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Nanotechnologies — Sample preparation for the characterization of metal and metal-oxide nano-objects in water samples

Nanotechnologies — Préparation des échantillons pour la caractérisation de nano-objets métalliques et d'oxydes métalliques dans les échantillons d'eau

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Foreword

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This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

With the increasing use of manufactured nano-objects in commercial products and applications, such as consumer and healthcare products, solar panels, batteries, surface coatings, and water treatment, it is likely that these nano-objects will eventually be released to the environment, especially in aquatic environments. There are, however, limited technical data available on the occurrence/transport/fate of manufactured nano-objects after they are released to the aquatic environment. Together with the current global shortage of water supply and an increasing demand for water recycling, concerns for the potential health impacts of manufactured nano-objects in water will increase.

Related to nano-objects in aqueous matrices, knowledge of environmental parameters like natural organic matter content, pH, ionic strength (IS) etc., is important since these may influence particle size, fate, stability and chemical composition. An aqueous sample can be a complex mixture of particles of different nature, size, reactivity, composition, agglomeration state and shape. Hence the initial preparation of the samples, such as pre-treatment and size fractionation, are critical steps for any subsequent analysis of the nano-objects. A consolidated table listing common fractionation techniques is given by Simonet, et al.[1] and Hassellov, et al.[2].

Although several methods for the detection and characterization of manufactured nano-objects in aqueous matrices are described in ISO/TR 18196:2016, the methods are at various stages of development into technical specifications or standards. Most importantly, there is no accepted standard as yet on pre-analysis treatment (i.e. collection, storage and size fractionation) of manufactured nano-objects in water. This document can contribute to the development of a future international standard for the analysis and characterization of metal and metal-oxide nanoparticles in aqueous matrices. This will allow interlaboratory comparison of results and contribute to future studies of commercial products containing manufactured nano-objects, thus, finally, support the growth of nanotechnology related industries.

Nanotechnologies — Sample preparation for the characterization of metal and metal-oxide nano-objects in water samples

1 Scope

This document provides an overview of approaches of sample preparation (i.e. pre-treatment and size-fractionation) for analytical measurements applied to surface and drinking water, potentially containing relevant amounts and types of metal and metal oxide nano-objects, including collection from source and storage of samples, pre-concentration of analytes, and their fractionation.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

3.1
manufactured nanomaterial
nanomaterial intentionally produced to have selected properties or composition

[SOURCE: ISO/TS 80004-1:2015, 2.9]

3.2
measurand
quantity intended to be measured

[SOURCE: ISO/IEC Guide 99:2007, 2.3, modified — The notes to entry have been deleted.]

3.3
nano-object
discrete piece of material with one, two or three external dimensions in the nanoscale

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

[SOURCE: ISO/TS 80004-2: 2015, 2.2]

3.4
nanostructured material
material having internal nanostructure or surface nanostructure

Note 1 to entry: If external dimensions are in the nanoscale, the term *nano-object* (3.2) is recommended.

[SOURCE: ISO/TS 80004-1:2015, 2.7, modified — Note 1 to entry has been replaced.]

3.5

surface water

water which flows over, or rests on, the surface of a land mass

[SOURCE: ISO 13164-1:2013, 3.1.20]

3.6

drinking water

water either in its original state or after treatment, intended for drinking, cooking, food preparation, or other domestic purposes, regardless of its origin

Note 1 to entry: Also known as potable water.

[SOURCE: ISO 5667-5:2006, 2.2, modified]

4 Symbols and abbreviated terms

HDPE	high density polyethylene
FFF	field-flow fractionation
Flow FFF	flow field-flow fractionation
UF	ultrafiltration
SEC	size exclusion chromatography
GPC	gel permeation chromatography
SDS	sodium dodecyl sulfate
DLS	dynamic light scattering
UV-vis	ultraviolet–visible spectroscopy
RC	regenerated cellulose
PES	polyethersulfone

5 Types of metal and metal oxide-based manufactured nano-objects

Based on the relevance of existing commercial products[1], the following metal and metal oxide-based nano-objects were considered in the development of this document:

- Titanium dioxide (TiO_2): One of the most commonly used nanomaterial ingredients in many common consumer products (e.g. sunscreens, antifungal paints) and medical products;
- Zinc oxide (ZnO): Also widely used in sunscreens and medical products;
- Silver (Ag): Commonly used as an antibacterial agent in consumer textiles and medical products;
- Gold (Au): Widely used in biomedical applications, including bioassays, drug delivery and hyperthermal therapy.

6 Types of water matrices of interest

Two types of water matrices were considered in this document, surface water and drinking water. Surface water is the primary water source receiving waste streams, and thus it is prone to receive

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[1] Industry-wide applications: http://www.understandingnano.com/nanoparticles.html.

manufactured nano-objects used in various commerical products. Drinking water in urban cities is produced by water treatment plants to remove all particulates which can include nano-objects. However, Kaegi and coworkers have found trace quantities of nano-objects in water samples after treatment[13]. Thus, with increasing use of manufactered nano-objects in commercial products, it is likely that monitoring of nano-objects in drinking water will become important in the future.

7 Sample collection and storage

7.1 General

To minimize changes in the state of water samples from the point of sample collection to analysis, precaution should be taken during collection, transport and storage. Currently there is no standard in collection, transport and storage of surface water samples containing nano-objects, but the following ISO publications on water quality – Sampling, should be used as a starting point for the development of nano-object specific procedures:

- ISO 5667-1 on guidance on the design of sampling programmes and sampling techniques;
- ISO 5667-3 on preservation and handling of water samples as well as the use of sample containers;
- ISO 5667-4 on guidance on sampling from lakes;
- ISO 5667-5 on guidance on sampling of drinking water from treatment works and piped distribution systems;
- ISO 5667-6 on guidance on sampling of rivers and streams.

Since nano-objects in surface water may undergo continuous physicochemical changes, it is useful to carry out the analysis of the samples with minimal delay.

7.2 Containers for sample collection and storage

Besides taking guidance from ISO 5667-3, common types of containers used in collection and storage of freshwater samples for nano-object analysis include high density polyethylene (HDPE)[8][9][10] and borosilicate glass[11]. The containers, especially HDPE, are typically pre-washed with dilute acid before use. For physicochemical analysis of nano-objects, the collected samples are typically stored at ambient or sub-ambient temperatures but without freezing. Very often, water samples require stabilization and addition of anti-microbial agents to allow storage.

8 Sample pre-treatment

8.1 Introduction

Raw water samples collected from an industrial or environmental source often require pre-treatment to (i) remove large particulates and/or (ii) concentrate the nano-objects of interest. Together with dilution techniques (not discussed here), these are the main pre-treatment methods before the sample can be used for further size fractionation and/or analysis.

WARNING — Since nano-objects can adsorb onto large particulates, the following pre-treatment methods may potentially remove nano-objects to some extent.

8.2 Sedimentation and centrifugation

8.2.1 Sedimentation

In sample pre-treatment, sedimentation can be used to reduce large particulates from the raw water samples, leaving the nano-objects in the water suspension so that the subsequent sample treatment / analysis can function effectively.

Gravitational sedimentation is a process to allow large and/or dense particles to settle out of the water column due to earth gravity. It is a simple process and suitable for processing from small to large quantities of samples (e.g. 1 ml to 20 l). The process usually takes a few hours to complete and particles > 5 µm in diameter can readily be removed from the water sample[12]. The advantage of gravitational sedimentation is simplicity without the need for use of expensive instrumentation. It may be used as an initial treatment step, to remove the largest (massive) materials quickly and conveniently, not intended to remove sub-micrometre particles.

8.2.2 Centrifugation

Besides sedimentation, centrifugation is another way to remove large particulates from the raw water samples. In addition, centrifugation is capable of concentrating nano-objects in the case where the number of nano-objects in the water sample is too low for the downstream analysis.

Centrifugation is a process that uses the centrifugal force to differentially separate constituents in heterogeneous mixtures according to their effective mass (density and size), as the denser or larger constituents of the mixture migrate away from the axis of the centrifuge more rapidly and form a sediment. The sedimentation rate is specified by the angular velocity usually expressed as revolutions per minute (RPM), or acceleration expressed as gravitational force (g-force). The conversion factor between RPM and g depends on the radius of the centrifuge rotor. A particle's settling velocity in centrifugation is a function of size and shape of the particles, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid medium, and the viscosity of the liquid. The sedimentation rate is zero when the density of the particle and liquid are the same (i.e. no density contrast exists).

8.2.3 Stepwise sedimentation and centrifugation

Kaegi and coworkers developed this technique for water samples containing different sizes of nano-objects[13]. First, the water sample (surface or drinking water) was allowed to stand for 2 h to remove large particles. The top layer (top 2 cm, equivalent to 1 l or 15 % v/v) of the water sample was then extracted and centrifuged at 330 g for 30 min to sediment micrometre particles. The top layer (top 2 cm or 15 % v/v) of the centrifuged sample was extracted and subjected to a second centrifugation at 2 700 g for 1 h to sediment particles >200 nm in diameter. The supernatant after the second centrifugation should be highly enhanced in nano-objects, which can be further concentrated via higher speed centrifugation.

8.2.4 Factors affecting centrifugation and sedimentation

8.2.4.1 Sedimentation rate

Metal-based nano-objects separated by centrifugation can be obtained either in a supernatant or as a sediment. Collecting nano-objects as sediment is more often practiced since it may separate the nano-objects of interest from the dissolved constituents in the water sample. In general, the smaller nano-objects require larger acceleration (larger g-force) or faster rotation and longer centrifugation time. For example, centrifugation at 7 000 g to 10 000 g for 20 min to 30 min has been employed successfully for sedimentation of gold nano-objects 15 to 20 nm in diameter, while 15 000 g to 18 000 g for 30 min has been employed for gold nano-objects 5 to 10 nm in diameter[14][15]. Collecting nano-objects in the supernatant is useful when there are unwanted larger size particulates in a sample. For metal-based nano-objects, this is usually applicable for samples with < 20 nm diameter centrifuged at < 5 000 g for less than 15 min. For nano-objects < 5 nm, ultracentrifugation for very long periods of time may be necessary to completely remove the particles from the water column. Surface waters spiked with nano-objects like ZnO have been processed via centrifugation for aggregation and dissolution studies of the nano-objects for different periods of time[16][17]. It was found that the size of ZnO nano-object aggregates increased between 2 h to 8 h incubation time before reaching steady-state size.

8.2.4.2 Temperature

The operational temperature for commercial refrigerated centrifuges typically ranges from 4 °C to 25 °C (ambient). For metal-based nano-objects > 20 nm in diameter, the operational temperature has little effect for sediment collection. For nano-objects < 10 nm, lower operation temperature typically allows more stable sediment because of the suppression of Brownian motion and the associated diffusion, i.e. to suppress the re-dispersion of the sediment.

8.2.4.3 Density gradient

Centrifugation with a density gradient medium can be used to separate nano-objects of different size as well as shapes. The largest nano-objects sediment, while the smallest nano-objects remain near the top of the gradient medium after centrifugation. Glycerol is commonly used for generating density gradients[18]; however, discontinuous density gradients generated by multiphase density media have been employed for separating nano-objects of different sizes and shapes. For example, Whitesides and coworkers used surfactant-based three-phase media (8,7 % v/v Brij-35, 10 g/l poly(2-ethyl 2-oxazoline), and 11,7 g/l Ficoll) to separate a mixture of gold nanoparticles, rods and larger particulates[19].

8.2.5 Advantages and limitations of centrifugation

Centrifugation is a facile method for nano-object separation in aqueous samples. It is relatively independent of the composition of the buffer and compatible with all downstream characterization techniques. With the current range of commercial centrifuges, a quantity of 300 ml to 500 ml samples can be processed each time. Also, minimal training is needed for operation. The main disadvantage of centrifugation is that it may induce agglomeration of nano-objects collected as sediment, especially at high g-forces. In addition, nano-objects of similar sizes and density may not be separated readily with this method.

8.3 Filtration

Filtration is an operation used to remove solid particles from liquids by interposing a semipermeable barrier (usually a filter membrane) in which only liquids or small dissolved species can pass through. A broad range of filter pore sizes (from sub-micrometre to micrometre) and versatile membrane materials (e.g. PTFE, regenerated cellulose, nylon, cellulose nitrate, polycarbonate, etc.) make it a common technique for raw water pre-treatment. The pore size is usually selected according to the application or objective. A 0,1 µm pore size filter is capable of removing mycoplasma, while removal of bacteria and fungi can be performed with ~0,2 µm pore size filter membranes. Coarse particle removal or prefiltration is generally accomplished with 0,45 µm or larger pore size filter membranes. Raw water sample pre-treatment by filtration is a commonly adopted step in nano-object analysis, e.g. to analyse pre-existing nano-objects in the filtrate of the water samples[10][20], or spiked manufactured nanomaterials for downstream analysis[21][22].

9 Size fractionation techniques

9.1 Introduction

After sample pre-treatment, water samples containing the nano-sized objects can be subjected to further treatment / analysis. Size fractionation is one of the commonly used techniques to isolate nano-objects into different size populations, and other properties of each size population within the water sample can be obtained post- fractionation. With the combination of other techniques, size groups collected from the fractionation can be further analysed and more information such as the shape, structure, and chemical composition etc. of the particles can be obtained.

In this section three commonly used size fractionation techniques, field-flow fractionation (FFF), ultrafiltration, and size exclusion chromatography, are discussed. Annex A compiles a list of techniques applicable for characterizing/analysing the nano-objects after size fractionation. Technical Specification 21362 Nanotechnologies: Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation" was recently published on the application of FFF on detection and identification of nano-objects in aqueous media.

9.2 Field-flow fractionation (FFF)

9.2.1 Introduction

FFF is a chromatography-like separation technique that requires no stationary phase. The FFF separation process is achieved by the balance of forces involving an external field applied perpendicular to the direction of sample flow through a thin channel. A laminar parabolic flow profile develops in the channel with flow velocity increasing from near zero at the channel wall(s) to a maximum at the centre. The perpendicular applied force field drives the particles toward the accumulation wall. Diffusion associated with Brownian motion, in turn, produces a counteracting motion for nano-objects. Smaller particles with higher diffusion rates tend to reach an equilibrium position at higher (faster moving) channel flow streams. Thus, different size particles separate according to the velocity gradient in the channel, with smaller particles eluting early. FFF is suitable for size fractionation of nano-objects in the 1-100 nm range and colloids up to roughly 1 µm in "normal mode" for various types of samples.

Flow field-flow fractionation (Flow FFF) is the most widely used subset of techniques for the separation and characterization of both natural colloids[20][23] and manufactured nano-objects[23]-[27]. It has been applied for fractionation of nano-objects in surface water samples from rivers[10][28], lakes[9] and streams[29][30]. In a recent study, colloidal particles from three different freshwater sites showed three size ranges: < 4,2 nm, 4,2 nm to 15,8 nm and 15,8 nm to 32,4 nm as determined by Flow FFF[9]. In another study, analysis of size and composition of the nano-objects in river samples, revealed 3 colloid populations: 0,5 nm to 4 nm (binding with most elements), 3 nm to 8 nm (protein-like colloids) and 5 nm to 40 nm (Fe-rich colloids)[28]. Apart from the size information, estimates of the concentrations of the different fractions can also be obtained. To date, however, there is no reference available for similar analysis of manufactured nano-objects in surface water from rivers, lakes and streams. This is due in part to the difficulty in differentiating naturally occurring nano-objects from manufactured nano-objects.

9.2.2 Advantages and limitations

By using programmed elution with flow FFF, it is possible to fractionate nano-objects with a very wide range of sizes. However, resolution of the peaks may be compromised if the size range is large. A wide range of detection techniques can be coupled online with Flow FFF and collection of flow FFF sample fractions can be further analysed offline. Online and offline detectors can provide a wealth of information on particle properties including size, shape, concentration, structure, chemical composition and optical properties. To analyse water samples of low particle concentration, preconcentration or on-channel concentration are often necessary prior to the fractionation process. Online techniques like ultraviolet–visible spectroscopy (UV-vis) and dynamic light scattering (DLS) have limitations for analysis of samples with low particle concentrations, while inductively coupled plasma mass spectrometry (ICP-MS) is effective if the nano-objects contain metals, non-metals and metalloids.

In theory, any aqueous or non-aqueous phase of any ionic strength and a pH between 2 and 11 can be used as a carrier. This gives versatility in terms of selecting the carrier composition to favour colloidal stability, thus minimizing the potential change in chemistry of the medium surrounding the nano-objects from the sample to the Flow FFF carrier during fractionation. The optimized carrier would have miminal impact on aggregation, disaggregation or conformational changes of the nano-objects and sample-membrane interaction and adsorption. Moreover, researchers have demonstrated that a functionalized membrane could minimize the accumulation of nano-objects on the membrane surface to improve the recovery of nano-objects.

The retention/elution time of nano-objects with the same size but different coatings may vary due to the different interactions between particles and the membrane in the channel. Even if the surface properties of nano-objects are identical, retention time can differ based on the core material[31]. The ability to separate same particles with different coatings would be a benefit for nano-objects fractionation for Flow FFF. The accuracy of the size results may be affected by using retention time for size analysis[32], however, this problem can be overcome by use of direct online size measurement rather than dependence on retention data either theoretically or using size calibration.

9.3 Ultrafiltration

Ultrafiltration (UF) is a technique used to separate nano-objects from small dissolved molecules in solution. UF separates based on molecular pore size. The advantage of UF is that it works directly with environmental samples and there may not be a need to pre-concentrate nano-objects before separation. UF has been used to investigate the influence of colloidal particles in trace metal partitioning for different types of water samples. However, possible limitations are coagulation and clogging at the membrane surface and so the choice of a high efficiency membrane becomes important.

Unlike other techniques like FFF and size exclusion chromatography, which require the use of chemical buffers or mobile phases and could potentially render dissolution or agglomeration of the nano-objects, UF concentrates target species without a specific buffer requirement. It can also handle a high volume of samples. However, artefacts should carefully be assessed as they can greatly influence the analytical results. Using two commercially available membranes polyethersulfone (PES) and regenerated cellulose (RC), trace metallic colloids in fresh water were analysed by Armstrong and coworkers[33]. This study on membranes helped in determining the efficiency of UF and concluded that RC outperformed PES membranes in low ionic strength waters and is a preferred choice for mercury partitioning under ambient fresh water conditions.

9.4 Size exclusion chromatography

Size-exclusion chromatography (SEC), also known as gel permeation chromatography (GPC), is a chromatographic technique that separates molecules and particles according to their hydrodynamic size as they pass through a porous stationary phase. Smaller particles are able to enter the pores of the stationary phase and therefore remain longer in the column, whereas particles with a size larger than average pore size of the stationary phase are excluded and eluted out of the column first.

SEC separation involves the selection of a suitable stationary phase of proper pore size and suitable mobile phase compatible with the nano-objects of interest. However, irreversible adsorption of the nano-objects onto the stationary phase may occur due to the high surface area of the stationary phase and the high surface energy of the nano-objects. This undesirable effect of adsorprtion of nano-objects can be overcome by introducing surfactants into the mobile phase since the surfactants stablilize the nano-objects and prevent them from agglomerating inside the column. With gold nano-objects as an example, a mixture of 5 nm and 38 nm nano-objects can be separated with a 100 nm pore size polymer-based stationary phase and 1 mmol/l to 5 mmol/l of sodium dodecyl sulfate (SDS)[34].

Annex A
(informative)

Relevant nano-object characterization techniques2)

Technique	
Acoustic spectroscopy	
Auger electron spectroscopy	AES
Condensation particle counter	CPC
Differential mobility analysis system	DMAS
Dynamic light scattering	DLS
Electron energy loss spectroscopy	EELS
EM based X-ray spectrometry	EDX/EDS/WDS
Fluorescence spectroscopy	FL
Fourier transform infrared spectroscopy/Imaging	FTIR
Induced grating method	IG
Inductively coupled plasma – mass spectrometry and single particle ICP-MS	ICP-MS
Laser diffraction	
Mass spectrometry	MS
Particle tracking analysis	PTA
Optical absorption spectroscopy	UV-Vis-NIR
Quartz microbalances	QCM
Raman spectroscopy/Imaging	
Resonant mass measurement	RMM
Scanning electron microscopy	SEM
Scanning probe microscopy	SPM
Secondary ion mass spectrometry	SIMS
Small angle X-ray scattering	SAXS
Static light scattering photometer	SLS
Single particle light interaction methods	
Transmission electron microscopy	TEM
X-ray diffraction	XRD
X-ray photoelectron spectroscopy	XPS
Laser-induced breakdown spectroscopy	LIBD

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2) Refer to ISO/TR 18196:2016.