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Detection and characterization of manufactured nano-objects in complex matrices – Guide

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Summary of pages

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Foreword

Publishing information

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The PAS process enables a specification to be rapidly developed in order to fulfil an immediate need in industry. A PAS may be considered for further development as a British Standard, or constitute part of the UK input into the development of a European or International Standard.

Relationship with other publications

Application of PAS 139 can support the monitoring of process waste for the presence of unbound manufactured and/or engineered nano-objects, guidance on the disposal of which is given in PAS 138.

Presentational conventions

The provisions in this standard are presented in roman (i.e. upright) type. Its recommendations are expressed in sentences in which the principal auxiliary verb is "should".

Commentary, explanation and general informative material is presented in italic type, and does not constitute a normative element.

Contractual and legal considerations

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a PAS cannot confer immunity from legal obligations.

Introduction

The novel properties of materials produced on the nanoscale have led to enormous research and development activity and significant markets in “nano-enabled” consumer products [1]. Many of the nano-objects¹⁾ used in these products have already been incorporated into a range of complex matrices [2]. Such incorporation can be distinguished for the purposes of detection and characterization into:

- a) intentional incorporation (e.g. silica or titanium dioxide in food or cosmetics);
- b) accidental addition (e.g. silver and titanium dioxide in the environment).

For the purposes of this PAS, the distinction is important because intentional incorporation means that there is likely to be more information on the nature of the nano-object in its pristine form prior to incorporation. In addition, it is possible that the nano-objects could be present at reasonably high concentrations relative to the sensitivity of the detection systems discussed in Clause 6. Therefore, intentional incorporation is considerably easier to investigate than accidental incorporation for the purposes of detection and characterization, given the wider array of available techniques. These techniques are able to produce a greater amount of data, in which more confidence can be placed. In many instances, our understanding and our ability to detect accidental incorporation is currently severely limited. In both cases, likely nano-object inhomogeneity within the complex matrix means that uncertainties in sampling and sample handling are increased.

Although nano-objects have many beneficial uses, there are concerns over possible ecotoxicological and toxicological hazards that some nano-objects might present. In some reports, toxicity has been demonstrated although there remains considerable uncertainty [3] about dose measurement, biological uptake and mechanisms of action. In view of this potential hazard, regulatory and scientific assessment requires an understanding of exposure to both humans and the environment. Exposure could occur through nano-objects present in complex matrices such as biological fluids, cosmetics, environmental systems, food and pharmaceutical ingredients. Ideally such exposure can be quantified. However, in many cases, especially in accidental incorporation, a qualitative indication of nano-object presence might be all that can be determined. Current difficulties in the quantification of nano-objects primarily stem from two issues. Firstly, there could be an intricate set of interactions that nano-objects might undergo with components in complex matrices, changing the properties of the nano-object from its pristine nature. Secondly, the complex matrices are likely to intrinsically contain elements of which the manufactured nano-objects are made and to contain nano-objects from other (non-manufactured) sources.

1 Scope

This Publicly Available Specification (PAS) provides guidance on the detection and characterization of manufactured nano-objects dispersed in complex matrices. The guidance given is relevant to:

- a) nano-objects with at least two dimensions between approximately 1 nm to 100 nm including:
 - 1) nano-objects with inorganic cores (such as metals, metal oxides, quantum dots that can be present as single metals, alloys and core-shell materials);
 - 2) selected carbonaceous nano-objects (carbon nanotubes);
 - 3) nano-objects with a range of geometries and morphologies (including spherical, pyramidal, fibrillar, tubular and other shapes);

¹⁾ The term nano-object, as used in this PAS, refers to objects with one or more external dimensions between approximately 1 nm and 100 nm (see 2.4 and 3.2)

b) complex matrices including:

- 1) a range of environmental systems such as sediments and soils, surface and groundwater;
- 2) biological tissues and fluids;
- 3) food and drink;
- 4) cosmetics and healthcare, personal care and pharmaceutical products;
- 5) solid and liquid phases in b)1) to 3).

This PAS is not applicable to polymeric nano-objects, C60 and related fullerenes, biologically derived nano-objects and nano-objects with only one dimension in the nanoscale.

This PAS addresses appropriate sampling, sample handling and treatment and analysis.

Whilst the procedures described might be applicable to the detection and analysis of manufactured nano-objects in commercially produced nano-composites, such bulk materials are not the intended focus of this PAS.

It is recognized that this is a new and complex area; this PAS could be of value to those who need to simply recognize the issues (e.g. policy makers, regulators) and those who need to address them for analysis and metrology of specific nano-object containing matrices (laboratory workers in industry or elsewhere).

This PAS is not intended to address issues of detection and characterization of incidental or natural nano-objects.

2 Terms and definitions

For the purposes of this PAS, the following terms and definitions apply.

NOTE DD CEN ISO/TS 27687 and DD ISO/TS 80004-1 list terms and definitions related to core terms in the fields of nano-objects and nanotechnologies respectively.

2.1 agglomerate

collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components

NOTE 1 The forces holding an agglomerate together are weak forces, for example van der Waals forces, or simple physical entanglement.

NOTE 2 Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[DD CEN ISO/TS 27687:2009, 3.2]

2.2 aggregate

particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components

NOTE 1 The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement.

NOTE 2 Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[DD CEN ISO/TS 27687:2009, 3.3]

2.3 characterization

measurement of the physical and/or chemical properties of a nano-object and its dispersion

2.4 nano-object

material with one, two or three external dimensions in the nanoscale

NOTE Generic term for all discrete nanoscale objects.

[DD CEN ISO/TS 27687:2009, 2.2]

2.5 nanoscale

size range from approximately 1 nm to 100 nm

NOTE 1 Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.

NOTE 2 The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures, which might be implied by the absence of a lower limit.

[DD CEN ISO/TS 27687:2009, 2.1]

3 Measurands**3.1 General**

Due to the small size of nano-objects combined with their low concentration and the complexity of the matrices under consideration, detection and characterization of nano-objects is complicated. However, certain properties of nano-objects can potentially be used to detect and characterize them in complex matrices. These properties include size and size distribution, elemental composition, elemental and isotopic ratios, surface charge and chemistry, shape and crystal structure [4] (see 3.2 to 3.6). While in some cases it might be possible to accurately and precisely quantify nano-object concentration, in many cases this remains impossible. Where such nano-object quantification is possible, it is not routine or trivial. In other cases, it might be possible to give an upper limit to concentrations or to qualitatively detect, characterize and identify nano-object presence and properties. Such cases are discussed below.

3.2 Size and size distribution

For the purposes of this PAS, nano-objects are defined as having at least one external dimension between approximately 1 nm and 100 nm (see DD CEN ISO/TS 27687 and DD ISO/TS 80004-1) and this is the criterion that makes them “nano”.

NOTE Beyond this PAS, the term “nano” is also applied to some objects below 1 nm and exceeding 100 nm. An object might be defined as “nano” based on other criteria, for example, “novel” properties (such as behaviour) or particle number concentration. The definition used here should not prejudice the use of the term “nano” when defining certain pharmaceutical products and medical devices [5].

Nano-objects can also aggregate or agglomerate (see DD ISO/TS 27687) into larger structures; these aggregates or agglomerates might have dimensions larger than 100 nm but can still be considered to have nano-related properties. Current recommendations explicitly consider these to have nano properties, especially for loosely bound agglomerates, where loss of SSA is minimal [5]. Aggregates of nano-objects where there is still a large SSA and an internal structure might be differentiated, in principle, from material of similar size with no appreciable internal structure and lower specific surface areas (SSAs).

3.3 Elemental composition and elemental and isotopic ratio

Nano-objects are often composed of either one or a small number of elements. Along with size, the elemental composition of the core material is one of the nano-object's main characteristics. For example, a nano-object can be composed of materials, such as gold, silver, a gold/silver alloy or it could have a gold-silver core-shell structure. Similarly, titanium dioxide, zinc oxide, zerovalent or multivalent iron nano-objects are all distinct nano-objects, having different composition, but within the size range defined in 2.5.

Elemental composition alone is not a sufficient criterion to distinguish nano-objects, as a certain element of the nano-object might also be present as dissolved material or as a larger particle. Therefore, the total elemental concentration is indicative of an upper limit on the nano-object mass concentration. For some requirements, this information might be sufficient. For example, if the upper limit of the nano-object concentration indicates that risk to the environment or to human health is minimal, from a regulatory-based risk assessment, then further discrimination between nano-objects and their larger counterparts or dissolved elements is not necessary.

Nano-objects designed and produced for specific purposes can have specific ratios of elements or their isotopes. Such signatures have been used for identification purposes in other fields (e.g. geochemistry for source apportionment and similar studies [6]). Certain nano-objects in sun creams, energy production and other areas use specific dopants to modify function [7]. Thus these specific applications give elemental ratios between narrowly defined limits. Examples include the doping of titania with manganese or tungsten [8] for specific applications and these elemental ratios can be used to distinguish the manufactured nano-objects from natural or incidental nano-objects with different elemental ratios. Where these limits are known (e.g. where a specific source is being investigated), or where the ratios can be obtained (e.g. by a large scale survey of physico-chemical properties of samples), then these ratios can be exploited for detection and characterization.

Similarly, isotopic ratios for certain elements might provide a means of distinguishing manufactured nano-objects from other sources where industrial, biological or environmental processes result in isotopic fractionation (see also 6.2). Although as yet little utilized commercially, it is also possible to label nano-objects [9] with stable isotopes of elements to make their quantification possible with limits of detection several orders of magnitude lower than without the use of labels. In the absence of specific labels, it might be possible to exploit differences in isotopic ratios between the nano-object and the complex matrix. For example, the stable or radiogenic carbon ratios in carbon nanotubes might be sufficiently different from the ratios in relevant complex matrices to allow discrimination between the nano-object and the complex matrix. This possibility needs further examination before it can be utilized in practice.

3.4 Surface charge

Surface charge and similar (although not identical) properties, such as electrophoretic mobility and zeta potential, are properties that play a substantial role in dispersion and stabilization of certain types of nano-objects. However, other materials found in complex matrices, such as protein and humic substances, can also have a surface charge (or similar), complicating measurement by:

- a) not being unique to manufactured nano-objects; and
- b) causing changes to nano-object surface charge [10, 11].

Surface charge can be informative where nano-objects are characterized in a simple medium (e.g. water or low ionic strength media) followed by mixing with a relevant complex matrix. This provides information specifically about how the nano-objects interact with components in complex matrices (e.g. for investigating the formation of protein coronas on nano-objects in biological fluids or similar interactions with

natural organic macromolecules, such as humic substances, in environmental systems). The methods provide quantification of the changes of nano-objects as they move from their “as-prepared” state into complex matrices. Such data can provide understanding of dispersion and aggregation, interaction with biomolecular systems, and fate and behaviour in the environment. However, for low level contamination of complex media by nano-objects, the current methods available are difficult to implement for detection and characterization because of both a lack of specificity and sensitivity. Routinely, methodologies require $\text{mg}\cdot\text{L}^{-1}$ nano-object concentrations when using commercially available measurement systems.

3.5 Shape

Nano-objects have specific two and three dimensional shapes that can be resolved by appropriate methods. Particularly for the dispersed materials, shape can be a property that allows their identification in complex matrices. Most particles are spherical or roughly so. Data analysis to quantify shape depends on the analytical methods used. However, many nano-objects have complex shapes including cubes, pyramids and flower shapes. If the shape of the nano-object doesn't change after dispersion in the complex matrix due to dissolution and other processes, the regularity of the shape itself could indicate the presence of a manufactured nano-object. Distinction from the complex matrix is most obvious in the case of carbon nanotubes and other nano-objects with a high aspect ratio (nanofibres, nanotubes and nanorods made of carbon or of various metal and metal oxides). The unusual shape leads to an easier recognition of the nano-object separate from the background, although some complex matrices also contain such high aspect ratio materials of similar size. (For example, eutrophic waters where bacterial and algal growth is high, produce high concentrations of fibrils made of polysaccharides or mucopolysaccharides [12] which have similar shapes to nanofibres, nanotubes and nanorods.)

In addition, agglomerates and aggregates of nano-objects also have specific shapes, which can be quantified. How this quantification is achieved is again dependent on the metrology and analysis performed. Fractal dimension analysis can be used for quantifying agglomeration/aggregation where intentional additions of nano-objects are made and there is sufficient background data on the nano-object in its pristine form. Fractal dimensions can provide information about how the nano-object shape changes from its pristine state to its state in the complex matrix [13]. However, the methods are unlikely to unambiguously identify nano-objects in complex matrices from low level contamination, based on shape.

3.6 Crystal structure

Crystal structure relates to the regular and repeating three dimensional arrangement of atoms in a material including nano-objects, while amorphous material exhibits no such regularity. Crystal structure is frequently discussed in relation to titanium dioxide nano-objects and a small number of other nano-objects, as different crystal forms that they exhibit can affect behaviour and toxicity [14]. As with charge, crystal structure can be used in determining the presence of prepared nano-objects in simple media and can provide a signature in high concentrations with substantial prior knowledge of the nano-object. However, crystal structure is otherwise less useful in complex matrices.

3.7 Specific surface area (SSA)

SSA is the operationally defined surface area (usually measured by nitrogen sorption) normalized to mass. In general, nano-objects have a small surface area but a large SSA. SSA is inversely related to size, for non-porous and spherical nano-objects and, as such, in principle can be used in a similar manner to size (see 4.1). However, given practical considerations (see 5.7), its utility for