PAS 139:2012

BSI Standards Publication

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

bsi.

...making excellence a habit.™

Publishing and copyright information
The BSI copyright notice displayed in this document indicates when the document was last issued.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012.

ISBN 978 0 580 75718 1

ICS 07.030

No copying without BSI permission except as permitted by copyright law.

Publication history
First edition 2012

Amendments issued since publication

Date	Text affected

Contents

Summary of pages
This document comprises a front cover, an inside front cover, pages i to iv, pages 1 to 26, an inside back cover and a back cover.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

This page deliberately left blank

Foreword

Publishing information

This Publicly Available Specification (PAS) was commissioned by the Department for Business Innovation & Skills (BIS) and its development facilitated by BSI Standards Limited and published under licence from The British Standards Institution. It came into effect on 31 May 2012.

Acknowledgement is given to Jamie Lead, Professor of Environmental Nanoscience, University of Birmingham, as the technical author, and the following organizations that were involved in the development of this PAS as members of the steering group:

- Campden BRI
- Department for Environment, Food and Rural Affairs (DEFRA)
- Food and Drink Federation (FDF)
- Food and Environment Research Agency (FERA)
- Food Standards Agency (FSA)
- GlaxoSmithKline Research and Development Ltd
- Ionbond UK Ltd
- LGC Limited
- NanoRISK
- Natural History Museum
- University of Birmingham

Acknowledgement is also given to the members of a wider review panel who were consulted in the development of this PAS.

The British Standards Institution retains ownership and copyright of this PAS. BSI Standards Limited as the publisher of the PAS reserves the right to withdraw or amend this PAS on receipt of authoritative advice that it is appropriate to do so. This PAS will be reviewed at intervals not exceeding two years, and any amendments arising from the review will be published as an amended PAS and publicized in *Update Standards*.

This PAS is not to be regarded as a British Standard. It will be withdrawn upon publication of its content in, or as, a British Standard.

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-objects [1)] 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**).

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

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

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

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).

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

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

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

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 $mg \cdot L^{-1}$ 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 is 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

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

determination of nano-objects in complex media is reduced. However, as with other properties discussed above, SSA can be used to quantify the change in properties after interaction with complex matrices (e.g. after sorption of proteins).

3.8 Surface chemistry

Surface chemistry is a frequently mentioned property and differs from the properties described in **3.3**. The surface chemistry might include more detailed analysis such as oxidation, coordination chemistry and nature of any capping agent, as well as the surface elemental composition. In practice, analysis of the surface (defining the surface becomes increasingly difficult at smaller sizes) is a considerable technical challenge, although in principle the measurement of surface properties can be used in detecting and identifying nano-objects.

3.9 Agglomeration and aggregation

Nano-objects have a natural tendency towards agglomeration and aggregation, and a considerable amount of development in nanotechnology has aimed at maintaining dispersion. Agglomerates and aggregates do not always form and nano-objects can be found in both a dispersed and agglomerated/aggregated form. In complex matrices and where agglomeration/aggregation occurs, nano-objects are unlikely to be subject to homoaggregation (aggregation between two or more of the same type of nano-objects) but are more likely to be subject to heteroaggregation. In this case, heteroaggregation would involve interaction with components of the complex matrix, for example, proteins, polymers and humic substances. Deagglomeration (and possibly disaggregation) can also occur after interaction with similar complex matrix components [15]. In addition, nano-object agglomeration/aggregation can be influenced by other matrix components, such as salts.

Agglomeration and aggregation (and their reverse) can be measured and quantified in complex media by calculation of size, fractal dimension and other properties. However, knowledge of certain nano-object properties, such as size of the dispersed nano-object, is generally required to ensure this measurand provides sufficient information to unambiguously detect and characterize nano-objects in complex media.

3.10 Combination of measurands

Given the complexity of nano-objects and complex matrices, their different properties and different situations (as covered in **3.2** to **3.9**), the use of a single measurand (often determined by a single measurement technique) is unlikely to result in unambiguous detection and characterization of nano-objects in complex matrices. Indeed, the literature contains numerous examples [16] where a multi-method approach and the determination of a number of measurands is recommended, even in relatively simple media. In complex media, this approach is essential.

A number of the methods are applicable only where there is information about the nano-object prior to incorporation within the complex matrix. These methods provide information about changes on the nano-object properties within the complex matrix. For the more challenging scenario, where little or no prior information is available and there is low level contamination of the nano-objects, it might only be possible to gain upper limits on concentration or qualitative detection. Although not fully tested, the combination of sizing and element/isotope detection provides the most promising mechanism for full quantification of nano-objects under such conditions. Methods for combined size and element/isotope measurements are given in Clause **6**.

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

4 Sampling, storage and transport

4.1 Sampling

4.1.1 General

This PAS does not cover the decision-making process required to select appropriate samples; this PAS covers the sampling, storage and transport of specific samples once they have been selected. This exclusion is best explained by means of an example: planning a sampling programme in the environment to determine whether a specific point source (e.g. a sewage treatment works) is discharging nano-objects. This PAS does not cover decisions regarding whether to sample up or down stream, how far up and down stream to sample, or at what spatial and temporal frequency to sample. Discussion of relevant sampling procedures are covered elsewhere [BS 6001 (series), BS 6002 (series)]. This PAS covers decisions required once the sample has been identified.

In determining the appropriate sampling strategy, the following interlinking factors should be considered together:

a) stability of the complex matrix (see **4.1.2**);

b) stability of the nano-object (see **4.1.3**); and

c) stability of the nano-object in the complex matrix (see **4.1.4**).

4.1.2 Stability of the complex matrix

The matrix is stable over a certain amount of time dependent on the sample handling required, the analysis required and the analytical methods employed. Sampling should be conducted in such a way to ensure that changes to the complex matrix are kept to a minimum. The following changes should be considered when sampling:

a) prevention of ingress or egress of gases or volatile components;

NOTE For example, loss of carbon dioxide from drinks and environmental samples, with consequent change in pH, might lead to changes in particle surface charge and therefore changes in matrix chemistry and in some cases in microbiology. In addition, ingress of oxygen to anoxic waters and sediments cause rapid changes in matrix chemistry leading to the formation of new nano-scale objects which might cause interferences.

b) increase in turbulence produced in the sample (i.e. nano-object in complex matrix) leading to greater mixing and changes in agglomeration and deagglomeration of complex matrix components or other loss of structure;

c) minimization of temperature changes or reduction in temperature – temperature changes can alter the physico-chemistry of the matrix through increased agglomeration, changed pH or increased microbiological activity;

d) minimization of (changes in) sample exposure to light in order to reduce photo-oxidation of organic material or microbial photosynthetic processes.

4.1.3 Stability of the nano-object

The links between nano-object and matrix behaviour should be considered, as should the nature of information required. For example, if quantification of the total amount of nano-object in the complex matrix is needed, then **4.1.2** becomes largely redundant. However, if an understanding of the interactions between the nano-object and matrix is required, then careful consideration of both **4.1.2** and this subclause should be given.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

The stability of nano-objects is dependent on sample handling, and the analytical procedures used. For example, changes in matrix to nano-object ratio might affect the agglomeration state in suspension. Sampling should be conducted in such a way to ensure that changes to the nano-object are kept to a minimum. The following should be considered when sampling:

a) prevention of ingress or egress of gases, moisture or volatile components;

NOTE For example, loss of carbon dioxide from drinks and environmental samples, with consequent change in pH, could lead to changes in particle surface charge. In addition, ingress of oxygen to anoxic samples could cause changes in oxidation state of nano-objects.

b) increase in turbulence produced in system leading to greater mixing and changes in aggregation and disaggregation of nano-objects;

c) minimization of temperature changes or reduction in temperature – temperature changes can alter nano-object diffusive movement and enhance agglomeration/aggregation;

d) minimization of (changes in) sample exposure to light in order to reduce photo-oxidation of many different types of nano-objects;

e) prevention of loss of nano-object through sorption to sampling containers walls;

This issue is particularly relevant at low levels of nano-object contamination from complex matrices and appropriate choice and pre-treatment of plastic- and/or glass-ware should be considered. No particular nano-specific guidelines are available, but the plastic- and/or glass-ware surface chemistry and porosity should be considered, as they are likely to influence losses.

f) prevention of loss of nano-objects through dissolution – especially for nano-objects such as silver and zinc oxide where dissolution is relatively substantial and rapid;

Rapid dissolution of nano-objects indicates that sampling should be rapid to ensure minimal changes. Both the loss of nano-object through sorption to sampling containers walls and through dissolution can lead to an underestimation of nano-object presence and/or concentration.

g) prevention of contamination during sampling;

Contamination during sampling should be avoided in order to reduce incorrectly high values. Contamination can take two forms. Firstly, the element of which the nano-object is composed could be a contaminant. Zinc is a particularly well-known contaminant as it is used in plasticizers and could be present in settling atmospheric dust. Secondly, contamination of particles in the nanoscale range might result from, e.g. plastic- and/or glass-ware, the atmosphere or elsewhere. While this contamination might not be severe in many cases, it is worth noting that even ultra high purity waters used in research laboratories contain nano-objects that are measurable by the most sensitive methods [17].

4.1.4 Matrix heterogeneity

Complex matrix heterogeneity is likely to lead to an increased measurement uncertainty.

NOTE 1 Measurement uncertainty is defined in ISO/IEC Guide 98 and EURACHEM/CITAC's publication, Quantifying uncertainty in analytical measurements *[18].*

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

Samples with complex matrices can contain:

a) both liquid and solid materials, perhaps intimately interlinked (soils in the saturated zone, sediments and bedrock with associated interstitial water, milk, suntan lotions, etc.);

b) largely liquid (surface waters, carbonated drinks); or

c) largely solid phase (soils in the unsaturated zone, certain foods).

Prior to sampling, it should be decided whether or not the solid phase, liquid phase or both, either separately or together are of interest.

For environmental samples, there are well established protocols for sampling soils, waters, sediments and other systems for chemical and pollutant analysis [12]. Although few of these have been tested or validated for manufactured nano-objects analysis, it is likely that such protocols (e.g. taking cores of anoxic sediments and maintaining anoxic conditions), are transferable to manufactured nano-objects. However, other protocols have been investigated for sampling unmodified natural nano-objects and these also are likely to be appropriate for sampling manufactured nano-objects.

NOTE 2 Given the wide variety of potential complex matrices and methods, it is beyond the scope of this PAS to discuss all scenarios and the reader is referred to other published literature [4].

In addition, within each phase, there might be substantial heterogeneity on a spatial scale which might affect the characterization of nano-objects (see Clause **5** on sample preparation).

4.2 Transport and storage

Both transport and storage are considered together and transport is, in effect, only relevant as part of the storage process. Although current developments suggest that there is scope for the development of in-situ methods for sampling and/or analysis [19], these are not available to date to quantify nano-objects themselves. Therefore, storage and transport from the place of sampling to the place of analysis are always required.

Since both nano-objects and complex matrices are often highly unstable and changeable through processes such as agglomeration/aggregation and dissolution, the primary requirement is simply to minimize the time between sampling (see **4.1**) and sample preparation to stabilize the sample (see Clause **5**) or analysis (see Clause **6**). Where this is possible, sample preparation and stabilization should be performed at the time of sampling. The addition of chemical stabilizers (e.g. antimicrobial chemicals) should not be used as they can result in sample modification.

In addition to time, the conditions of storage can affect the nano-object and its complex matrix. Key changes that can occur during storage are dissolution, agglomeration/aggregation and microbial growth which affect both the complex matrix and the nano-object (see also **4.1.2** and **4.1.3**) and all are affected to some extent by temperature. Low temperatures can reduce microbial activity substantially, while freezing essentially prevents growth. In addition, low temperatures can slow agglomeration although changes in dissolved gas and related microbial and chemical conditions may be exacerbated by reduced temperatures. Combination with dark conditions, preventing any photosynthetic microbial activity, can be useful in stabilizing samples in some cases. In other cases, changes in sampling might be immediate or precipitated by changes such as lowered temperatures including freezing. In such cases, analysis should be undertaken as soon as possible and any delays might result in substantial uncertainty and likely change. The length of time between sampling and analysis before change might occur is sample dependent and

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

should be considered on a case-by-case basis. In general, temperatures below 0 °C are not recommended due to the serious alterations to nano-object and matrix which might occur. However, where the complex matrix is already frozen (e.g. frozen food, environmental ice), the sample should be maintained under these conditions. The validation of storage procedures should be performed before routine use.

During transport and other parts of storage, turbulent motion of the samples, especially liquid samples, should be minimized. The primary reason for this is to reduce turbulent mixing of samples which might impact deleteriously on both nano-object and complex matrix. In particular, agglomeration/aggregation rates of both might be enhanced causing uncontrolled changes to the sample.

Where possible, samples should be transported and stored in the dark due to the possibility of photo-oxidation and dissolution [20], which might change the nature of the nano-objects and reduce their concentration. Impacts on the complex matrix are also likely due to photochemistry, free radical formation and microbial photosynthetic activity.

5 Sample preparation

5.1 General

In this Clause, a number of important generic sample preparation methods are discussed, primarily filtration and ultrafiltration (see **5.2**) and field flow fractionation (see **5.3**). Ultracentrifugation preparation and sample preparation for electron microscopy (EM) and atomic force microscopy (AFM), is also discussed (see **5.5**). Five specific sample preparation methods are discussed for:

a) inductively coupled plasma mass spectrometry (ICP-MS, see **5.4**);

b) electron and atomic force microscopy (see **5.5**);

c) dynamic light scattering (DLS, see **5.6**);

d) Brunauer-Emmett-Teller (BET) SSA (see **5.7**); and

e) X-ray photoelectron spectroscopy (XPS) (see **5.8**).

These techniques are in turn covered in Clause **6**.

5.2 Filtration and ultrafiltration (UF)

Filtration and UF potentially allow quantitative permeation of nano-objects in liquid through the membrane of material smaller than the nominal pore size and retention of material larger than the nominal pore size. In the case of filtration, the relevant nominal pore sizes are ca 0.05 μm to 1.0 μm and for UF they are usually quoted in units of kDa (1 000 atomic mass units) and are approximately 1 kDa to 50 kDa, with the smallest UF pore sizes being roughly equivalent to 1 nm in size. However, using pore size data directly for interpretation of size should be performed with care, since the membranes are calibrated against retention of globular proteins and other reference materials which might behave differently to the nano-objects considered in this PAS. Suitable standards for nano-objects which might be used to calibrate separation and analytical methods are being developed [21]. Both filtration and UF can be used together or separately. Possible joint use would provide a separation at ca 0.05 μm to 0.1 μm and at 1 nm, giving size separation for dissolved (<1 nm), dispersed nano-aggregates (1 nm to 50 nm or 1 nm to 100 nm) and larger particles or nanoaggregates (>50 nm or 100 nm).

Filtration is a relatively simple method, although inaccuracies can be introduced from incorrect choice and pre-treatment of membranes or incorrect filtration conditions giving rise to non-size based separations [22]. The charge of the

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

membrane and species to be separated should be considered; for example, opposite charges on membranes and dissolved chemical species could lead to their unwanted retention on the membrane. Charge-based interactions can be offset by treating the membranes with reasonably high concentration salt solution (e.g. 0.1 M calcium nitrate) followed by rinsing with water before nano-object separation. In addition, pre-treatment with detergents and dilute (0.1 M) acid could help to prevent contamination from the membrane. Non-size separations can occur because of membrane clogging or due to formation of a "gel" layer above the membrane surface. Both clogging and gel layer formation reduce the effective membrane pore size. Clogging and gel layer formation can be minimized by filtering small volumes and using low flow rates. A simple check on membrane operation can be performed by measuring the flow rate of pure water before and after sample filtration. In an unobstructed membrane the flow rates are comparable.

In UF, filtration occurs through very small pore sizes (~1 kDa), permeation is slow and significant pressure is required to force solvent and solute through the membrane, in order to separate them from the nano-objects. Problems with gel layer formation and clogging are generally less severe than filtration because of this slow permeation [23]. As with filtration, membrane-solvent, membrane-solute and membrane-nano-object interactions mean that there can be non-size-based separation under non-ideal conditions. The choice of the physical and chemical properties of the membrane and pre-treatment are again essential to accurate characterization and the choice of their exact nature depends on both the nature of the nano-object and matrix.

For both filtration and UF, the flow of liquid is generally perpendicular to the membrane surface, leading to the problems of gel layer formation described and resulting in concentrations at the membrane surface which could be many orders of magnitude higher than concentrations in the bulk suspension. Cross-flow filtration, where the flow of solvent is across the membrane surface, can be used in order to reduce these issues; separation is the result of the pressure difference on either side of the membrane. Cross-flow (ultra)filtration is much used in the separation of proteins and environmental nanoscale materials. However, cross-flow (ultra)filtration has rarely been used for the nano-objects considered in this PAS, so validation is required.

Appropriate detection methods are required to analyse both filtrates and retentates from both filtration and UF. As the separation can be non-destructive, a wide variety of methods can be used for analysis. Electron and force microscopy methods have been used for analysis of complex matrices after filtration, with a further preparation step as discussed in **6.3**, although generally such samples have not included manufactured nano-objects. Inductively coupled plasma mass spectrometry (ICP-MS) has also been used extensively (see **6.2**), sometimes with manufactured nano-objects [24]. An issue with sample stabilization pertains to the analysis by ICP-MS and this is discussed in **6.4**.

5.3 Field flow fractionation (FFF)

Field flow fractionation (FFF) [25] is a family of separation methods, of which flow FFF (FlFFF) is the most used and appropriate for separation of nano-objects in complex matrices, although sedimentation FFF (SdFFF) can also be used. Depending on the analytical parameters used, FFF can size and separate in the nanoscale (ca 1 nm) to micro (ca 10 μm) range. In FlFFF, separation occurs in a thin channel where the velocity of liquid flowing through the channel is parabolic, with quickest flow in the centre. A cross flow of liquid pushes particles towards one wall and the concentration gradient created causes a diffusive movement of particles to the centre of the channel, where flow rates are higher. Therefore, smaller (more diffusive) particles elute from the channel first and the FlFFF is capable of high resolution separations at the nanoscale.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

The technique therefore measures hydrodynamic size, which is not identical to the core size measured by electron microscopy. Accuracy depends on the manner of calibration, either theoretically or experimentally, and comparison with other methods has often yielded good agreement. However, mass balances can be less good, with losses to membranes a particular potential problem. A more quantitative treatment of FFF theory is provided elsewhere [4, 25].

The FlFFF system facilitates a high resolution separation at very small scales , and produces an intrinsic sizing, along with separation. As FFF is a non-destructive technique, it can be used along with the detection methods discussed in Clause **6** either off-line or on-line. ICP-MS, TEM and AFM can be used as detection methods along with FFF to provide detailed information on nanoscale components in complex matrices [26]. Similar detection methods that are used for filtration and UF can be used for FFF, although the inherent sample dilution (due to mixing the sample with eluent) means that there is a need for highly sensitive sample detection methods, preconcentration or both. In more modern systems, on-channel preconcentration can be used which is less intrusive than off-line preconcentration such as addition of coagulants or filtration [27]. Nevertheless, 100 fold to 1 000 fold preconcentration of sample can lead to an increased rate of agglomeration/aggregation and preconcentration should be validated prior to routine use.

5.4 Sample preparation for inductively coupled plasma mass spectrometry (ICP-MS)

When using ICP-MS alone or after separation by methods such as FIFFF (see **6.2**), appropriate sample stabilization between sampling and sample introduction could be required. Firstly, dissolved metal components (e.g. the filtrate from UF) should be stabilized with appropriate acidification and this is well documented [28]. In general, low concentrations (0.1 M) of nitric acid are advisable, although specific metal analytes might require more stabilization. The acid prevents sorptive loss of the metal to the container walls, which occurs readily at low concentrations, while the use of nitric acid (rather than hydrochloric, sulfuric or similar) does not add new elements to the ICP-MS which might cause isobaric or polyatomic interference [29]. Secondly, some nano-objects such as zinc oxide [30] could also be readily and quantitatively dissolved by use of low concentrations (ca 0.1 M) of strong acid. Stabilization is therefore identical as for dissolved metals. However, for nano-objects that do not fully dissolve under these conditions, acidification could be problematic when using ICP-MS, since lowering the pH might cause nano-object surface charge to approach zero, leading to enhanced aggregation [10] and a lower tendency for the nano-object to be fully ionized in the ICP-MS. In such conditions, aggressive digestion (high temperature and pressure) by concentrated acids or oxidants might be required. Recovery from digestion should be quantitatively assessed by the use of controls and mass balance calculations. Suitable reference materials (RMs) are currently available [21], to help with this process. Another option might be to add further or better stabilizing agents (polymers or surfactants) to fully disperse the nano-objects allowing ICP-MS to more effectively ionize them. Validation with appropriate controls and standards should always be performed for quantitative analysis. For nano-objects, the efficiency of ionization as a function of nano-object size and core material, might be assessed by monitoring instrument signal as a function of plasma temperature.

The coupling of FlFFF to ICP-MS has been developed and is in the process of being validated for nano-objects in complex matrices. Sampling and sample treatment is often required prior to introduction into the coupled system.

If unfractionated by the methods in **5.2** and **5.3**, dissolved materials and nano-objects might exist together. Therefore, sample preparation could be difficult for reasonably soluble, charge stabilized nano-objects. Separation between the dissolved and suspended fractions can be achieved by the use of UF (see **5.2**).

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

For use of ICP-MS to generate particle number concentrations, rapid analysis and a lack of degradation of the sample should be ensured. There are no published methods for separation of a complex matrix from a nano-object and analysis of the nano-object. However, in the absence of detectable concentrations of non-manufactured nano-objects of the composition of interest, there is sufficient specificity to provide quantitative data. As with other uses of ICP-MS, it is necessary to quantify the ionization efficiency as a function of size and composition.

5.5 Sample preparation for electron microscopy (EM) and atomic force microscopy (AFM)

Either on whole samples or after separation, a number of preparation methods are suitable for EM. For EM where liquid can be present (e.g. environmental scanning EM, ESEM; or environmental transmission EM, ETEM) samples can be imaged in situ, i.e. without extensive drying and with the sample under ultra-high vacuum (UHV) conditions. The sample remains wholly or partly hydrated and little sample preparation is required. However, environmental EMs are expensive, still relatively rare and suffer from lower spatial resolution (1 to 2 orders of magnitude lower) than their high vacuum counterparts. Cryo-preparation methods (low temperature) allow visualization of minimally perturbed samples. The optimal uses for cryo-preparation have been well described in the life sciences literature [31]. For environmental samples, both cryo-methods and stabilization via a hydrophilic resin which polymerizes as the sample is dried have been shown to minimize sample disturbance for TEM analysis in comparison with traditional methods of simply drying a sample onto a grid. This preparation method is sometimes called drop deposition [32]. The issues with sample perturbation relate, in part, to increased salt concentration on drying. These are also avoided by ultracentrifugation of a sample onto a grid followed by careful but extensive washing to remove salts [32]. Ultracentrifugation also ensures an excellent coverage of nano-objects on the grid. If this method is used and the samples are still dried unprotected under UHV, changes could occur to any hydrated organic material in the complex matrix. However, it is most likely this change would not occur to any inorganic nano-objects present.

For AFM great care should be taken with choice of preparation method as underestimation or overestimation of aggregation and poor distribution on surfaces such as mica or highly ordered pyrolytic graphite (HOPG) can occur. However, ultracentrifugation and sorption methods are minimally perturbing in many cases [33]. It is currently not clear whether AFM imaging on air-dried samples or on wet samples is optimal; *a priori* the analysis of wet samples should be preferred, but this imaging mode is more difficult, slower, and might lead to sorption of organics to the AFM cantilever, affecting data interpretation [34]. Optimal sample introduction methods are likely to be dependent on the nature of both the complex matrix and the nano-object.

5.6 Sample preparation for dynamic light scattering (DLS)

Although DLS has limited use in highly complex and heterogeneous samples, there are two key considerations to improve data accuracy:

a) the availability of data on the nano-object prior to incorporation within a complex media; and

b) reduction of polydispersity [35].

The algorithms behind DLS size data produce non-unique solutions and therefore size data derived from DLS in complex systems is not accurate but can give an indication of agglomeration. Preparation of complex matrices via filtration or similar methods to reduce polydispersity is always required.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

5.7 Sample preparation for Brunauer-Emmett-Teller (BET)

BET requires presentation of a dry powder under UHV conditions. No validated methods for nano-object preparation exist and problems with drying of hydrated, organic complex matrices exist, as with EM. Different methods for SSA analysis are being investigated (see **6.6**).

5.8 Sample preparation for X-ray photoelectron spectroscopy (XPS)

XPS requires presentation of a dry powder under UHV conditions and, where this occurs, problems with drying of hydrated, organic complex matrices exist, as with EM. Some systems can present cryo-samples (i.e. at low temperature) to the XPS, reducing such problematic changes. However, cryo-XPS has attendant losses in analytical signal. There is little work in this area on nano-objects in complex matrices.

6 Separation, measurement and analytical techniques

6.1 General

This Clause provides a non-definitive list of techniques that:

a) potentially could be used in complex media for analysis on the measurands described in Clause **3**; and

b) a user might reasonably be able to access.

Techniques are excluded if their use in complex matrices is questionable (XRD), they have not been used extensively to date for this purpose (MALDI-ToF) or where access is limited (X-ray spectroscopy from synchrotron sources). They are not grouped in a particular order as in many cases they determine more than one measurand. For example, inductively coupled plasma mass spectrometry (ICP-MS) can be used to quantify total elemental and isotopic composition and ratio, along with particle number concentration [36]; while electron microscopy can measure size distribution, aggregation, shape, crystal structure, and, with associated spectroscopy methods, elemental concentration and speciation. With suitable sample preparation (see Clause **5**), further measurands can be determined.

6.2 Inductively coupled plasma mass spectrometry (ICP-MS)

ICP-MS is a viable technique used to quantify total elemental mass concentrations of metals and metalloids at extremely low limits of detection (parts per trillion to parts per billion), with "clean" preparation methods deployed (see Clause **5**). ICP-MS works by stripping away any complex matrix and ionizing metals at high temperature, before the mass spectrometry detection system is used to quantify the concentration of remaining ions against prepared standards. Validated calibration strategies, including the use of internal standards, post-separation addition of a calibrant, and the addition of an isotopically enriched nano-object for isotope dilution purposes, are not fully available. However, they are in development [37]. Advantages of ICP-MS include multi-element and multi-isotope detection, a very wide linear range, low limits of detection and ability to work at high temperatures. Isotopic ratios of certain elements might be used to discriminate between the manufactured nano-objects and natural and incidental nano-objects in complex matrices.

In operation, ICP-MS heats a sample to about 6 000 °C and it ionizes and therefore measures all the dissolved metal of interest and a fraction of the nano-objects. As nano-objects become larger, less and less of the sample is amenable to ionization. The size at which this happens has not been fully quantified and varies, dependent on the refractory nature of the relevant materials, and so complete ionization

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

needs to be validated on a case-by-case basis. The decrease in sensitivity produced is dependent on:

a) the ICP-MS operating systems;

b) the nature of the nano-object or agglomerate/aggregate; and

c) the refractory nature of the elemental composition.

For larger material (e.g. aggregates of nano-objects), digestion by acid and/or oxidants is required prior to sample introduction. Suitable controls (ionization efficiency as a function of temperature; comparison of fully digested and non-digested samples) should also be run to ensure accuracy after the digestion, making the whole analytical procedure more laborious and slower.

In certain modes of data collection, ICP-MS can be used as a single particle detector and provide information on particle number concentration, i.e. numbers of particle per unit volume [38]. In this mode, the dwell time is long enough to detect a single nano-object reaching the mass spectrometer, resulting in a pulse of ions to the detector, which is detected as a significant peak against the baseline reading. The production of such data might be useful, as number rather than mass concentration might be an important metric driving toxicological responses. However, this method is not routinely used and still requires development.

ICP-MS can be coupled off-line with the separation techniques such as UF (see **5.2**) to provide greater information. Calculation of mass balances is always essential. For example, used with UF, dialysis or ultracentrifugation, the methods can produce information relating to solubility and dialysis. More specifically, for characterization, the use of a size-based separation method along with ICP-MS allows the total particulate phase to be separated from the dissolved material. ICP-MS alone is generally unable to quantify elemental concentrations of nano-objects specifically, unless solubility is extremely low, although it has been used in this way in the literature. Where solubility is low, ICP-MS concentration could approach the nano-object (mass) concentration.

ICP-MS has also been used off- and on-line with field flow fractionation (FFF, see **5.3**) to produce combined information on particle size and concentration in environmental and other systems [39]. This method exhibits a great deal of potential for further quantitative analysis of nano-objects in complex media. However, on-line operation is only accurate where full ionization of samples is achieved. At present, this should be established on a case-by-case basis.

6.3 Electron microscopy (EM)

EM consists of a group of well-known imaging methods that can be operated in different modes, including scanning, transmission and scanning transmission (SEM, TEM and STEM, respectively). Traditionally these methods have been used in UHV which presents challenges for sample preparation (see Clause **5**). However, more recent developments have allowed them to be used to examine hydrated samples (e.g. environmental or cryo-TEM and SEM). These developments have both advantages and disadvantages for application to nano-objects in complex matrices. The main advantage is the reduced perturbation of the sample and the potential to investigate a complex matrix without drying; the main disadvantage is the concomitant reduction in spatial resolution. The various EM methods provide different information, with the STEM and TEM generally having sufficient spatial resolution to image the smallest nano-objects (i.e. ca 1 nm). In contrast, SEM is generally more suitable for imaging larger nano-objects or their aggregates (>50 nm). The highest quality UHV TEMs and STEMs can provide near-atomic resolution through developments such as aberration correction.

Information can be extracted on a single particle basis for size distribution, aggregation, morphology and crystallinity (X-ray diffraction for TEM), along with

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

elemental quantification and speciation with suitable spectroscopy attachment (X-ray energy dispersive spectroscopy, X-EDS; electron energy loss spectroscopy, EELS). As discussed in Clause **3**, multiple measurands are useful if (a) there is a degree of prior knowledge about the nano-objects and (b) they are present at high concentrations. Without prior knowledge and at lower concentrations, morphology for some nano-objects (e.g. those with high aspect ratios), or elemental composition give the requisite information for detection and characterization. Therefore, X-EDS becomes a necessary addition to EM in complex matrices. Without the ability to detect elemental information, image contrast and morphology alone provide very uncertain evidence of the presence of nano-objects.

EM (particularly TEM and STEM) have great importance in the characterization of nano-objects in complex matrices. However, the advantage of having a method that can be used to interrogate a single particle in great detail is off set by the difficulty of analysing sufficient particles for statistical rigour. Given the general need for elemental composition data also, a major issue with EM is the long analysis time.

6.4 Atomic force microscopy (AFM)

AFM is one method from a group of scanning probe microscopy techniques. These techniques are all based on interactions between the sample and a probe. In AFM, interaction forces are measured between a sharp (nm scale) tip and the sample which has been sorbed onto a suitable surface. The AFM has seen a rapid development because it (a) can be used in wet or dry (i.e. non UHV) conditions and (b) has a (sub-nm scale) size resolution high enough to be used both for imaging and force measurements. Generally, from imaging, size and selected material properties can be measured without the specificity available to EM and associated methods.

The lack of specificity in material and chemical analysis is a major limitation of AFM. However, AFM has a high spatial resolution in non-UHV conditions and is suitably for measurement of single particles. Height measurements from the surface should always be used to quantify size at the nanoscale. In lateral measurements there is lack of accuracy, which can be partially corrected. Typical AFM tips of silicon nitride are somewhat too large to perform appropriate measurements. However, the recent use of carbon nanotubes might reduce problems seen with "large" tip sizes, tip wear and complex tip geometry [16].

6.5 Dynamic light scattering (DLS)

DLS is a technique based on measuring the fluctuations over time of scattered laser light on interaction with small particles in random Brownian motion. Mathematical treatments of these fluctuations provide quantification of diffusion coefficients and, from that, hydrodynamic size. DLS is a very popular method and provides high quality data when used on monodisperse samples. Its application to complex matrices is limited due to difficulties with bias towards large particle size and the general inaccuracy of mathematical algorithms for calculations involving polydisperse and or complex systems. However, as with several other methods discussed in this Clause, DLS provides information relevant to nano-objects in complex media, if (a) relatively high concentrations of nano-objects are used and (b) there is some information about the nano-object prior to its incorporation within the complex matrix. For example, knowledge of the nano-object DLS-based size in simple solutions, along with DLS measurement of the complex matrix alone, can be contrasted with DLS measurements of the nano-object in the complex matrix.

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

6.6 Brunauer-Emmett-Teller (BET) SSA

BET measurement of SSA is a routine measurement of a property relevant to nano-objects removed from complex media. This technique uses the sorption of nitrogen gas onto a solid surface to calculate the SSA. BET is widely used for powders and solids and often reported as equivalent size, based on a spherical geometry. The amount of material required for testing is generally larger than the material available. This together with the requirement for dry samples means that its use for the characterization of nano-objects in complex matrices is limited. A number of other measurement methods have been developed including titrations with suitable sorbent (e.g. dye molecules or nuclear magnetic resonance, NMR). The ability of these methods to quantify the SSA of nano-objects in complex matrices is largely unproven.

6.7 X-ray photoelectron spectroscopy (XPS)

XPS is a method that excites a surface with X-rays of a certain energy and measures the kinetic energy distribution of the resulting emitted electrons. This method provides elemental and chemical information of materials in the top few nms of a surface. XPS gives similar data as the spectroscopy methods attached to TEM but with lower lateral resolution but better depth resolution. XPS is a UHV method and suffers the same potential problems as EM does when analysing complex media, i.e. changes in structure of components of the complex media, potentially affecting the nano-object of interest.

6.8 Dialysis

Dialysis works in a similar manner to filtration, i.e. through separation via a porous membrane, although the separation is driven by diffusion rather than by pressure. Thus, equilibrium solubility measurements of the nano-object are given from dialysis. Longer periods of time for equilibrium can be required, compared to UF. For nano-objects, current data suggests that minutes to days are required although ionic strength and stirring alter the time to equilibrium. The differences in the two measurements, the application and the outputs from ultrafiltration and from dialysis are quite different, although superficially similar. Dialysis is solely a separation method and requires further analysis in order to produce quantitative data.

6.9 Field flow fractionation (FFF)

FFF is an important analytical and separative tool discussed in **5.3** and thus, is not further considered here.

6.10 Ultraviolet-visible (UV-Vis) spectroscopy

UV-Vis spectroscopy is used for characterization, for example, for understanding surface plasmon effects in metal nano-objects, which are related to surface properties, shape and aggregation state, and for determining oxidation state in metal oxide nano-objects [40]. The method is cheap and rapid and can be applied to complex matrices, assuming absorption of the complex matrix does not overlap with the absorbance signal from the nano-object. UV-Vis spectroscopy has been applied to environmental samples [41] and to investigate protein interactions with nano-objects [42] amongst other uses.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

6.11 Fourier transform infra-red spectroscopy (FTIR)

FTIR has been used in practice for nano-object characterization [43, 44]. For instance, it has been applied to investigation of Ag nano-object interactions with cell walls [45] and proteins [46] amongst other uses.

6.12 Raman spectroscopy

Raman spectroscopy involves interactions of molecular systems and their vibrations. The method has been applied to understanding the crystal structure and chemistry of nano-objects [47] and has potential uses in complex matrices. Variants of the method such as surface enhanced Raman spectroscopy and coherent anti-Stokes Raman scattering (CARS) microscopy are also used. Surface enhanced Raman spectroscopy increases sensitivity by orders of magnitude while coherent anti-Stokes Raman scattering has been used to identify nano-object aggregates within biological cells.

6.13 Multi-method approaches to characterization

The requirement to use a range of measurands for the accurate detection and characterization of nano-objects in complex matrices is covered in **3.10**. In general, a single analytical method can provide data for several measurands as discussed in this Clause. Nevertheless, the use of several analytical and metrological methods provides improved information and reduced bias [16], especially for nano-objects in complex matrices. The advantage of a multi-method approach is due to the often heterogeneous and polydisperse nature of the nano-objects, especially in complex matrices; single methods used alone have a number of limitations for such samples. The disadvantages of this multi-method approach are the additional time and cost. There are numerous properties of nano-objects (see Clause **3**) which may be used to detect and characterize them in complex matrices. These properties might easily have large distributions of values, especially for high production volume and commercially available nano-objects. A range of techniques provides information on different parts of the distributions and different properties. For example, TEM provides detailed information on small particles, whereas DLS provides data on aggregates; TEM provides a sizing of the core of the nano-object which is very electron dense, while DLS provides information on diffusion coefficient and the hydrodynamic diameter; finally TEM is a single particle technique, which provides detailed information on a small number of particles, whereas DLS provides data averaged over a large ensemble of particles. Similar arguments could be made for other types of techniques.

Table 1 summarizes the limitations and advantages of the techniques used in the separation and analysis of nano-objects in complex matrices.

7 Reporting of results

Full reporting of results is required to ensure confidence in data. Information is, at least in part, derived from Clauses **3** to **6**. Recorded information should include but is not limited to:

a) sample and nano-object procurement (either separately or together);

b) any treatment or storage before sample received;

c) sampling methods;

d) sample storage including storage time;

e) sample treatment and preparation for analysis;

f) analytical and metrological techniques used and how they were used;

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

g) QA/QC procedures used in b) to f);
h) descriptive statistics and statistical tests used for data interpretation;
i) results and major conclusions;
j) summary of all relevant information.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

Table 1 **Summary of separation and analytical techniques which can be used for the detection and/or characterization of nano-objects in complex matrices**

Instrument and method		Property measured	Limitations	Advantages
Cross-flow ultrafiltration	CFUF	Size	• Poor size resolution (quantification of resolution rarely performed)	• Can be used to concentrate sample • Good recovery of sample • Minimal sample change(s)
Sedimentation field flow fractionation	Sed-FFF	Buoyant mass	• Generally requires a pre-concentration step	• Non-destructive • Good size resolution (accurate separation of materials of ca 10% difference in size) • Fast (1 to 2 samples per hour)
Flow field flow fractionation	Flow-FFF	Diffusion coefficient	• Generally requires a pre-concentration step • NP-membrane interactions, relatively low (~70%) nano-object recovery	• Non-destructive • Good size resolution (accurate separation of materials of ca 10% difference in size) • Fast (1 to 2 samples per hour)
Centrifugation		Sedimentation coefficient	• Low size resolution (quantification of resolution rarely performed)	• Minimal sample change(s)
(Ultra)filtration	UF	Size	• Binding with UF membrane, aggregation • Poor size resolution (ca 4 to 5 size fractions between 1 nm and 10 μm)	• Minimal sample change(s) • Quick and little sample preparation
Electron microscopy	EM	Size, shape and agglomeration/ aggregation	• Artefacts due to sample preparation • Generally, ultra high vacuum and dry sample	• High resolution (sub 1 nm) • 2D or 3D images
Atomic force microscopy	AFM	Size, shape and aggregation	• Poor lateral accuracy	• High resolution (sub 1 nm) • Minimally perturbing
Inductively coupled plasma mass spectrometry	ICP-MS	Total elemental composition in bulk mode	• Limited NP information for some nano-object types	• Simultaneous multi-elemental analyses • ICP-MS in particular, very low limits of detection (parts per trillion to parts per a billion) and isotope ratio measurement • Possibility for coupling with separation techniques • Elemental specificity
Inductively coupled plasma optical emission spectrometry	ICP-OES			

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

Table 1 **Summary of separation and analytical techniques which can be used for the detection and/or characterization of nano-objects in complex matrices** *(continued)*

Instrument and method		Property measured	Limitations	Advantages
Atomic absorption spectroscopy	AAS	Total elemental composition	• Limited NP information for some nano-object types • Single metal analysed at one time	• Possibility for hyphenation, less than ICP techniques • Elemental specificity
X-ray energy dispersive spectroscopy (X-EDS)	EDX	Semi-quantitative elemental composition for single particles	• Semi-quantitative information • Ultra high vacuum and dry sample	• Accurate and fast • Single particle elemental information
Electron energy loss spectroscopy	EELS	Elemental and chemical information	• Time consuming • Ultra high vacuum and dry sample	• Single particle chemical information
X-ray photoelectron spectroscopy	XPS	Elemental and chemical information in surface layer	• Ultra high vacuum and dry sample • Possible perturbation	• Detailed chemical information
Dynamic light scattering	DLS	Hydrodynamic diameter	• Poor accuracy for polydisperse and agglomerated samples and for complex matrices	• Fast (several samples per hour, data analysis can take longer) • Gives an indication of the aggregation state
Brunauer-Emmett-Teller	BET	Specific surface area/ porosity	• Artefacts due to sample preparation • High vacuum and dry sample	• High precision • Direct measure of surface area • Direct data on charge
X-ray diffraction	XRD	Crystal structure and crystallite size	• Powdered samples • Artefacts due to sample preparation	• Information on size and crystallinity

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

Bibliography

For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

Standards publications

BS 6001 (series), *Sampling procedures for inspection by attributes*

BS 6002 (series), *Sampling procedures for inspection by variables*

DD CEN ISO/TS 27687:2009, *Nanotechnologies – Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate*

DD ISO/TS 80004-1, *Nanotechnologies – Vocabulary – Part 1: Core terms*

ISO/IEC Guide 98-1, *Uncertainty of measurement – Part 1: Introduction to the expression of uncertainty in measurement*

ISO/IEC Guide 98-2, *Uncertainty of measurement – Part 2: Concepts and basic principles*

ISO/IEC Guide 98-3, *Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement*

PAS 138, *Disposal of manufacturing process waste containing manufactured nano-objects – Guide*

Other publications

[1] HER MAJESTY'S STATIONERY OFFICE. *UK Nanotechnologies strategy: Small technologies, great opportunities*. URN 10/825. HMSO, 2010.

[2] Klaine, S.J., et al. Nanomaterials in the environment: Behavior, fate, bioavailability, and effects*. Environmental Toxicology and Chemistry*. 2008, **27**(9), 1825-1851. ISSN 1552-8618.

[3] Fabrega, J., et al. Silver nanoparticles and their behaviour and effects in natural waters. *Environment International*. **37**, 517-531.

[4] Hassellöv, M. and R. Kaegi. *Environmental and human health impacts of nanotechnology.* Chapter: *Analysis and characterization of manufactured nanoparticles in aquatic environments*. 211-266. John Wiley & Sons Ltd, 2009.

[5] SCIENTIFIC COMMITTEE ON EMERGING AND NEWLY IDENTIFIED HEALTH RISKS (SCENIHR). *Scientific basis for the definition of the term "nanomaterial"*. Brussels: European Commission, 2010. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_032.pdf

[6] Gulson, B. and H. Wong, Stable isotopic tracing – A way forward for nanotechnology*. Environmental Health Perspectives*. 2006, **114**(10), 1486-1488.

[7] Bui, D.-N., et al. Effect of Si doping on the photocatalytic activity and photoelectrochemical property of TiO_2 nanoparticles*. Catalysis Communications*. 2011, **13**(1), 14-17. ISSN 1566-7367.

[8] Stengl, V., et al. New generation photocatalysts: How tungsten influences the nanostructure and photocatalytic activity of TiO_2 in the UV and visible regions. *ACS Applied Materials & Interfaces*. 2011, **3**(10), 4014-4023.

[9] Dybowska, A.D., et al. Synthesis of isotopically modified ZnO nanoparticles and their potential as nanotoxicity tracers*. Environmental Pollution*. 2011, **159**(1), 266-273. ISSN 0269-7491.

[10] Diegoli, S., et al. Interaction between manufactured gold nanoparticles and naturally occurring organic macromolecules*. Science of The Total Environment*. 2008, **402**(1), 51-61. ISSN 0048-9697.

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

[11] Jamison, J., et al. Altering protein surface charge with chemical modification modulates protein–gold nanoparticle aggregation. *Journal of Nanoparticle Research*. 2011, **13**(2), 625-636. ISSN 1388-0764.

[12] Santschi, P.H., et al. Fibrillar polysaccharides in marine macromolecular organic matter as imaged by atomic force microscopy and transmission electron microscopy. *Limnology and Oceanography*. 1998, **43**(5), 896-908.

[13] Baalousha, M., et al. Aggregation and surface properties of iron oxide nanoparticles: Influence of ph and natural organic matter. *Environmental Toxicology and Chemistry*. 2008, **27**(9), 1875-1882. ISSN 1552-8618.

[14] Jiang, J., et al. Does nanoparticle activity depend upon size and crystal phase? *Nanotoxicology*. 2008, **2**(1), 33-42.

[15] Li, D., et al. Effect of soil sorption and aquatic natural organic matter on the antibacterial activity of a fullerene water suspension. *Environmental Toxicology and Chemistry*. 2008, **27**(9), 1888-1894. ISSN 1552-8618.

[16] Domingos, R.F., et al. Characterizing manufactured nanoparticles in the environment: Multimethod determination of particle sizes. *Environmental Science & Technology*. 2009, **43**(19), 7277-7284. ISSN 0013-936X.

[17] Bundschuh, T., et al. Detection of biocolloids in aquatic media by Nano-Particle Analyzer. *Spectroscopy*. 2005, **19**(1), 69-78.

[18] EURACHEM/CITAC. *Quantifying uncertainty in analytical measurements*. Guide CG 4. 2nd Edition. EURACHEM/CITAC, 2000. Available from: http://www.eurachem.org.

[19] Veeken, P.L.R.v.d. and H.P.v. Leeuwen. DGT/DET gel partition features of humic acid/metal species. *Environmental Science & Technology*. 2010, **44**(14), 5523-5527. ISSN 0013-936X.

[20] Zook, J., et al. Measuring silver nanoparticle dissolution in complex biological and environmental matrices using UV-visible absorbance. *Analytical and Bioanalytical Chemistry*. 2011, **401**(6), 1993-2002. ISSN 1618-2642.

[21] NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY (NIST). *Reference materials are 'gold standard' for bio-nanotech research*. NIST, 2008 [cited 1 November 2011]. Available from: http://www.nist.gov/pml/div683/gold_010808.cfm.

[22] Yoon, Y., et al. Effects of retained natural organic matter (NOM) on NOM rejection and membrane flux decline with nanofiltration and ultrafiltration. *Desalination*. 2005, **173**(3), 209-221. ISSN 0011-9164.

[23] Chang, Y.-J. and M.M. Benjamin. Modeling formation of natural organic matter fouling layers on ultrafiltration membranes. *Journal of environmental engineering*. 2003, **129**(1), 25-32.

[24] Farkas, J., et al. Characterization of the effluent from a nanosilver producing washing machine. *Environment International*. 2011, **37**(6), 1057-1062. ISSN 0160-4120.

[25] Baalousha, M., et al. Flow field-flow fractionation for the analysis and characterization of natural colloids and manufactured nanoparticles in environmental systems: A critical review. *Journal of Chromatography A*. 2011, **1218**(27), 4078-4103. ISSN 0021-9673.

[26] Rameshwar, T., et al. Determination of the size of water-soluble nanoparticles and quantum dots by field-flow fractionation. *Journal of Nanoscience and Nanotechnology*. 2006, **6**(8), 2461-2467.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

[27] Lyvén, B., et al. Optimisation of on-channel preconcentration in flow field-flow fractionation for the determination of size distributions of low molecular weight colloidal material in natural waters. *Analytica Chimica Acta*. 1997, **357**(3), 187-196. ISSN 0003-2670.

[28] Lead, J.R., et al. Trace metal sorption by natural particles and coarse colloids. *Geochimica et Cosmochimica Acta*. 1999, **63**(11-12), 1661-1670. ISSN 0016-7037.

[29] Yang, X. and G. Low. Validation of a digestion procedure for ICP-AES and dynamic reaction cell ICP-MS for trace elemental analysis in environmental samples. *Environmental Chemistry Letters*. 2009, **7**(4), 381-387. ISSN 1610-3653.

[30] Franklin, N.M., et al. Comparative toxicity of nanoparticulate ZnO, Bulk ZnO, and $ZnCl_2$ to a freshwater microalga (Pseudokirchneriella subcapitata): The importance of particle solubility. *Environmental Science & Technology*. 2007, **41**(24), 8484-8490. ISSN 0013-936X.

[31] Miot, J., et al. Preservation of protein globules and peptidoglycan in the mineralized cell wall of nitrate-reducing, iron(II)-oxidizing bacteria: a cryo-electron microscopy study. *Geobiology*. 2011, **9**(6), 459-470. ISSN 1472-4669.

[32] Lead, J.R., D. Muirhead and C. T. Gibson. Characterisation of freshwater aquatic colloids by atomic force microscopy. *Environmental Science and Technology*. 2005, **39**, 6930-6936.

[33] Balnois, E. and K.J. Wilkinson. Sample preparation techniques for the observation of environmental biopolymers by atomic force microscopy. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2002, **207**(1-3), 229-242. ISSN 0927-7757.

[34] Baalousha, M. and J.R. Lead. Characterization of natural aquatic colloids (<5 nm) by flow-field flow fractionation and atomic force microscopy. *Environmental Science & Technology*. 2007, **41**(4), 1111-1117. ISSN 0013-936X.

[35] Dukhin, A.S., et al. Monitoring nanoparticles in the presence of larger particles in liquids using acoustics and electron microscopy. *Journal of Colloid and Interface Science*. 2010, **342**(1), 18-25. ISSN 0021-9797.

[36] Bolea, E., et al. Size characterization and quantification of silver nanoparticles by asymmetric flow field-flow fractionation coupled with inductively coupled plasma mass spectrometry. *Analytical and Bioanalytical Chemistry*. 2011, **401**(9), 2723-2732. ISSN 1618-2642.

[37] von der Kammer, F., et al. Separation, and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation. *Trends in Analytical Chemistry*. 2011, **30**, 425-436.

[38] Dubascoux, S., et al. Field-flow fractionation and inductively coupled plasma mass spectrometer coupling: History, development and applications. *Journal of Analytical Atomic Spectrometry*. 2010, **25**(5), 613-623. ISSN 0267-9477.

[39] Huang, W., et al. Dissolution and nanoparticle generation behavior of Be-associated materials in synthetic lung fluid using inductively coupled plasma mass spectroscopy and flow field-flow fractionation. *Journal of Chromatography A*. 2011, **1218**(27), 4149-4159.

[40] Heckert, E.G., et al. The role of cerium redox state in the SOD mimetic activity of nanoceria. *Biomaterials*. 2008, **29**(18), 2705-2709.

[41] Cumberland, S.A. and J. R. Lead. Particle size distributions of silver nanoparticles at environmentally relevant conditions. *Journal of Chromatography A*. 2009, **1216**, 9099-9016.

[42] Dong, Z., et al. Phase behavior of poly(sulfobetaine methacrylate)-grafted silica nanoparticles and their stability in protein solutions. *Langmuir: The ACS Journal of Surfaces and Colloids*. 2011, **27**(24), 15282-91.

 © The British Standards Institution 2012. Published by BSI Standards Limited 2012

[43] Srivastava, R., et al. 4-(Ethoxycarbonyl) phenyl-1-amino-oxobutanoic acid-chitosan complex as a new matrix for silver nanocomposite film: Preparation, characterization and antibacterial activity. *International Journal of Biological Macromolecules*. 2011, **49**(5), 863-870.

[44] Aydin, M., et al. Synthesis, magnetic and electrical characteristics of poly(2-thiophen-3-yl-malonic acid)/Fe_3O_4 nanocomposite. *Journal of Alloys and Compounds*. 2012, **514**, 45-53.

[45] Salunkhe, R.B., et al. Studies on Silver Accumulation and Nanoparticle Synthesis By Cochliobolus lunatus. *Applied Biochemistry and Biotechnology*. 2011, **165**(1), 221-234.

[46] Sennuga, A., et al. Ferroxidase activity of apoferritin is increased in the presence of platinum nanoparticles. *Nanotechnology*. 2011, **23**(3), 035102.

[47] Pottier, A., et al. Size tailoring of TiO_2 anatase nanoparticles in aqueous medium and synthesis of nanocomposites. Characterization by Raman spectroscopy. *Journal of Materials Chemistry*. 2003, **13**, 877-882.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

This page deliberately left blank

This page deliberately left blank

NO COPYING WITHOUT BSI PERMISSION EXCEPT AS PERMITTED BY COPYRIGHT LAW

British Standards Institution (BSI)

BSI is the national body responsible for preparing British Standards and other standards-related publications, information and services.

BSI is incorporated by Royal Charter. British Standards and other standardization products are published by BSI Standards Limited.

About us

We bring together business, industry, government, consumers, innovators and others to shape their combined experience and expertise into standards -based solutions.

The knowledge embodied in our standards has been carefully assembled in a dependable format and refined through our open consultation process. Organizations of all sizes and across all sectors choose standards to help them achieve their goals.

Information on standards

We can provide you with the knowledge that your organization needs to succeed. Find out more about British Standards by visiting our website at bsigroup.com/standards or contacting our Customer Services team or Knowledge Centre.

Buying standards

You can buy and download PDF versions of BSI publications, including British and adopted European and international standards, through our website at bsigroup.com/shop, where hard copies can also be purchased.

If you need international and foreign standards from other Standards Development Organizations, hard copies can be ordered from our Customer Services team.

Subscriptions

Our range of subscription services are designed to make using standards easier for you. For further information on our subscription products go to bsigroup.com/subscriptions.

With **British Standards Online (BSOL)** you'll have instant access to over 55,000 British and adopted European and international standards from your desktop. It's available 24/7 and is refreshed daily so you'll always be up to date.

You can keep in touch with standards developments and receive substantial discounts on the purchase price of standards, both in single copy and subscription format, by becoming a **BSI Subscribing Member**.

PLUS is an updating service exclusive to BSI Subscribing Members. You will automatically receive the latest hard copy of your standards when they're revised or replaced.

To find out more about becoming a BSI Subscribing Member and the benefits of membership, please visit bsigroup.com/shop.

With a **Multi-User Network Licence (MUNL)** you are able to host standards publications on your intranet. Licences can cover as few or as many users as you wish. With updates supplied as soon as they're available, you can be sure your documentation is current. For further information, email bsmusales@bsigroup.com.

BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK

Revisions

Our British Standards and other publications are updated by amendment or revision.

We continually improve the quality of our products and services to benefit your business. If you find an inaccuracy or ambiguity within a British Standard or other BSI publication please inform the Knowledge Centre.

Copyright

All the data, software and documentation set out in all British Standards and other BSI publications are the property of and copyrighted by BSI, or some person or entity that owns copyright in the information used (such as the international standardization bodies) and has formally licensed such information to BSI for commercial publication and use. Except as permitted under the Copyright, Designs and Patents Act 1988 no extract may be reproduced, stored in a retrieval system or transmitted in any form or by any means – electronic, photocopying, recording or otherwise – without prior written permission from BSI. Details and advice can be obtained from the Copyright & Licensing Department.

Useful Contacts:

Customer Services
Tel: +44 845 086 9001
Email (orders): orders@bsigroup.com
Email (enquiries): cservices@bsigroup.com

Subscriptions
Tel: +44 845 086 9001
Email: subscriptions@bsigroup.com

Knowledge Centre
Tel: +44 20 8996 7004
Email: knowledgecentre@bsigroup.com

Copyright & Licensing
Tel: +44 20 8996 7070
Email: copyright@bsigroup.com

bsi.

...making excellence a habit.™