
| | Composite | No | because of solid matrix |
| | Other | No | |
| Composite (particles) | Core/shell | Yes ⚠ | Particle outer size |
| | Multiple coatings | Yes ⚠ | Particle outer size |
| | A mix of two or more different materials | No | Yes, particle outer size, if mixture is known ⚠ |
| Number of nanoscaled dimensions | 1 | No | |
| | 2 | No | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods | No | |
| | Flakes and discs | No | |

| | | | |
|--|------------------------------------|-------|---|
| | Other | | |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | Difficult for NPs if broad PSD |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | Difficult for NPs if broad |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | Yes ⚠ | Reflects internal lengths (e.g. pore size, inter-particle distance) |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes ⚠ | Reflects internal lengths (e.g. pore size, inter-particle distance) |
| | Nanoparticles are not agglomerated | Yes | |
| counting, separative | Single particle counting | No | |

| | | | |
|--|---|---------------|--|
| or ensemble techniques | Calculate number or concentration from ensemble methods | Yes | |
| | Method combination (hyphenated methods) | No | |
| Working range | Size range | 1 nm – 100 µm | |
| | Concentration range | >1 vol.-% | |
| | Minimum needed sample amount | 100 mL | |
| | Linearity/proportionality | No Yes | No, with regard to size Yes, with regard to quantity (volume) for dilute suspensions |
| | Limits of detection/quantification | N/A | Depends on density/ thermo-acoustic contrast and size |
| | Sensitivity (Counting efficiency) as a function of size | N/A | |
| Limits of detection/quantification | What is the lower limit to detect | N/A | |
| Trueness | Indicate the trueness of this CM | N/A | Size: “falseness” if wrong model parameters (e.g. viscosity, sound speed) or if too a high particle concentration (>> 10 vol.-%) |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | N/A | Volume weights: “falseness” if frequency range too narrow or PSD too broad (low information content below 5 µm) |
| Robustness | Specify the robustness of this CM | good | |
| Precision | Specify the precision of the CM | N/A | PSD average (modes, median, (power) mean): high PSD width: high |
| Resolution | Specify the resolution of this CM | poor | For NPs |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | Yes ⚠ | If similar acoustic properties |
| | discrimination from nanoparticles of another composition | Yes ⚠ | If similar acoustic properties |

| | | | |
|--|--|---|---|
| | Impurities | N/A | |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | No | |
| Measures individual particles | Does this CM measure individual particles? | No | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | Yes  | see "internal lengths" above |
| Composition | Does this CM analyse composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | "acoustophoretic diameter" | For inorganic particles in water (i.e. high-frequency hydrodynamic diameter) which is approx. the specific surface area |
| Destructive method or not | Is it a destructive method? | No | |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 24: Template for characterisation of USSp

9.4.3 Small-angle X-ray scattering (SAXS)

9.4.3.1 Measuring principle

Small-angle X-ray scattering (SAXS) is a technique based on the interaction between X-rays and matter to probe the structure of materials. Light passing through a medium that is not completely homogenous is forced to deviate from the straight light. The scattering angle, the deviation from the straight line, depends on the wavelength of light and the size of the particles on which it is scattered. The processed data are the intensity, I , of X-ray scattered by a sample as a function of angular position of a detector.

The intensity is expressed in absolute scale, cm^{-1} , independent from test parameters such as X-ray wavelength, experimental background, time of acquisition and sample thickness. 2D raw data images are converted into diffractograms displaying the scattered intensity I as a function of scattering vector q defined by:

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

λ : X-ray wavelength

The intensity pattern of the scattered radiation can give information of the particle size, size distribution,

as well as of the shape of the particles and its nanostructure. Size and shape of the particles are obtained by fitting of the measured scattering curves (intensity vs angle).

Two main applications of SAXS are characterization of nanoparticles and determination of large surface areas. The SAXS theory is complete and based on fundamental physical⁶⁹. A study of six European metrology institutes proved recently that SAXS allows traceable size determination of monomodal, spherical nanoparticles⁷⁰.

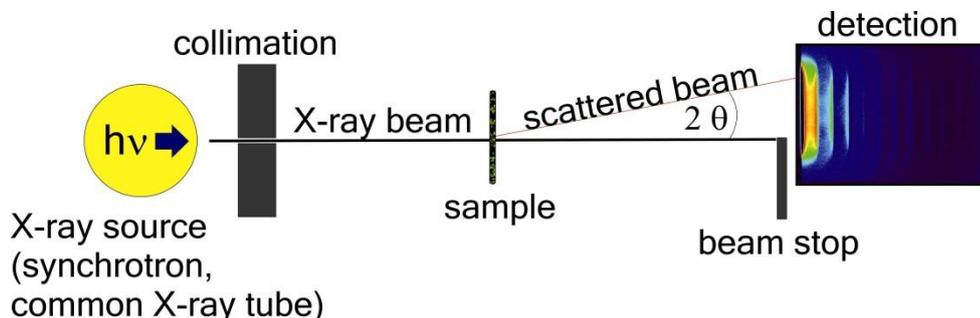


Figure 16: Measuring principle of SAXS

9.4.3.2 Performance – general remarks

The method is accurate, non-destructive and requires a minimum of sample preparation. SAXS covers only the lower range of interest from 1 to 100 nm. The experimental devices at synchrotrons and common laboratories are well developed. Numerous technical improvements have been made during the last five years. High throughput instruments were realized by using sample changing robots⁷¹ and new X-ray detectors for low noise data recording are available⁷² for synchrotrons and normal SAXS laboratories. Currently numerous SAXS manufacturers in Europe released improved SAXS instruments. These instruments are suitable for routine and standardized measurements in accordance with ISO standards.

9.4.3.3 General performance table. Small-angle X-ray scattering (SAXS)

| Main features | |
|----------------------------|---|
| Type of samples | nano-particles suspended in liquids, nanoparticles in viscous and solid matrices, nanoparticles in powders etc. (any two-phase systems where the phases have different density) |
| Type of sizing | scattering ensemble averaging technique |
| Particle property measured | Particle size as defined by the scattering from the particle surface; therefore density of particles must differ from that of their surroundings (e.g. water) |
| Type of quantity | size, shape and concentration |
| Size range | 1 to 100 nm (upper limit depends on specific instrument) |
| Concentration range | 10 ⁻⁴ to 100 vol.-% (lower limit depends strongly on size and density of particles, as well as on the quality of the instrument) |
| Information content | Size, shape, size distribution (intensity, volume and number-weighted) |

| |
|--|
| Main advantages |
| <ul style="list-style-type: none"> • particles can be detected with minimal sample preparation. No or little danger exists for sample preparation artefacts. • very good reproducibility for monodisperse and fairly spherical particles |
| Main disadvantages |
| <ul style="list-style-type: none"> • larger particles produce higher signals than smaller particles. Therefore, the determination of number-weighted size distributions makes a detailed analysis of the measuring uncertainties necessary. Procedures in determination of complete error budgets in SAXS data analysis is not yet established in the SAXS data evaluation. • upper size limit. • measurements for polymorphous and polydisperse samples are very difficult leading to a significant differences between instruments for the same sample • it does not distinguish between agglomerates and aggregates and primary particles |

Table 25: Performance table SAXS

9.4.3.4 Detailed performance table. Small-angle X-ray scattering (SAXS)

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/No) | Notes |
|---|--|---------------------------|--|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | Yes | |
| | Solid particulate form | Yes | |
| | Dispersed or embedded in different kinds of matrices | Yes ⚠ | as long as the X-rays transmit through the material |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | Yes ⚠ | In-situ measurement |
| | Dispersible in non-polar liquids | Yes ⚠ | In-situ measurement |
| | Dispersible in polar liquids | Yes ⚠ | In-situ measurement |
| | Dispersible in material-specific media | Yes ⚠ | In-situ measurement |
| | Can be aerosolized | No | aerosols can be measured with special set-ups (Shyjumon et al., 2008) |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | Yes ⚠ | size dependent absorption or fluorescence in the UV-Vis range does not affect the SAXS results |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |

| | | | |
|---------------------------------|--|-------|--|
| | Biological | Yes | |
| | Composite | Yes | |
| | Other | | |
| Composite (particles) | Core/shell | Yes ⚠ | core and shell must have different electron densities |
| | Multiple coatings | Yes ⚠ | multiple coatings must have different electron densities |
| | A mix of two or more different materials | Yes | |
| Number of nanoscaled dimensions | 1 | No | |
| | 2 | No | |
| | 3 | Yes | |
| Shape of nanoparticles | Sphere or similar | Yes | |
| | Equiaxial | Yes | |
| | Tubes, fibres, rods | No | Yes if the shape is known ⚠ |
| | Flakes and discs | No | Yes if the shape is known ⚠ |
| | Other | No | If different forms are mixed in the same sample SAXS analysis is possible only with additional information like shape information from imaging methods ⚠ |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes ⚠ | If polydispersity is above ~20 %, |

| | | | |
|---|---|--------------------|--|
| | | | data evaluation may become ambiguous without further info |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes ⚠ | if modes are too close to each other distinction of the modes is difficult |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | No | |
| | Nanoparticles are not agglomerated | Yes | |
| counting, separate or ensemble tech- niques | Single particle counting | No | |
| | Calculate number or concentration from ensemble methods | Yes | |
| | Method combination (hyphenated methods) | Yes | |
| Working range | Size range | > 1 nm | Range varies in dependence on instrument type |
| | Concentration range | 0.01 to 100 vol. % | the concentration range depends strongly on the electron density of the particles. The higher the electron density is, the more sensitive SAXS is. |
| | Minimum needed sample amount | 1 to 10 μ L | depending on the instrument and sample holder used |
| | Linearity/proportionality | Yes | |
| | Limits of detection/quantification | > 1 nm | |
| | Sensitivity (Counting efficiency) as a function of size | good | sensitivity increases with size |
| Limits of detection/quantification | What is the lower limit to detect | 1 nm to 100 nm | depends on instrument |
| Trueness | Indicate the trueness of this CM | good | SAXS is a metrologically traceable method (Meli et al., 2012) |

| | | | |
|--|---|------------------|--|
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | good | Can be quantified if uncertainties are considered properly (Pauw et al., 2013) |
| Robustness | Specify the robustness of this CM | good | Results are robust for defined SOPs of data processing |
| Precision | Specify the precision of the CM | 0.1 nm to 1.0 nm | Depending on the particles size. The larger the particles the smaller the absolute precision |
| Resolution | Specify the resolution of this CM | 0.1 nm to 5 nm | The larger the particles the smaller is the absolute resolution |
| Size distribution | Is it possible to measure size distribution? | Yes | |
| Selectivity | discrimination from non-nanoparticles of the same composition | Yes | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | Yes | |
| | discrimination from nanoparticles of another composition | Yes | with contrast variation techniques |
| | Impurities | N/A | |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | Yes | Restricted to small agglomerates/aggregates (typically <100nm) |
| Measures individual particles | Does this CM measure individual particles? | No | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | Yes | |
| Composition | Does this CM analyse composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | physical length | based on density differences |
| Destructive method or not | Is it a destructive method? | No | only if a synchrotron is used as a high flux X-ray source |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | No | Yes, if required ⚠ |
| Sample support | Does this CM need preparation on | No | |

suited supports?

Table 26: Template for characterisation of SAXS

9.5 Integral methods

9.5.1 BET for determination of specific surface area

9.5.1.1 Measuring principle

The Brunauer-Emmett-Teller (BET) theory was derived in 1938 to explain the physical adsorption of gas molecules on a solid surface⁷³. BET serves as the most often applied technique for the measurement of the specific surface of a material – typically porous. BET explains mono- and multilayer adsorption of gas molecules on a solid and dry material. Nitrogen and argon gas are widely used for measurements. BET is based on three hypotheses:

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

The resulting BET equation is applied for fitting experimental gas adsorption isotherms and gives the adsorbed monolayer gas quantity. Knowledge of gas quantity, adsorption cross section of the adsorbing gas and the molar gas volume allows calculation of the specific surface area of the material⁷⁴.

The BET equation is

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

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

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

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

The equation is an adsorption isotherm and can be plotted as a straight line with the y-axis showing $1/v[(P_0/P)-1]$ and $\varphi = P/P_0$ on the x-axis according to experimental results (BET plot). P is the equilibrium pressure and P_0 is the saturation pressure. The value of the slope, A , and the y-intercept, I , of the line are used to calculate the monolayer adsorbed gas quantity v_m and the BET constant c . The following equations are used:

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

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

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

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

9.5.1.2 Performance – general remarks

The BET method is widely used and accepted in industry, academia and (governmental and regulatory) research institutes. For example, the National Institute of Standards and Technology (NIST, US) and BAM provide a practical guide for its application which is available without charge from NIST⁷⁵. BET is also standardized by ISO (ISO 9277: 2010). BET can be applied easily. The BET theory is based on expansive assumptions (see above), and therefore, the results obtained by BET are method defined⁷⁶. Also different values for the same material can be obtained if different gases are used. As a consequence the specific surface area values should be named BET surface area and must often be considered as apparent. Nevertheless, Round-Robin tests for the development of reference materials for BET as performed by BAM proved good accuracy of the BET method.

9.5.1.3 General performance table. BET for determination of specific surface area.

| Main features | |
|---|---|
| Type of samples | dry solid |
| Type of sizing | BET is not a sizing method; for non-porous nanoparticles of known density it is possible to calculate an average size |
| Particle property measured | surface area |
| Type of quantity | surface per volume ratio as integral value over all particles in a test sample |
| size range | all size ranges |
| concentration range | only 100 % (pure, dried material is needed, normally as powder) |
| information content | relatively low, requires dry samples |
| Main advantages | |
| <ul style="list-style-type: none"> • Certified reference materials are available for a wide range of specific surfaces up to 1300 m²/g, | |
| Main disadvantages | |
| <ul style="list-style-type: none"> • Particles and non-particulate porous materials cannot be distinguished • Materials must be free of any volatile compounds, for example, water-free • Measurement times can be in the range of hours and increase with increasing surface area | |

Table 27: Performance table BET

9.5.1.4 Detailed performance table. BET for determination of specific surface area

| Criteria (generally) | Criteria (more specific) | Characterisation (Yes/ No) | Notes |
|---|--|----------------------------------|---|
| Nanoparticles in powder or liquid suspensions or embedded in a matrix | Dispersed in liquids | No | |
| | Solid particulate form | Yes | |
| | Dispersed or embedded in different kinds of matrices | No | |
| Dispersibility by dispersion protocols | Dispersible in aqueous media | No | |
| | Dispersible in non-polar liquids | No | |
| | Dispersible in polar liquids | No | |
| | Dispersible in material-specific media | No | |
| | Can be aerosolized | No | |
| Substance Nature | Inorganic | Yes | |
| | Size-dependent absorption / fluorescence | No | |
| | Carbon based | Yes | |
| | Organic, particulate | Yes | |
| | Organic, non-particulate | Yes | |
| | Biological | No | |
| | Composite | Yes | |
| | Other | | |
| Composite (particles) | Core/shell | Yes ⚠ | Particle outer size |
| | Multiple coatings | Yes ⚠ | Particle outer size |
| | A mix of two or more different materials | Yes ⚠ | Particle outer size |
| Number of nanoscaled dimensions | 1 | No | |
| | 2 | No | |
| | 3 | Yes | Size derived from measured surfaces as available for gas sorption |
| Shape of nanoparticles | Sphere or similar | Yes | Size derived from measured surfaces as available for gas sorption |
| | Equiaxial | Yes | Size derived from measured sur- |

| | | | |
|--|-----------------------------------|-----|-------------------------------------|
| | | | faces as available for gas sorption |
| | Tubes, fibres, rods | No | |
| | Flakes and discs | No | |
| | Other | | |
| Thermal degradation sensitivity | Above 0°C | No | |
| | Sensitivity above 25°C | Yes | |
| | Sensitivity above 37°C | Yes | |
| | Sensitivity above 50°C | Yes | |
| | Sensitivity above 100°C | Yes | |
| | Sensitivity above 150°C | Yes | |
| | Sensitivity above 500°C | Yes | |
| | Sensitivity above 1000°C | Yes | |
| Cooling degradation sensitivity | Sensitive below 25 °C | Yes | |
| | Sensitive below 0 °C | Yes | |
| | Sensitive below -18 °C | Yes | |
| | Sensitive below -35 °C | Yes | |
| | Sensitive below -78 °C | Yes | |
| | Sensitive below -195 °C | Yes | |
| E- beam sensitivity | e- beam sensitive | Yes | |
| | Not e-beam sensitive | Yes | |
| Sample dispersity and modality | Monodisperse sample | Yes | |
| | Polydisperse sample | Yes | |
| | Monomodal sample | Yes | |
| | Multimodal sample | Yes | |
| Conductivity properties | Conductive | Yes | |
| | Semiconductive | Yes | |
| | Insulator | Yes | |
| Magnetic properties | Magnetic | Yes | |
| | Non magnetic | Yes | |
| Functionalization / no functionalisation | Functionalised | Yes | |
| | Not functionalised | Yes | |
| Agglomeration/ ag- gregation state | Nanoparticles are aggregated | No | |
| | Nanoparticles are not aggregated | Yes | |
| | Nanoparticles are agglomerated | Yes | |
| | Nanoparticles are not agglomerat- | Yes | |

| | ed | | |
|---|---|----------------|---|
| counting, separate or ensemble techniques | Single particle counting | No | |
| | Calculate number or concentration from ensemble methods | No | |
| | Method combination (hyphenated methods) | No | |
| Working range | Size range | 1 nm to ~10 µm | |
| | Concentration range | only 100 % | pure dried material is needed, normally as powder |
| | Needed sample amount | ~100 mg | |
| | Linearity/proportionality | Yes | |
| | Limits of detection/quantification | 1 nm | |
| | Sensitivity (Counting efficiency) as a function of size | good | |
| Limits of detection/quantification | What is the lower limit to detect | 1 nm | |
| Trueness | Indicate the trueness of this CM | mean | the values are often apparent BET surfaces. The surfaces are not real surfaces for large specific surfaces. The measured surface depends often on the type of gas used for measurement. |
| Trueness in weighting the size fractions | Specify the trueness in weighting the size fractions of this CM | poor | |
| Robustness | Specify the robustness of this CM | good | for a defined SOP or the ISO standard ISO 9277:2010. |
| Precision | Specify the precision of the CM | good | |
| Resolution | Specify the resolution of this CM | good | |
| Size distribution | Is it possible to measure size distribution? | No | |
| Selectivity | discrimination from non-nanoparticles of the same composition | No | |
| | discrimination from non-nanoparticles of another composition (matrix particles) | No | |
| | discrimination from nanoparticles of another composition | No | |

| | | | |
|--|--|---|--|
| | Impurities | N/A | |
| Measures aggregation | Is it possible to measure aggregation or agglomeration of particles? | No | |
| Measures individual particles | Does this CM measure individual particles? | No | |
| Counting constituent particles in aggregations | Is the method able to count constituent particles in aggregates? | Yes  | surfaces are measured as long as the surface is available for gas sorption |
| Composition | Does this CM analyse composition? | No | |
| Specification of the type of size (diameter) | Specify: for example hydrodynamic... | specific surface areas | surface areas in square meters per gram of particles |
| Destructive method or not | Is it a destructive method? | No | as long as the particles are stable in high vacuum |
| Other Specificity | | | |
| Vacuum | Does the method operate under vacuum? | Yes | |
| Sample support | Does this CM need preparation on suited supports? | No | |

Table 28: Template for characterisation of BET for VSSA

9.5.2 Porosimetry for determination of the outer volume-specific specific surface area (VSSA)

This chapter will be up-dated in the 7.7 version of the NanoDefine Methods Manual. The results reported in the deliverable D 3.5 are going to be discussed along with the evaluation of the T-Plot method as a support for VSSA measurements.

Annex C.7 (to be included after being delivered by NANoREG WP2a)

9.6 International standards on particle sizing

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

9.6.1 Sizing

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

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

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

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

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

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

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

9.6.2 Sampling and sample preparation

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

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

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

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

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

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

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

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

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

9.6.3 Electron microscopy

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

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

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

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

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

9.6.4 PTA/DUM

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

9.6.5 TRPS / ESZ / n-Coulter-counter

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

9.6.6 ICP-MS

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

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

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

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

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

9.6.7 DMAS / DEMS = SMPS aerosol sizing

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

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

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

9.6.8 AC

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

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

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

[ISO 15825:2004](#), Rubber compounding ingredients – Carbon black – Determination of aggregate size distribution by disc centrifuge photosedimentometry.

9.6.9 DLS

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

[ISO 22412:2008](#), Particle size analysis – Dynamic light scattering (DLS).

9.6.10 ALS – laser diffraction

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

[ISO 24235:2007](#), Fine ceramics (advanced ceramics, advanced technical ceramics) – Determination of

particle size distribution of ceramic powders by laser diffraction method.

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

9.6.11 SAXS

[ISO/TS 13762:2001](#), Particle size analysis – Small angle X-ray scattering method. **WITHDRAWN**

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

9.6.12 USSp

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

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

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

9.6.13 XRD

[EN 1330-11:2007](#), Non-destructive testing - Terminology - Terms used in X-ray diffraction from polycrystalline and amorphous materials.

[EN 13925-1:2003](#), Non-destructive testing - X-ray diffraction from polycrystalline and amorphous material - Part 1: General principles.

[EN 13925-2:2003](#), Non-destructive testing - X-ray diffraction from polycrystalline and amorphous materials - Part 2: Procedures.

[ISO 24095:2009](#), Workplace air – Guidance for the measurement of respirable crystalline silica.

9.6.14 BET

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

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

10 Decision Support Flow Scheme

To be included in the final version of the NanoDefine Methods Manual (v7.7)

This chapter will illustrate and discuss a decision support flow scheme that will guide the user logically through a sequence of tasks, decision nodes and options in order to decide whether a material is a nano-material according to the EC Definition. The framework will also allow for expert judgement at every decision node.

11 Conclusions

To be included in the final version of the NanoDefine Methods Manual (v7.7)

12 Annexes

All annexes to be included in the final version of the NanoDefine Methods Manual (v7.7).

12.1 Sonication

In order to more provide the project partners with the means to dispersing the priority materials the following list of protocols have been developed for the dispersion of the selected priority materials. These protocols, where indicated as adequate, are considered to be effective and reproducible for producing particle dispersions. These protocols should be feasible in most laboratories equipped with most common probe or vial tweeter sonicators which have a nominal power rating of at least 100W. Since there can be variation in the power and efficiency of different sonicators it is recommended that, where possible, users of the protocols should attempt to optimize the sonication time for their own particular sonicator equipment. To do this it is recommended that the dispersion procedure be applied using different sonication times with the mean particle size being measured by CLS or DLS. The most suitable sonication time should be chosen as the time when either a minimum size is reached or when it can be judged that further decreases in size require excessively longer time period of sonication. In the case of probe sonication where no access to DLS/CLS is possible then users should adopt the sonication times recommended in the procedures.

It should be noted that studies for the harmonization of sonication procedures are on-going within WP2 and more detailed procedures related to the harmonization of sonication equipment and protocols will be reported in the Deliverable 2.3- Standardised dispersion protocols for high priority materials groups

At this time procedures have been experimentally tested for 7 of the 11 materials available in the project. For one material, IRMM-382 – MWCNT a well-known and generally effective dispersion protocol from the NanoGenoTox project has been proposed although at this time it has not been tested specifically with this material. However, since the IRMM-382 is expected to be very similar to the widely studied NM400 MWCNT materials it is highly probable that the dispersion protocol will be effective. The 3 materials which have yet to be examined are IRMM-389 – Basic methacrylate copolymer particles, BAM-11 – Zeolite powder and IRMM-383 – Nano-steel.

In developing these protocols it has been found that agglomerated materials cannot be adequately dispersed by the use of low energy mixing (stirring/shaking) or by the use of ultrasonic bath (USB). Instead it has been necessary to apply the high intensity methods of probe sonication (USP) or Vial-Tweeter (VT) sonication. The methods developed by JRC have been based on the use of vial tweeter while the studies done by DTU have used a probe sonicator.



Figure 17 (a): Ultrasonic Vial Tweeter sonicator(VT) (b) Ultrasonic Probe sonicator (USP)

Sonication at JRC: At this time preliminary trials at JRC show that vial-tweeter and probe sonication can produce comparable particle size distributions using similar sonication times although with different sample volumes. The probe sonicator at JRC (Hielscher UP200S) is equipped with 200W

source a 3mm probe operating at 75% amplitude and a 0.5 cycle time. Samples are held in 15ml plastic centrifuge tubes filled with 4ml of solution. The probe tip is position centrally in the tube with the end of the probe being held at a depth of 1-1.5cm below the surface of the liquid. In the case of Vial-tweeter the instrument is equipped with 250W source which is operated at 75% amplitude and a 0.5 cycle time to reduce sample heating. The samples to be treated are held in closed 2ml Eppendorf tubes mounted in the highest energy positions of the vial tweeter bar. The Eppendorf tubes are normally loaded with 2ml of sample liquid. No sample cooling is done with the vial tweeter system. Under these conditions the two systems produce similar dispersions (as assessed by CLS) when using similar treatment times.

Sonication at DTU: For comparison the sonicator system (Microson XL 2000, QSonica, LLC) used by DTU is equipped with a 100W source and a 6.4mm tip operating at 22,5 kHz fixed frequency. The sonication probe was placed at approximately one third into the dispersion. Sonication was done using 66% amplitude and the samples were continuously cooled in an ice-water bath to minimize heat development during sonication. The surface of the ice-water was ~1 cm above the surface of the suspension inside the vial to ensure proper cooling.

A detailed comparison of the two methods will be done and included in deliverable D2.3 as a part of the study which will attempt to provide procedures to verify and harmonize the performance of different sonicator systems

12.2 Preliminary dispersion procedures

At this time the temporal stability of the dispersions has not been evaluated and so dispersions should be used as soon as possible after completion of the dispersion procedure. In all cases it is advisable to shake or vortex solution as most of the priority materials contain a fraction of primary particles or agglomerates which are sufficiently large as to sediment under gravity in a relatively short time.

In the case that is desired to use a sample more than 30 minutes after preparation it is recommended that the sample be treated to 5minutes sonication in an ultrasonic bath. Experience at JRC has shown that many common nanostructured materials which have been dispersed in liquid using high energy sonication– probe sonicator or vial tweeter- may re-agglomerate over hours or days but provided that the material is not dried this is often reversible with the input of low energy sonication in a laboratory bath sonicator. The low energy of a bath sonicator is sufficient to break the agglomerates and re-suspend sedimented particle without the risk of possible sample degradation (breaking of primary particles or irreversible fusion of free particulates) which can occur if high intensity ultrasonication is done for more than the optimal time.

12.2.1 Protocol for IRMM-382 – MWCNT

At this time no optimised protocol has been verified for the specific IRMM-382 MWCNT material which has been distributed in the NanoDefine project. This material, according to the manufacturer, should be compatible with the widely studied MWCNT materials denominated NM400 which has been widely studied in the nanotoxicology field. In particular the NM400 has undergone detailed study in the NanoGenoTox project which has been responsible for the development of a dispersion protocol based on the use of Bovine Serum Albumin (BSA) protein as an effective generic wetting/surfactant compound. This method is widely applicable to many nano-materials and its effectiveness for NM400 materials has been shown in the course of the NanoGenoTox project. It is therefore considered valid to propose that this protocol be adopted for the dispersion of the IRMM-382 – MWCNT materials although at this time the effectiveness has still to be verified by TEM. The protocol developed in the NanoGenTox project may be downloaded from the following web address.

1. Nanogenotox dispersion protocol: K. A. Jensen, Y. Kembouche, E. Christiansen, N. Jacobsen, H. Wallin, C. Guiot, O. Spalla, O. Witschger, The generic NANOGENOTOX dispersion protocol: final protocol for producing suitable manufactured nanomaterial exposure media, NANOGENOTOX Joint Action, 2011.
<http://www.nanogenotox.eu/files/PDF/web%20nanogenotox%20dispersion%20protocol.pdf>

Additional information regarding the use of the protocol and the results obtained on a variety of MWCNT materials including the NM400 materials are available in the following documents which are also available via internet.

2. Nanogenotox report deliverable 4.2:
http://www.nanogenotox.eu/files/PDF/Deliverables/d4.2_tem_characterisation.pdf
3. RASMUSSEN Kirsten et al.
Multi-walled Carbon Nanotubes, NM-400, NM-401, NM-402, NM-403: Characterisation and Physico-Chemical Properties, Publications Office of the European Union JRC91205, ISBN 978-92-79-39648-9 (pdf),
<http://publications.jrc.ec.europa.eu/repository/handle/JRC91205> (2014)

12.2.2 Protocol for IRMM-380 – Organic pigment yellow 83 (nano grade)

The procedure developed for the IRMM-380 – Organic pigment yellow 83 (nano grade) produces an aqueous dispersion of the product by the use of vial tweeter- sonication and requires the use of a chemical detergent. Dispersion of this materials as not effective in pure water without detergent with 100 nm being the minimum mean size achievable. To improve the results a number of different detergents were evaluated but the most effective was found to be the chemical detergent/wetting agent known commercially as NEKAL BX. This product is more correctly known as Butyl naphthalene sulpho-nate (CAS No. 52628-07-6). The procedure is detailed below. The particle size distribution achieved by this method is close to that found by TEM and as such this method is considered to be finalised

12.2.2.1 Optimised Procedure for IRMM-380

- Step 1 Prepare a mixture of MeOH and Nekal BX (0.5 mg/ml): solution A
- Step 2 Make a Nanomaterial solution in pure MeOH (1 mg/ml), vortex (2') and USB (2'): solution B
- Step 3 Add solution A to solution B (ratio 10 µl/ml), vortex (2') and USB (2')
- Step 4 Evaporate MeOH to leave surfactant coated particles
- Step 5 Add MilliQ water to get 10mg/ml solids in water and re-disperse again by vortex and 1' VT/USP sonication
- Step 6 Prepare aqueous dilution of sample to 0.1mg/ml
- Step 7 Treat diluted sample with high power sonication using vial tweeter or Probe sonicator. For the vial tweeter the optimized time of sonication was found to be 15 minutes.

12.2.2.2 DLS/CLS size measurements for dispersed IRMM-380

| Dispersion approaches | MilliQ | EtOH | MeOH | MeOH nekalBX 15' VT |
|---|--------|-------|-------|---------------------------|
| Mean particle size (IRMM-380) by CLS (weight-size distribution) | 100n m | 25 nm | 60 nm | 50 nm |
| Mean particle size (IRMM-380) by DLS (Z-average) | | | | 47 nm |

12.2.2.2.1 Weight based size distribution (CLS) for dispersed IRMM-380 – Organic pigment yellow 83 (nano grade)

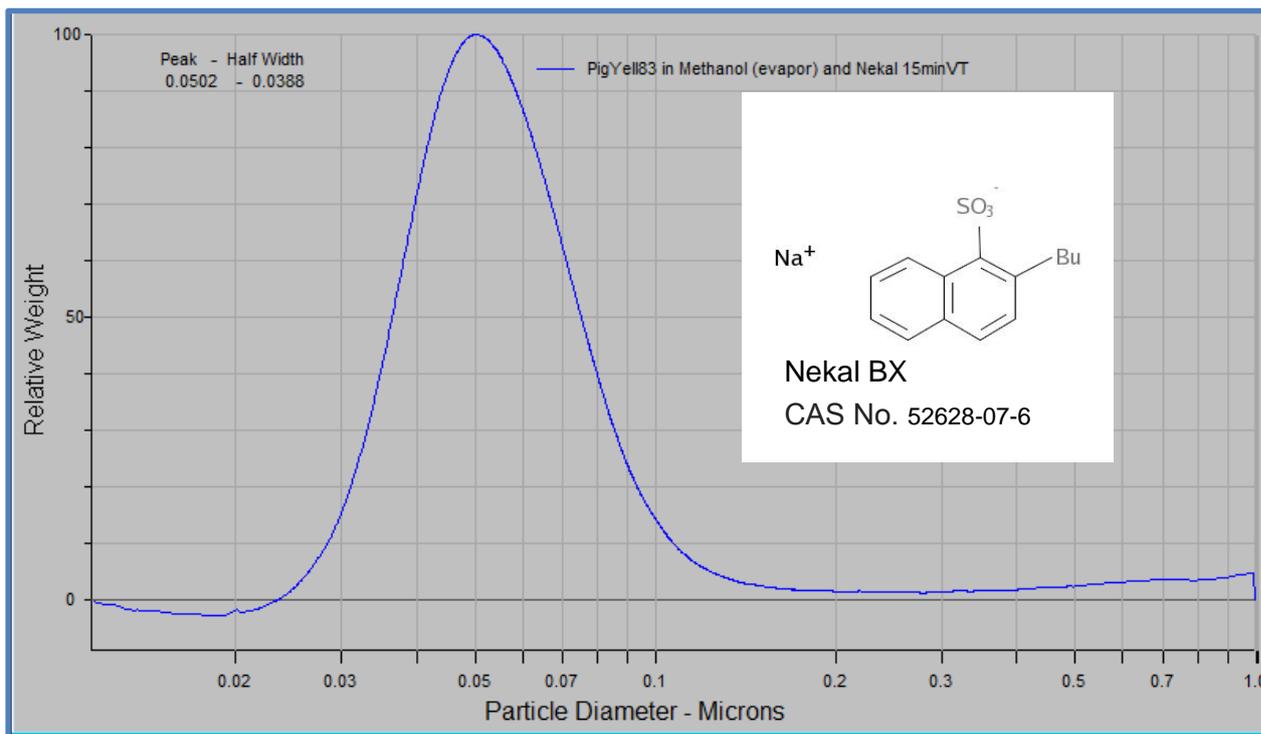


Figure 18: CLS* analysis of aqueous dispersion of Pigment Yellow 83 (IRMM380)

12.2.3 Protocol for IRMM-386 – Organic pigment yellow 83 (coarse grade)

The procedure developed for the IRMM-380 – Organic pigment yellow 83 (coarse grade) produces an aqueous dispersion of the product by the use of vial tweeter- sonication. In the initial trials with this material the procedure previously described for the nano-grade version of these materials (IRMM-380) was applied and found to give acceptable results. However further trials showed that comparable results (as assessed by CLS) could be achieved by a simpler procedure based on sonication in pure water. This ultrasonic dispersion times tested were: 1', 5', 15' and 30' with VT; 15' with USP. The optimise treatment was found to be 15' with vial tweeter.

12.2.3.1 Optimised Procedure for IRMM-386

- Step 1 Weight sufficient material to produce a final solution concentration of 1 mg/ml in MilliQ water. Homogenised it by vortexing (3") or by low power USB (3")
- Step 2 Dilute the solution to 0.1 mg/ml concentration in Milli-Q water
- Step 3 Treat diluted sample with high power sonication using vial tweeter or Probe sonicator. For the vial tweeter the optimized time of sonication for a 2ml sample was found to be 15 minutes at 75% amplitude and 0.5 cycle time

12.2.3.2 DLS/CLS size measurements for dispersed IRMM-386

| Addition of MeOH + Nekal BX and US | 15 min VT | 15 min USP |
|---|-----------|------------|
| Mean particle size (IRMM-386) by CLS (weight-size distribution) | 240 nm | 326 nm |

| Sonication Time (VT) | 1 min | 5 min | 15 min | 30 min |
|---|--------|--------|---------------|--------|
| Mean particle size (IRMM-386) by CLS (weight-size distribution) | 347 nm | 285 nm | 241 nm | 219 nm |

12.2.3.3 Weight based size distribution (CLS) for dispersed IRMM-386 – Organic pigment yellow 83

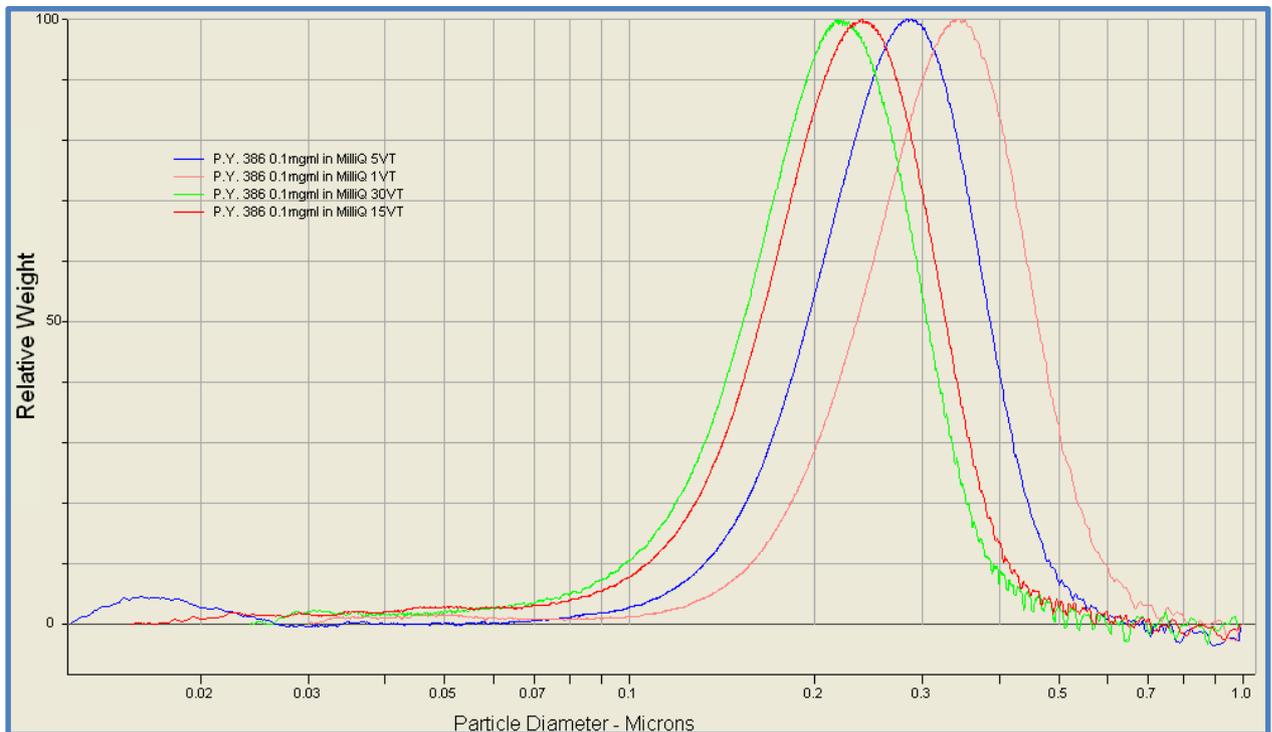


Figure 19: CLS analysis of aqueous dispersion of IRMM-386 Pigment Yellow 83 (coarse grade)

12.2.4 Protocol for IRMM-384 – CaCO₃ (fine grade)

The procedure developed for the IRMM-383.-CaCO₃ (fine grade) produces an aqueous dispersion of the product by the use of vial tweeter-sonication with addition of the stabilizing agent sodium hexametaphosphate (Calgon). In preliminary trials dispersion in pure water was tried and found to be poorly effective while other dispersing agents (Tergitol, Igepal and low molecular weight Polyacrylic acid) all were less effective than the sodium hexametaphosphate. It should be noted that the CaCO₃ has a low but non-negligible solubility in pure water and this may result in some loss of solids during dispersions. Some evidence from both TEM and CLS suggest that ultra-small particles which may be present in the solid material may be dissolving during dispersion in aqueous media. To limit the influence of the potential problem a larger than normal concentration of solids (50mg/ml) was used. This issue of possible dissolution will be the subject of a more detailed TEM based study which will be reported at a later date in deliverable D2.3- Standardised dispersion protocols for high priority materials groups.

An alternative strategy to avoid this problem could be to disperse the particles in a non-aqueous media which has lower solubility limit for CaCO₃ and so a number of trials were made using isopropanol rather than water as the dispersant liquid. In all cases the resulting mean size of particles in these distributions was significantly higher than that achieved in aqueous media.

It was concluded that the most effective method was to disperse the IRMM-384 in aqueous sodium hexametaphosphate by use of 10 minute vial tweeter sonication.

12.2.4.1 Preliminary procedure for IRMM-384

- Step 1 Prepare sodium hexametaphosphate solution (2mg/ml in Milli-Q water)
- Step 2 Add sufficient CaCO₃ to produce a concentration of 50 mg/ml and then homogenise this solution by vortexing or bath sonication for 5minutes.
- Step 3 Treat sample with high power sonication using vial tweeter or probe sonicator. For the vial tweeter the optimized time of sonication for a 2ml sample was found to be 10 minutes at 75% amplitude and 0.5 cycle time

12.2.4.2 DLS/CLS size measurements for dispersed IRMM-384

| Dispersion approaches | MilliQ | MilliQ + 10' VT | Calcon + 10' VT |
|---|-------------|-----------------|-----------------|
| Mean particle size (IRMM-384) by CLS (weight-size distribution) | 662 + 24 nm | 425 nm | 392 nm |

12.2.4.3 Weight based size distribution (CLS) for dispersed IRMM-384 – CaCO₃

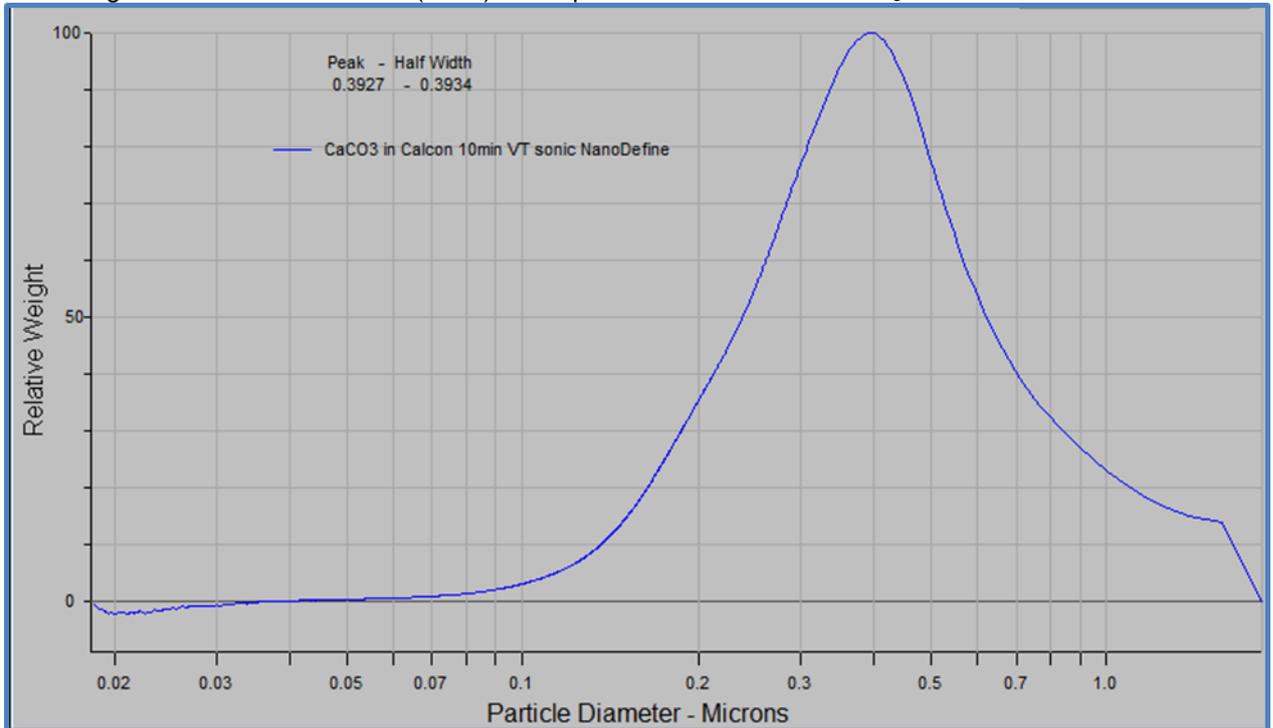


Figure 20: CLS analysis of aqueous dispersion of IRMM-384 – CaCO₃

12.2.5 Protocol for IRMM-387 – BaSO₄ (ultrafine grade)

BaSO₄ ultrafine grade (IRMM 387) in aqueous media

- Wetting agents tried - Milli-Q, EtOH (Bovine Serum Albumin (BSA) with NanoGenoTox protocol).
- Selection of stabilizing agents for materials - Sodium hexametaphosphate, and BSA tried (NanoGenotox protocol): Sodium hexametaphosphate effective and provides similar results to NanoGenotox protocol.
- Minimum US dispersion time- test 1min, 2min, 3min, 5min, 8 min and 16 min: 5min suitable.

12.2.5.1 Preliminary Procedure for IRMM-387

Step 1 Prepare stabilizing agent solution (2 mg/mL sodium hexametaphosphate) by dissolving the appropriate amount of sodium hexametaphosphate powder (e.g. Sigma Aldrich 305553) into Milli-Q water. Shake vigorously to ensure that all powder is solubilized. Subsequently, filter the stabilizer/dispersion medium using a 0.2 µm filter to ensure that no nanoparticles are present.

Step 2 Weight sufficient materials to produce a final solution with concentration of around 2.6 mg/mL BaSO₄ (e.g. 15.6 mg of BaSO₄ powder for 6 mL of stabilizing agent/dispersant)

Step 3 Add respective volume of stabilizing agent/dispersant (eg. 6 mL for 15.6 mg). Homogenize the solution by brief vortexing (20-30 s). Step 4 Treat sample with high power US (e.g. probe sonication). Optimized time of sonication was found to be 5 min (22.5 kHz probe sonicator, 66 % max. amplitude, 6.4 mm probe). After 5 min of sonication, the particle size continued to decrease but the PDI increased considerably and thereby extended sonication time is not advised.

12.2.5.2 DLS size measurements for dispersed IRMM-387

| Sonication Time | 0 min | 1 min | 2 min | 3 min | 5 min | 8 min |
|--|------------------|------------------|------------------|------------------|------------------|------------------|
| Intensity-weighted mean diameter: by DLS (Z _{ave} , cumulants method) | 127.8 ± 0.2 | 127.5 ± 0.7 | 125.9 ± 0.9 | 125.0 ± 1.0 | 124.3 ± 1.2 | 122.7 ± 0.6 |
| Polydispersity index by DLS (PDI, cumulants method) | 0.135 ± 0.006 | 0.133 ± 0.002 | 0.126 ± 0.012 | 0.132 ± 0.008 | 0.136 ± 0.006 | 0.151 ± 0.006 |

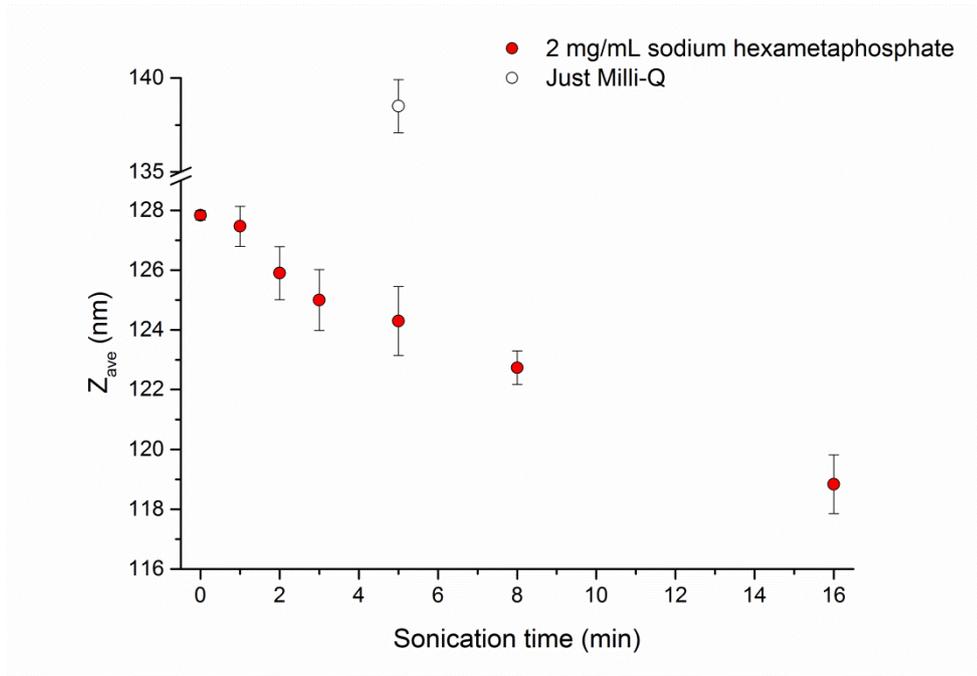


Figure 21: Z_{ave} values obtained by DLS for IRMM-387 – BaSO₄ (ultrafine grade) suspensions in Milli-Q water (N=2) and 2 mg/mL hexametaphosphate (N=3) prepared at different probe sonication times.

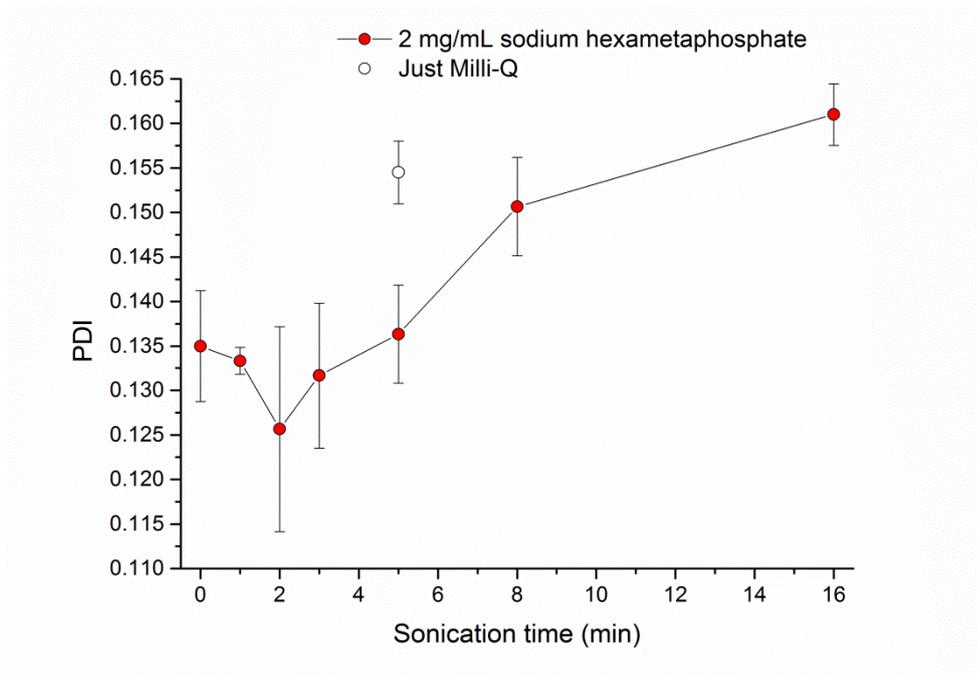


Figure 22: PDI values obtained by DLS for IRMM-387 – BaSO₄ (ultrafine grade) suspensions in Milli-Q water (N=2) and 2 mg/mL hexametaphosphate (N=3) prepared at different probe sonication times.

12.2.5.3 Intensity based size distribution (DLS) for dispersed IRMM-387– BaSO₄ (ultrafine grade)

| Sonication Time | 0 min | 1 min | 2 min | 3 min | 5 min | 8 min |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Peak mean | 148.6 ± 1.7 | 146.8 ± 2.0 | 144.3 ± 2.6 | 143.5 ± 2.1 | 143.5 ± 2.4 | 140.3 ± 0.7 |

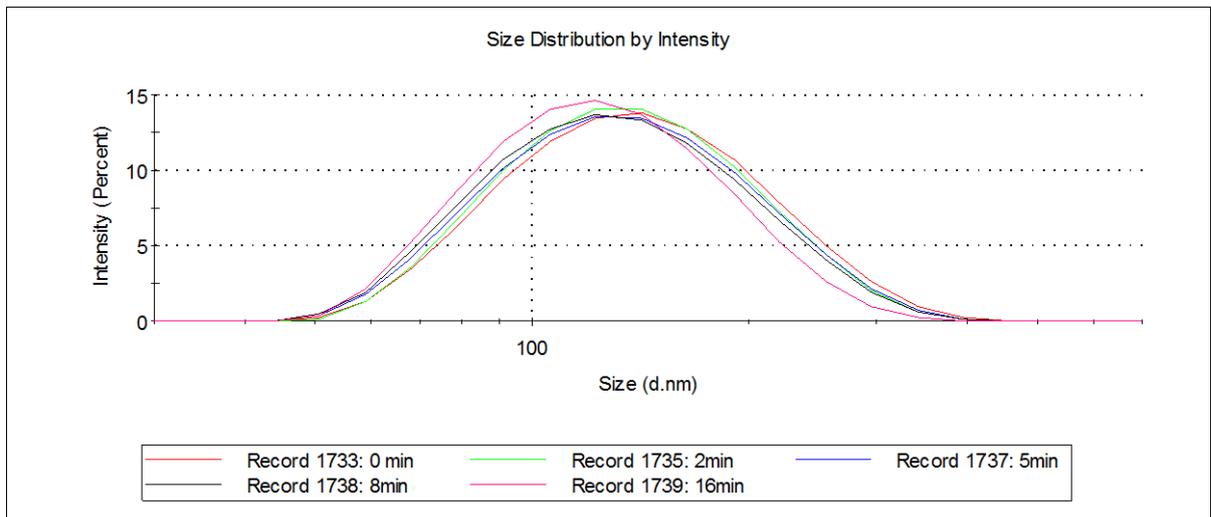


Figure 23: Intensity-weighted size distribution obtained by DLS for IRMM-387 – BaSO₄ (ultrafine grade) suspensions in 2 mg/mL hexametaphosphate (N=3) prepared at different probe sonication times.

12.2.6 Protocol for IRMM-381 – BaSO₄ (fine grade)

- BaSO₄ fine grade (IRMM 381) in aqueous media
- Wetting agents tried - Milli-Q, EtOH (NanoGenotox protocol).
- Selection of stabilizing agents for materials - Sodium hexametaphosphate, and BSA tried (NanoGenotox protocol): Sodium hexametaphosphate effective and provides similar results to NanoGenotox protocol.
- Minimum US dispersion time- test 2.5min, 5min, 10min, 15min, 20 min 25min and 30 min: 20min suitable.

12.2.6.1 Preliminary Procedure for IRMM-381

- Step 1 Prepare stabilizing agent solution (2 mg/mL sodium hexametaphosphate) by dissolving the appropriate amount of sodium hexametaphosphate powder (e.g. Sigma Aldrich 305553) into Milli-Q water. Shake vigorously to ensure that all powder is solubilized. Subsequently, filter the stabilizer/dispersion medium using a 0.2 µm filter to ensure that no nanoparticles are present.
- Step 2 Weight sufficient materials to produce a final solution with concentration of around 2.6 mg/mL BaSO₄ (e.g. 15.6 mg of BaSO₄ powder for 6 mL of stabilizing agent/dispersant)
- Step 3 Add respective volume of stabilizing agent/dispersant (eg. 6 mL for 15.6 mg). Homogenize the solution by brief vortexing (20-30 s).
- Step 4 Treat sample with high power US (e.g. probe sonication). Optimized time of sonication was found to be 20 min (22.5 kHz probe sonicator, 100 % max. amplitude, 6.4 mm probe). The minimum particle size is almost reached after 20 min probe sonication. In order avoid exceedingly long sonication times that risk damaging the probe, 20 min is advised as an appropriate sonication time.

12.2.6.2 DLS size measurements for dispersed IRMM-381 – BaSO₄ (fine grade)

| Sonication Time | 0 min | 2.5 min | 5 min | 10 min | 15 min | 20 min | 25 min |
|--|--------------|--------------|-------------|-------------|-------------|-------------|---------|
| Intensity-weighted mean diameter: by DLS (Z _{ave} , cumulants method) | 657.6 ± 17.1 | 493.8 ± 11.3 | 466.2 ± 1.0 | 415.6 ± 1.7 | 399.0 ± 1.6 | 377.4 ± 1.3 | 363 ± 2 |

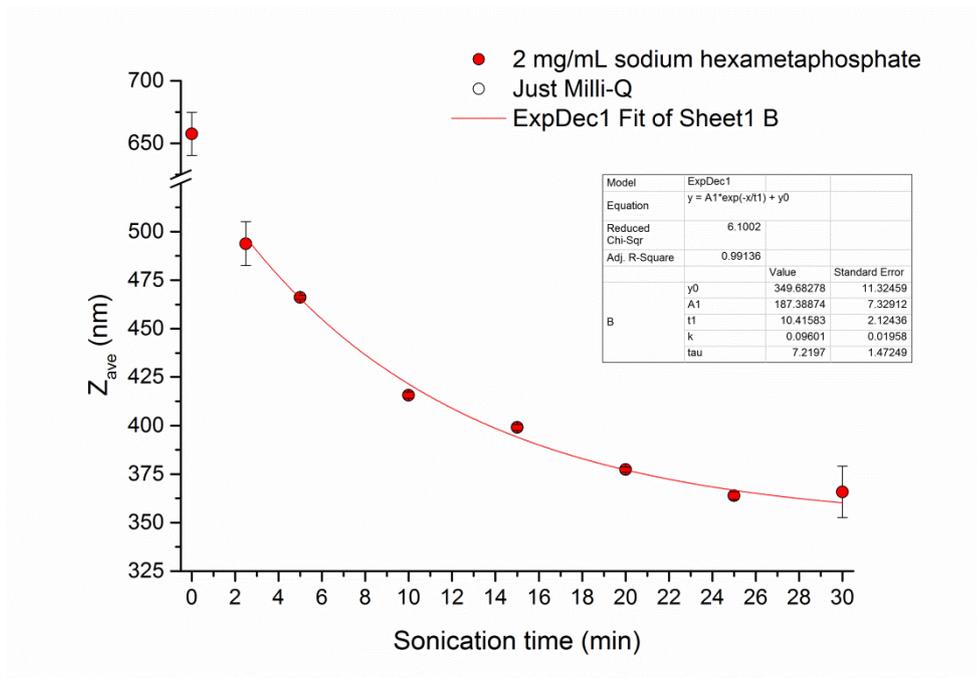


Figure 24: Z_{ave} values obtained by DLS for IRMM-381 – BaSO₄ (fine grade) suspensions in 2 mg/mL hexametaphosphate (N=2) prepared at different probe sonication times

12.2.6.3 Intensity based size distribution (DLS) for dispersed IRMM-387 – BaSO₄ (fine grade)

| Sonication Time | 0 min | 2.5 min | 5 min | 10 min | 15 min | 20 min | 25 min |
|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Peak mean (nm) | 661.8 ± 10.4 | 506.0 ± 7.3 | 489.0 ± 7.5 | 442.0 ± 4.5 | 429.1 ± 6.7 | 408.2 ± 5.1 | 392.2 ± 2.3 |

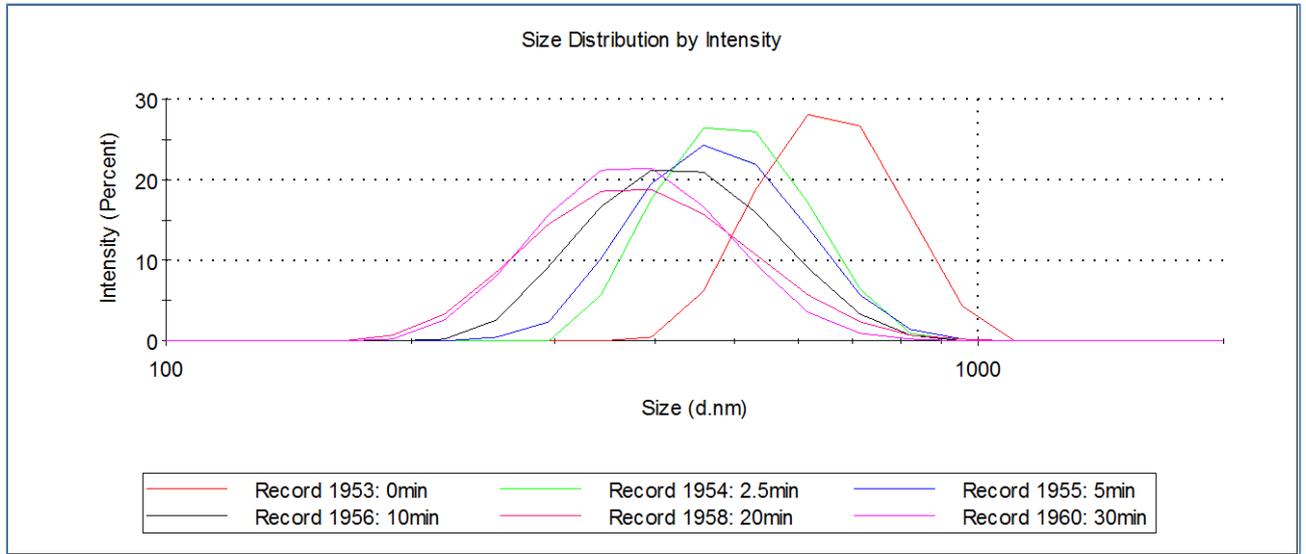


Figure 25 Intensity-weighted size distribution obtained by DLS for IRMM-381 – BaSO₄ (fine grade) suspensions in 2 mg/mL hexametaphosphate (N=2) prepared at different probe sonication times.

12.2.7 Protocol for IRMM-385 – Kaolin

Before starting the development of a dispersion protocol the Kaolin materials were examined to determine the surface charge using zeta-potential and obtained results are reported in table below.

| Material | Dispersion | State | pH | Z potential (mV) |
|-------------------|---------------------------|--|------|------------------|
| Nanodefine kaolin | 0.1 mg/ml in MilliQ water | native | 6.5 | -56 |
| Nanodefine kaolin | 0.1 mg/ml in MilliQ water | Modified by addition of 0.1 M HNO ₃ | 3.9 | -48 |
| Nanodefine kaolin | 0.1 mg/ml in MilliQ water | Modified by addition of 0.1 M NaOH | 12.1 | -71 |

From these results it seems that all materials have a strong negative charge across a large pH range from moderately acid to strongly basic. On this basis it was thought that this would confer a degree of colloidal stability in water without the need to use additional surfactants and so preliminary studies were carried out using only pure water at pH 6-7. The resulting particle size distributions showed a large proportion of material above 1µm and were not considered suitable. A second series of trials was done using method based on a protocol recommended by BASF designed for the preparation of particulate dispersion for analyses using gravitation sedimentation. In the original protocol sample dispersion was achieved by magnetic stirring but in current test the stirring has been replaced by the use of the ultrasonic vial tweeter or probe sonicator. Analysis of these dispersions showed a much lower average particle size clearly indicating the need for a chemical dispersant for effective de-agglomeration of the kaolin.

12.2.7.1 Preliminary procedure for IRMM-385 – Kaolin

- Step 1 Make a 6wt% solution of the kaolin by mixing dry clay with deionized water. Homogenise the solution by vortexing or bath sonication for 5minutes.
- Step 2 Make a 5% solution of tetra-sodium pyrophosphate (TSPP)- Na₄P₂O₇.
- Step 3 Add sufficient TSPP solution to the kaolin–water mix to produce a final TSPP concentration of 0.3mg/ml
- Step 4 Treat sample with high power sonication using vial tweeter or Probe sonicator. For the vial tweeter the optimized time of sonication for a 2ml sample was found to be 15 minutes at 75% amplitude and 0.5 cycle time

12.2.7.2 DLS/CLS size measurements for dispersed IRMM-385 – Kaolin

| Dispersion approaches | Probe Sonicator 15 | BASF DP + 15' VT |
|---|--------------------|------------------|
| Mean particle size (IRMM-385) by CLS (weight-size distribution) | 236 nm | 184 nm |

12.2.7.3 Weight based size distribution(CLS) for dispersed IRMM-385 – Kaolin

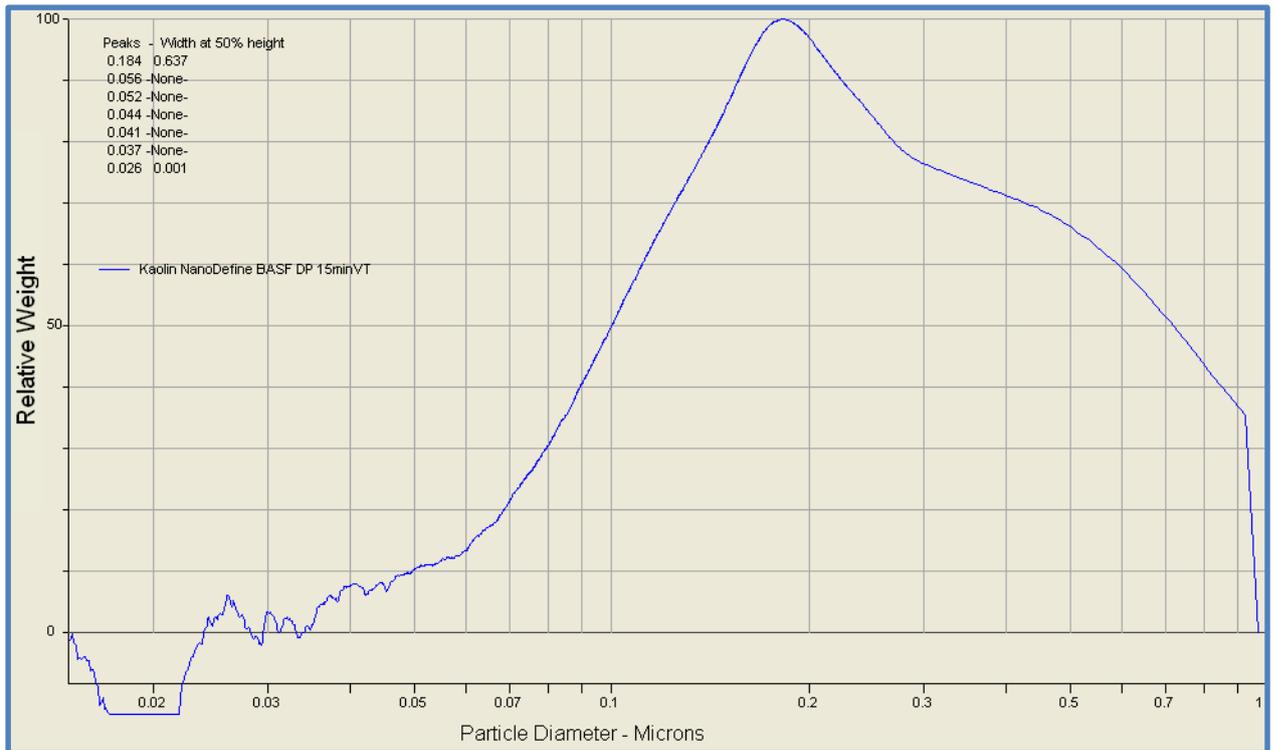


Figure 26 Weight based size distribution(CLS) for dispersed IRMM-385 – Kaolin

12.2.8 Protocol for BAM-11 – Zeolite powder

At this time no data is available for the dispersion of the BAM-11 – Zeolite powder

12.2.8.1 Protocol for IRMM-388-Coated TiO₂

The procedure developed for the IRMM-388- Coated TiO₂ produces an aqueous dispersion of the product by the use of vial tweeter- sonication and may be achieved with or without the addition of chemical stabilizers or wetting agents. After completing preliminary trials in pure water a number of attempts were made to improve on the results obtained by use of pre-wetting by ethanol and the addition of stabilizing agents (2mg/ml) including Calgon (sodium hexametaphosphate- SHMP), FL70 detergent mix and sodium dodecyl sulfate (SDS). In these cases a minor improvement was observed with Calgon being the most effective of the three additives tried. It was also noted that the dispersion of this materials was not influenced significantly by the VT sonication time with only minor improvements being observed when passing from the minimum time of 5 minutes to 15minutes. Trials up 60 minutes of sonication time were made with no further verifiable decrease in mean size occurring. It was concluded that optimised protocol should use SHMP but should it be desirable to avoid the use of additives a similar final size distribution can be achieved in pure water by a moderate increase in the sonication time.

Optimised Procedure

- Step 1 The TiO₂ material should be pre-wetted using small quantity of ethanol before being mixed with an aqueous solution of sodium hexametaphosphate (2mg/ml) to give a TiO₂ concentration of 1mg/ml
- Step 2 The solution should then be homogenised using an ultrasonic bath for 10minutes.
- Step 3 The sample should then be further diluted in aqueous sodium hexametaphosphate (2mg/ml) to produce a TiO₂ concentration of 0.1 mg/ml. The additional dilution step was done to reduce the concentration to a level better suited to direct use in CLS analysis and may be omitted if required.
- Step 4 Treat diluted sample with high power sonication using vial tweeter or Probe sonicator. For the vial tweeter the optimized time of sonication for a 2ml sample was found to be 15 minutes at 75% amplitude and 0.5 cycle time. Longer sonication times do not lead to any significant further any decrease in mean particle size.

12.2.8.2 DLS/CLS size measurements for dispersed dispersed IRMM-388- Coated TiO₂

| Dispersion approaches | MilliQ + 5' VT | MilliQ + 15' VT | MilliQ + 30' VT | Calcon + 5' VT | Calcon + 15' VT | |
|---|----------------|-----------------|-----------------|----------------|-----------------|--|
| Mean particle size (IRMM-388) by CLS (weight-size distribution) | 335 nm | 356 nm | 303 nm | 310 nm | 289 nm | |

12.2.8.3 Weight based size distribution(CLS) for dispersed IRMM-388- Coated TiO₂

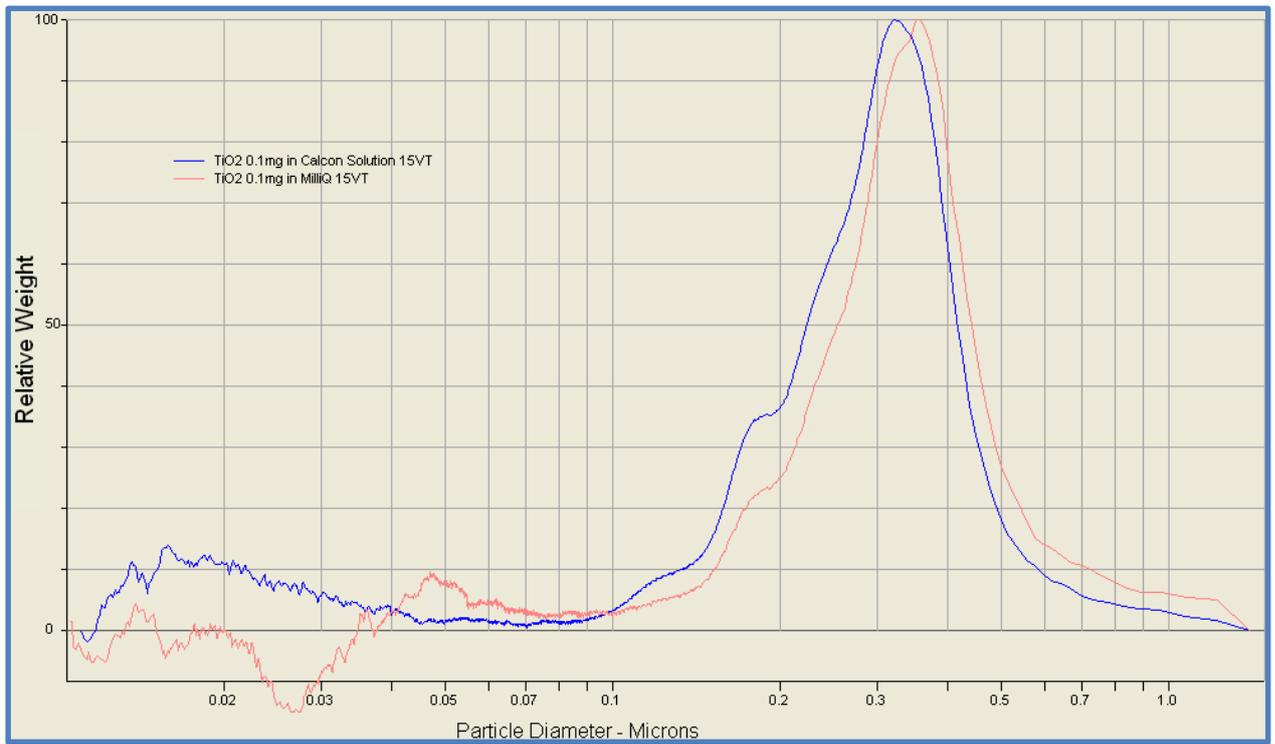


Figure 27 Weight based size distribution(CLS) for dispersed IRMM-388- Coated TiO₂

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