
Nanotehnologija - Navodilo za odkrivanje in identifikacijo nanopredmetov v kompleksnih matrikah

Nanotechnologies - Guidance on detection and identification of nano-objects in complex matrices

Nanotechnologien - Leitfaden für die Detektion und Identifizierung von Nanoobjekten in komplexen Matrizen

Nanotechnologies - Guide pour la détection et l'identification des nano-objets dans des matrices complexes

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Nanotechnologies - Document d'orientation pour la
détection et l'identification des nano-objets dans les
matrices complexes

Nanotechnologien - Leitfaden für die Detektion und
Identifizierung von Nanoobjekten in komplexen
Matrizen

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CEN/TS 17273:2018 (E)**European foreword**

This document (CEN/TS 17273:2018) has been prepared by Technical Committee CEN/TC 352 “Nanotechnologies”, the secretariat of which is held by AFNOR.

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Introduction

Nanotechnology is a rapidly developing field of science and technology that focuses on processes and materials at the nanoscale size (particle dimensions that are approximately 1 nm to 100 nm). It is a highly multidisciplinary field with a wide range of materials and applications, e.g. health care, information and communication technologies, energy production and storage, materials science/chemical engineering, manufacturing, environmental protection, consumer products (e.g. food, cosmetics, etc.). Therefore, the resulting products containing nanoscaled materials are very diverse and different in their properties.

CEN/TS 17010 provides guidelines for the identification of measurands to characterize nano-objects, and their agglomerates and aggregates and to assess specific properties relevant to the performance of materials that contain them. This document describes the measurands for characterizing nano-objects based on popular current techniques for characterizing nano-objects. Due to variable matrix interferences, a method-specific sample preparation protocol to separate particles of interest from their respective matrices is mandatory.

The production and use of nanomaterials may lead, among others, to an increasing release of nano-objects into the environment e.g. by liquid waste and production streams. To ensure sustainable use and development of nanotechnology there is a need for control and monitoring of nanomaterial systems according to their application (e.g. risk assessment). For that reason, it is essential to identify useful measurement techniques for the detection and characterization of nano-objects in so-called complex matrices, such as natural liquids, waste water, food and cosmetics [1]. It is important that specific characteristics of the nano-objects are known to be able to identify them.

There are numerous techniques for fractionation of nano-objects based on, e.g. Centrifugal Liquid Sedimentation (CLS) or flow based separation methods, such as Field-Flow Fractionation (FFF), hydrodynamic chromatography (HDC) and size exclusion chromatography (SEC). Generally, particle size distributions are obtained by the measurement of the particle concentration from the different size fractions.

Imaging techniques such as Electron Microscopy (EM) after appropriate sample preparation allow the detection/imaging of single particles according to several features, e.g. projection area, longest or shortest external dimension.

In case of counting techniques, after a high and known dilution of a particle stream only single particles are present in the detection zone and, e.g. particle volume or particle projection area dependent signals can be measured and related to the particle numbers by light scattering counting methods.

When many different particles are present in the detection zone, ensemble techniques such as static or dynamic scattering techniques can be used, provided that the size polydispersity of the particles is limited. Dynamic light scattering (DLS) analyses, for example, generate signal spectra with size dependent components. DLS can deconvolute these spectra into primary intensity-weighted particle size distributions, but only for relatively simple sample systems, with well-separated particle size modes and with the help of advanced algorithms.

The well-established particle size analysis techniques mentioned so far do not cover the chemical identification of the nano-objects. This document addresses the detection of nano-objects in complex liquid matrices which might contain an elevated level of inorganic salts, organic contaminants and larger organic and inorganic particles as well as natural background nano-objects. Therefore, it is important that for each particle two measurands are combined: not only the size is needed (for classification as a nano-object) but also the elemental composition (to discriminate the target particles with an a priori known elemental composition or morphology, from the matrix and background particles). The aim of this document is to guide the users how to combine size measurement with chemical identification for each particle.

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The document proposes the usage of 3 main characterization methods:

- Field Flow Fractionation combined with multiple detection systems delivering size related information and additionally material identification;
- Electron Microscopy equipped with Energy Dispersive X-ray Spectroscopy (EDX) to determine the elemental composition of the particles, additionally to their geometrical measures;
- Single particle Inductively Coupled Plasma – Mass Spectrometry as an elemental specific detection system gives as well size related information.

For the identification of nano-objects, this document requires a priori knowledge of their nature, e.g. their elemental composition.

All proposed methods currently do not allow *in situ* but only *ex situ* characterization.

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1 Scope

This document sets requirements for sampling and treatment of the complex matrices in order to obtain a liquid dispersion with sufficiently high concentration of the nano-objects of interest.

This document provides guidelines for detection and identification of specific nano-objects in complex matrices, such as liquid environmental compartments, waste water and consumer products (e.g. food, cosmetics). This document requires for the identification a priori knowledge of the nature of the nano-objects like their chemical composition. The selected detection and identification methods are based on a combination of size classification and chemical composition analysis. Identification can also be supported, e.g. by additional morphology characterization. Currently only Field Flow Fractionation, Electron Microscopy and single particle Inductively Coupled Plasma – Mass Spectrometry fulfil this combination condition.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CEN/TS 17010:2016, *Nanotechnologies — Guidance on measurands for characterising nano-objects and materials that contain them*

EN ISO/IEC 17025:2017, *General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2017)*

CEN ISO/TS 80004-1:2015, *Nanotechnologies — Vocabulary — Part 1: Core terms (ISO/TS 80004-1:2015)*

CEN ISO/TS 80004-2:2017, *Nanotechnologies — Vocabulary — Part 2: Nano-objects (ISO/TS 80004-2:2015)*

ISO 9276-2, *Representation of results of particle size analysis — Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions*

ISO 9276-3, *Representation of results of particle size analysis — Part 3: Adjustment of an experimental curve to a reference model*

ISO 9276-4, *Representation of results of particle size analysis — Part 4: Characterization of a classification process*

ISO 9276-5, *Representation of results of particle size analysis — Part 5: Methods of calculation relating to particle size analyses using logarithmic normal probability distribution*

ISO 9276-6, *Representation of results of particle size analysis — Part 6: Descriptive and quantitative representation of particle shape and morphology*

ISO 13322-1, *Particle size analysis — Image analysis methods — Part 1: Static image analysis methods*

ISO 14488, *Particulate materials — Sampling and sample splitting for the determination of particulate properties*

ISO/DIS 19749, *Nanotechnologies — Measurements of particle size and shape distributions by scanning electron microscopy*

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ISO/TS 21362, *Nanotechnologies — Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation*

ISO/DIS 21363, *Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in CEN ISO/TS 80004-2:2017 and CEN ISO/TS 80004-1:2015 and the following apply.

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

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

3.1**nanoscale**

length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from a larger size are predominantly exhibited in this length range.

[SOURCE: CEN ISO/TS 80004-1:2015, 2.1]

3.2**nano-object**

discrete piece of material with one, two or three external dimensions in the nanoscale (3.1)

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

[SOURCE: CEN ISO/TS 80004-1:2015, 2.5]

3.3**reference material**

RM

material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: RM is a generic term.

Note 2 to entry: Properties can be quantitative or qualitative, e.g. identity of substances or species.

Note 3 to entry: Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.

Note 4 to entry: ISO/IEC Guide 99:2007 (VIM) has an analogous definition (5.13), but restricts the term “measurement” to apply to quantitative values. However, Note 3 of ISO/IEC Guide 99:2007, 5.13, specifically includes qualitative properties, called “nominal properties”.

[SOURCE: ISO Guide 30:2015, 2.1.1]

3.4

certified reference material CRM

reference material (RM) characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

Note 1 to entry: The concept of value includes a nominal property or a qualitative attribute such as identity or sequence. Uncertainties for such attributes may be expressed as probabilities or levels of confidence.

Note 2 to entry: Metrologically valid procedures for the production and certification of RMs are given in, among others, EN ISO 17034 and ISO Guide 35.

Note 3 to entry: ISO Guide 31 gives guidance on the contents of RM certificates.

Note 4 to entry: ISO/IEC Guide 99:2007 has an analogous definition (5.14).

[SOURCE: ISO Guide 30:2015, 2.1.2, modified — One reference at the end of Note 2 to entry was altered.]

4 Symbols and abbreviations

For the purposes of this document, the following symbols and abbreviations apply.

Symbol	Quantity	SI Unit
c_n	Nano-object number concentration	m^{-3}
D	Diffusion coefficient	m^2s^{-1}
l	Mean thickness of the particle cloud	m
f	Friction coefficient	dimensionless
k	Boltzmann's constant	$\text{J}\cdot\text{K}^{-1}$
R	Retention ratio (F4)	dimensionless
m/z	mass-to-charge ratio	dimensionless
r_g	Radius of gyration	m
r_h	Hydrodynamic radius	m
T	Temperature	K
t_{elution}	Elution time (F4)	s
t_0	Channel void time of the carrier flow	s
V_0	Channel volume/channel void volume	m^3
\bullet V_c	Cross-flow rate	$\text{m}^3\cdot\text{s}^{-1}$
V_{elution}	Elution volume (F4)	m^3
V_{void}	Channel void volume of the carrier flow	m^3
w	Channel height	m
λ	Retention parameter (F4)	dimensionless

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Abbreviation	Term
AAS	Atomic Absorption Spectrometry
AF4	Asymmetric Flow-Field Flow Fractionation
BF	Bright Field
BSA	Bovine Serum Albumine
BSE	Back Scattered Electrons
CCD	Charge-Coupled Device
CLS	Centrifugal Liquid Sedimentation
DLS	Dynamic Light Scattering
DRI	Differential Refractive Index
ECD	Equivalent circular diameter
EDX	Energy-Dispersive X-ray Spectrometry
EELS	Electron Energy Loss Spectrometry
EM	Electron Microscopy
FFF	Field Flow Fractionation
F4	Flow-Field Flow Fractionation
HAADF	High-Angle Annular Dark-Field
HDC	Hydrodynamic Chromatography
hF5	Hollow-Fibre Flow-Field Flow Fractionation
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
ICP-ToFMS	Inductively Coupled Plasma – Time of flight Mass Spectrometry
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectrometry
LC	Liquid Chromatography
LIBD	Laser-Induced Breakdown Detection
LOD	Limit of detection
LOQ	Limit of quantification
MALS	Multi-Angle Light Scattering
MWCO	Molecular Weight Cut-Off
NOAA	Nano-objects and their aggregates and agglomerates
MS	Mass Spectrometry
PTA	Particle tracking analysis
RSD _R	Relative Standard Deviation for Reproducibility
RSD _r	Relative Standard Deviation for Repeatability
SDS	Sodium Dodecyl Sulphate
SE	Secondary Electrons

Abbreviation	Term
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
spICP-MS	single particle Inductively Coupled Plasma – Mass Spectrometry
STEM	Scanning transmission electron microscopy
TEM	Transmission Electron Microscopy
TOC	Total organic carbon
UPW	Ultrapure water
UV-vis	Ultraviolet and visible light
XRF	X-ray fluorescence

5 Possible tasks and measuring techniques

5.1 Examples for detection and identification tasks in complex matrices

As risk assessment of nano-objects requires, among others, characterization of the nano-objects and their aggregates in several environmental compartments, appropriate analytical techniques should be applied enabling to determine the size-distribution and nano-object concentration.

According to the scope of this document already known properties, such as elementary composition and/or morphology of these manufactured nano-objects should be used to distinguish them from natural background nano-objects. Quantitative analytical methods are also required to determine nano-objects at environmental concentrations and enable both effect and exposure assessments.

It should be ensured that these methods are sufficiently sensitive in terms of minimal particle size and material concentration. An illustration of size and concentration ranges for several sample materials is given in Annex A.

While studying product safety or risk evaluation, several examples of detection of (nano)materials in complex matrices have been investigated, e.g. silica in tomato soup, titanium dioxide in sun-lotion, silver nanoparticles in waste water, pigment and filler nanoparticles in coatings or polymer composites as well as carbon nanotubes in composites.

Several general approaches to prepare a complex sample for FFF analysis are summarized in Annex B.

The informative Annexes C and D provide examples of analysis of silver nanoparticles in decoration of pastry (by EM) and in chicken meat (by spICP-MS).

5.2 Overview of measurement techniques

The methods Field-Flow Fractionation (FFF), Electron Microscopy (EM) and single particle Inductively Coupled Plasma – Mass Spectrometry (spICP-MS) described in this document in Clause 7 are amongst the most established approaches able to detect and identify nano-objects in a number of complex matrices (e.g. waste water, environmental compartments). These methods are well known but the applications are still under development, especially for the detection of newer types of nano-objects in e.g. water and food. Experience can be gained from fields such as environmental chemistry, food chemistry, natural nanomaterial research and fundamental colloid chemistry. The methods are based on different physical phenomena and principles and for the particle size analysis different results can be obtained (e.g. hydrodynamic diameter, equivalent spherical diameter, length/radius).

FFF is a family of flow-based separation techniques able to physically separate macromolecules and particles from each other according to their molecular weight and size. FFF is applied to measure the

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particle size distribution; specific discrimination of particles due to their chemical composition is only achieved in conjunction with detection systems following the separation in FFF.

Provided that nano-objects can be representatively transferred from a stable dispersion to a suitable sample carrier (e.g. TEM-grids), Transmission and Scanning Electron Microscopy (TEM/SEM) can be applied to visualize these objects based on elastic and inelastic scattering of a parallel or convergent electron beam. Analysis of the EM images allows estimating the number-based distributions of the external dimensions of the 2D projections of the nano-objects. Specific nano-objects can be identified based on characteristic properties such as morphology, crystallographic structure, and, in combination with methods such as EDX or EELS, elemental composition with high spatial resolution.

Nano-objects in aggregates and agglomerates (NOAA), which are not dispersed by the sample preparation, can nevertheless in specific cases be detected by EM without physical separation.

spICP-MS can be used for the detection and characterization of nano-objects with elemental tags visible to ICP-MS in aqueous suspensions. spICP-MS has the exquisite advantage to allow the determination of the particle number concentration, an estimation of the particle size and number-based size distribution. Particle number concentrations that can be determined in aqueous suspensions range from 10^6 to 10^9 particles l^{-1} , and therefore sample dilution is often required with the benefit that dilution also reduces the impact of matrix interference and background signals.

Beside well known techniques also less established alternative methods or methods which are still under development are described in Annex E. These methods might be applied for special needs as they are not fully focusing on particle concentration, particle sizes or particle composition.

Some alternative detection methods and methods under development are designed for specific applications in the scientific field. In some cases the method is specific for special nano-objects which are easier to detect and trace. For example, special designed isotope-labelled nano-objects are used for bio-accumulation studies and dynamic environmental studies addressing the need to differentiate between the targeted nanomaterial content and the elemental background concentrations as well as allowing testing with environmentally realistic concentrations.

6 Guidance on sample preparation, particle detection and identification of nano-objects in complex matrices

6.1 Approach for “Detection and Identification of a relevant population of nano-objects based on a priori knowledge” (guidance chart)

The application of systematic development of sample preparation procedures and end-measurements for detection, characterization and quantification of the a priori known inorganic nano-objects requires a stepwise evaluation rather than an “all-in-once” evaluation. This stepwise evaluation as recommended in order to obtain desired information of particle sizes and concentrations joined with a relevant (measurement) uncertainty of each step or applied method. Sample preparation will likely be the most challenging part of the analytical process, despite the usage of robust analytical tools. Therefore, in order to evaluate each single preparative step and (multi) methodical approach implementing detection, characterization and quantification of the a priori known inorganic nano-objects, a protocol template can be used in order to achieve a sub-sequential evaluation for each single step [1].

In order to evaluate each single preparative step and the (multi-)methodical approach to detect, analyse the size and quantify the amount of the a priori known inorganic nano-objects, a protocol template can be used.

The user should be aware of the requirements in terms of e.g. particle concentration recovery, size distribution and requirements concerning sensitivity of the applied measurement method as well as the interpretation of obtained data during the sample preparation/stabilization process.

Based on the information on the physicochemical properties of the target particles and the matrix, a suitable sample preparation strategy is selected (Figure 1). Depending on the possible interaction of target particles with matrix components, a strategy of matrix destruction, physical separation and/or nano-object detachment and stabilization is chosen.

Stabilization of nanoparticles after digestion/separation is essential to obtain a stable dispersion. This might require the establishment of a specific pH value and/or addition of dispersing agents. The quality of the resulting suspension is optimized by maximizing the recovery of the target particles (via comparison of concentrations of a suitable identifier in the extract and in the original sample) while the matrix load is expected to be significantly reduced.

Additionally, the particle size distribution of the resulting suspension is measured, to identify the presence of particles in the micrometre and nanometre range, as well as the zeta potential of the particulate matter in the sample. Following the optimization of the dispersion (recovery, particle size range, stability) the best suited instrument(s) is(are) selected based on particle size range, concentration range and necessary pre-analysis treatment specific for each technique.

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