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Nanotechnologies — Considerations for the measurement of nano-objects and their aggregates and agglomerates (NOAA) in environmental matrices

Nanotechnologies — Considérations pour la mesure des nano-objets, et leurs agrégats et agglomérats (NOAA) dans les matrices environnementales

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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

There is an interest in determining the concentration of nano-objects and their aggregates and agglomerates (NOAA) in environmental matrices. Manufactured nanomaterials (NM) enter the environment via release from the manufacturing process and its waste streams, as well as via the use of commercial products and their recycle and disposal streams. Such measurement efforts require an understanding of the occurrence of natural materials that can interfere with the analysis or skew the results, knowledge of how the environment can interact with NM, and insights that require unique collection and analytical techniques specific to the composition of the particle. This document provides a review of published studies that report levels of NOAA in the environment and aspects of collection and sample preparation. The reader is also directed to the Further Reading section of this document for information regarding ISO guidance on sampling of air, water, and sediment, as well as a matrix of measurement techniques.

Not all manufactured NM are discussed here because there might not yet be published studies that examined them in the environment. On the other hand, collection methods and pre-analytical procedures might be similar for some or all NOAA in a given environmental matrix. Furthermore, NOAA isolated from the environment can be characterized using the same instruments and analytical techniques used for pristine NOAA. Thus, the lack of published studies does not preclude the ability to collect a specific NM from any environmental matrix and measure the NOAA present.

Although it is recognized that biota (i.e. living organisms) also can interact with NM by sequestering and/or transforming them, analysis of biota is intentionally excluded so that the scope of this Document does not become too broad. However, the impact of biota should not be overlooked. Such considerations could be part of a subsequent Technical Report.

Furthermore, when NM are used for environmental remediation, and there is interest in measuring residual levels of remediating NM after the environmental medium has been processed. It is anticipated that the considerations described here would be applicable to those investigations.

The audience for this document is expected to be scientists from the regulatory, academic, or industrial communities who wish to answer the question of how much manufactured NM is present in a specific environmental medium. The results could be used for environmental stewardship, for risk assessment, or to calibrate modelled exposure estimates, although these applications are not discussed here.

NOTE The term NM refers to the identity of the nanomaterial, whereas NOAA is a more inclusive term encompassing NM and aggregates that are the focus of the analyses described here.

Nanotechnologies — Considerations for the measurement of nano-objects and their aggregates and agglomerates (NOAA) in environmental matrices

1 Scope

This document provides some considerations for the collection of environmental samples to be analysed for manufactured NOAA, considerations to distinguish manufactured NOAA from background levels of naturally occurring nanoscale particles of the same composition, and preparation procedures to aid in the quantification of manufactured NM in environmental matrices.

2 Normative references

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

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

ISO/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-1 and ISO/TS 80004-2 and the following 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
environmental matrices
ambient air, surface water, sediment, soil, estuarine and marine waters and sediments

3.2
ambient air
outdoor air to which people, plants, animals or material may be exposed

Note 1 to entry: Workplace is excluded.

[SOURCE: ISO 4225:1994, 3.6]

3.3
surface water
water in overland flow and storage, such as rivers and lakes, excluding seawater

[SOURCE: ISO 14046:2014, 3.1.3]

3.4
sediment
bottom sediment
naturally-occurring solid material deposited by settling from suspension onto the bottom of bodies of water, both moving and static

[SOURCE: ISO 6107-2:2006, 13]

3.5
soil
upper layer of the Earth's crust composed of mineral particles, organic matter, water, air and living organisms

[SOURCE: ISO 18589-1:2005, 3.2.1]

3.6
estuarine water
water in the lower reaches of a river that is freely connected with the sea, subject to the influence of the tides and receiving an influx of salt water and fresh water supplies from upland drainage area

[SOURCE: ISO 772:2011, 1.20]

3.7
seawater
marine water
water in a sea or an ocean

[SOURCE: ISO 14046:2014, 3.1.4]

3.8
pore water
water occupying space between sediment particles in freshwater (including soil), brackish and marine environments

[SOURCE: ISO 11348-1:2007, D.2.4]

3.9
coastal lagoon
shallow body of water, such as a pond or lake, close to the sea and usually with a shallow, restricted inlet from the sea

[SOURCE: ISO 6107-8:1993, 29]

3.10
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 80004-1:2015, 2.5]

3.11
nanomaterial
NM
material with any external dimension in the nanoscale [as defined in 2.1 of ISO/TS 80004-1] or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of *nano-object* [as defined in 2.5 of ISO/TS 80004-1] and *nanostructured material* [as defined in 2.7 of ISO/TS 80004-1].

Note 2 to entry: See also definitions 2.8 to 2.10 of ISO/TS 80004-1.

[SOURCE: ISO/TS 80004-1:2015, 2.4]

3.12
euphotic zone
upper layer of a body of water where light penetration is sufficient to support effective photosynthesis

[SOURCE: ISO 6107-3:1993, 29]

4 Symbols and abbreviated terms

AAS	atomic absorption spectroscopy
CNT	carbon nanotube
ICP-MS	inductively coupled plasma mass spectroscopy
HDC	hydrodynamic chromatography
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
NOAA	nano-objects, their aggregates, and agglomerates
NOM	natural organic matter
PM2.5	particulate matter with a diameter of 2,5 µm and smaller
SWCNT	single-walled carbon nanotube
TEM	transmission electron microscopy
TEM-EDX	transmission electron miscroscopy equipped with energy dispersive X-ray detector
UHPLC	ultra-high performance liquid chromatography
UV-vis	ultraviolet-visible spectrometry

5 General considerations

5.1 General

As the use of NM becomes more widespread, questions about what concentrations exist in the environment from their use are likely to be raised. Nowack et al.[1], Mueller and Nowack[2], Liu and Cohen[3], and Keller et al.[4] have suggested potential pathways into the environment from manufacturing waste streams, end-of-use waste streams (e.g. NM in sunscreens that enter the waste stream), and release from products. In order to determine the concentrations of NM present in the environment, several issues/questions need to be addressed:

- What are the background levels of naturally occurring materials of the same or similar composition?
- How do concentrations of naturally occurring materials change over time/geography?
- Can the manufactured NOAA be distinguished from naturally occurring nano-objects?
- What are the instruments that have been used to quantify and characterize NOAA in the environment?
- What are the proper sample preparation methods? Do they vary with the NM of interest, or do they vary with the medium?

These questions are considered in the following document.

5.2 Considerations for determining background levels of NM

Many NM of the same chemical composition as manufactured NM can already occur naturally in the environment, for example substances such as TiO_2, SiO_2, and fullerenes, just to name a few. For manufactured NM, processes such as top-down manufacturing reduces the size of a large particle to a smaller one, while bottom-up manufacturing creates larger particles from smaller ones. Naturally-occurring NOAA can also be produced by top-down processes like erosion of mineral particles via wind or via weathering (e.g. UV irradiation and rain). Metals (e.g. gold and silver), oxides of iron, silicon, aluminium, and manganese, and sulphides (e.g. iron sulphide) occur via a bottom-up process from dissolved metal ions. The bottom-up processes may be abiotic or bio-assisted. In addition, NOAA can be produced from combustion of biological matter or from chemical precipitation. (Figure 1). Nanoscale particles can also be part of cosmic dust or be produced from volcanic activity[5].

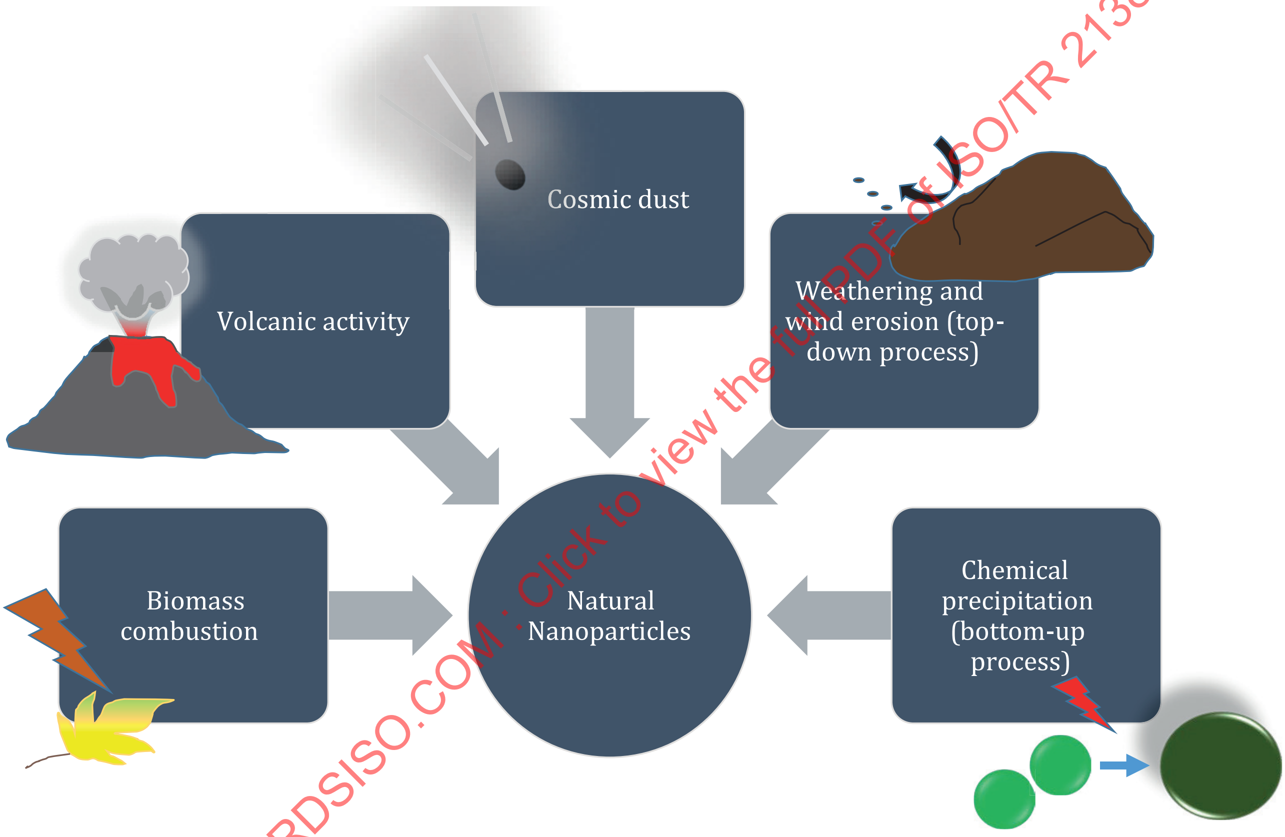

Figure 1 — Processes that create nano-objects in the environment. Adapted from Sharma et al.[5]

Therefore, some manufactured NM can be indistinguishable from naturally occurring nano-objects, making it impossible to assess the environmental exposure to these manufactured NM.

5.3 Distribution of NM in the environment

Where, in the environment, naturally occurring nano-objects or manufactured NM exist can depend on several factors, such as proximity to road surfaces, climate extremes, salinity of water, and the presence of objects onto which particles can adsorb. Liu and Cohen[3] predicted concentrations of various metal oxides in the environment based on global manufacturing, releases from production, and releases from disposal. Using the “MendNano” modelling program developed by Liu and Cohen (http://nanoinfo.org/mendnano), the concentrations of Ag, Al_2O_3, CeO_2, carbon nanotubes (CNTs), Cu-based NM (metal and metal oxides), Fe-based NM (metal and metal oxides), nanoclays, SiO_2, TiO_2, and ZnO were predicted for air, water, soil, and sediment in the Los Angeles (LA) region (USA) for a period of one year. The concentrations for any one matrix ranged more than 3 to fourfold, with TiO_2 at the highest concentration and silver or copper (as oxide) at the lowest concentration, depending on the matrix. Concentrations in air were predicted to be between 10^{-3} and 1 ng/m^3 for all substances, with the concentration of TiO2 predicted to be the highest (~1 ng/m^3) and copper oxide the lowest (~10^{-3} ng/m^3). The order of substances from highest concentration to lowest was TiO_2 > SiO_2 > Fe oxide = ZnO = Al_2O_3 > nanoclay = CeO_2 > CNT > Ag > Cu oxide. For water, concentrations ranged from 10^{-2} to 10^2 ng/L, with TiO_2 predicted at the highest concentration and ZnO at the lowest (Ag was predicted as 0 because it is insoluble in water). The ranking of substance by concentration in water was roughly the same as that in air, with TiO_2 > Al_2O_3 > SiO_2 = Fe oxide > nanoclay > CeO_2 > CNT > Cu oxide > ZnO > Ag. For soil, concentrations ranged from 10^{-3} to 1 µg/kg soil with the same relative ranking as that for air. Concentrations in sediment were several-fold greater than those for soil, ranging from 10^{-1} to 10^4 µg/kg sediment (Cu oxide was predicted to have undetectable concentrations in sediment). The ranking of substances by concentration was similar to that for water, with TiO_2 > Fe oxide > Al_2O_3 = SiO_2 > nanoclay > CeO_2 > CNT > Cu oxide > ZnO > Ag. Although these initial predictions represent the mass concentration of manufactured NM, it is not known if relative levels of naturally-occurring nano-objects might distribute in the same amounts or size distributions.

Furthermore, the levels of NOAA vary with time, season, and weather conditions. Liu and Cohen[3] predicted the daily and monthly variation in TiO_2 levels in various environmental media in LA County in California. The levels in air and water fluctuated by a factor of 10 over the course of the month and even during the course of a day in response to weather conditions. Concentrations in sediment and soil were predicted to be stable over a one-year time period. Daher et al.[6] showed that measured total particulate concentration in LA County varied depending on the season and location of sampling. In some locations, the total particulate in air was double that in winter compared to the concentration in the spring (16,1 ± 2,8 µg/m^3 [mean and standard error] in winter versus 7,9 ± 0,8 µg/m^3 in spring for Long Beach). These variations suggest that samples should be collected over extended weather conditions in order to eliminate seasonal fluctuations as a confounder in interpreting data.

5.4 Interaction with materials in environmental matrices

NOAA can be chemically transformed or adsorbed onto naturally occurring materials in the environment. The degree to which these phenomena happen and the extent of their impact differ based on the chemical properties of the NM and the matrix. A report from Denmark by Hartmann et al.[7] summarized the potential transformation processes in the four major environmental compartments and the relevance of such transformations for modelling. They describe how photochemical degradation, oxidation, reduction, dissolution, precipitation, speciation/complexation, sedimentation, adsorption, and biotransformation can impact or modify several different NM (metal oxides, metals, and carbon-based NOAA) in the environment[7]. The extent to which the concentration or sequestration of NOAA occurs in a matrix is dependent on the physical/chemical nature of the NM and the chemical nature of the environmental matrix. This report focuses on the impact of these processes on modelling of life cycle and fate, but they are also applicable to measurement of NOAA and, perhaps more importantly, to the metric used to describe concentration.

According to the report by Hartmann et al.[7], photochemically induced reactions (i.e. oxidation, reduction, or transformation) occur to the greatest extent in air, to a lesser extent in water, and not at all in soil or sediment (Table 1). NM with a particular molecular composition might be more subject to modification than others; thus, metals (e.g. silver and iron) or metal oxides (e.g. TiO_2 and perhaps CeO_2) that have bound to organic matter can be modified by oxidation. In addition, carbon-based NOAA (e.g. CNTs and carbon black) can be oxidized in air. The results of these changes can alter the surface properties (if not the chemical identity, such as for silver and iron), thereby leading to changes in adsorption or agglomeration. Hartmann and coworkers assessed the impact of photochemical reaction in air on modelling as “high” for these NM (metals such as silver and iron, metal oxides such as TiO_2 and to some extent CeO_2, and carbon-based NOAA), but non-existent for other NM that are not prone to such photo-induced reactions.

Several physical phenomena with NM can occur in the environment, including dissolution, aggregation, agglomeration, sedimentation, and adsorption. Dissolution occurs primarily in water and sediment, and to some extent soil depending on the presence of pore water. The impact of dissolution on the concentration of the NOAA can vary from low to high depending on the NM; thus, dissolution is a key transformation process of ZnO, Ag, and CuO and plays a significant role in their binding to natural organic matter (NOM) or larger particles. Therefore, for these metals and metal oxides dissolution in the presence of water could have a high impact on modelling and measurement. Of course, the ionic strength, pH, and presence of other materials also play roles in the extent of dissolution. Aggregation and agglomeration also occur primarily in water, but have an impact on NM in air, soil, and sediment as well. Thus, aggregation and agglomeration in water tends to have a high impact on the ability to quantify NM, and a medium impact in air, soil and sediment. Surface charges can change in water with ionic strength and pH playing a role. The nature of the NM, whether metal, metal oxide, or carbon-based NM, seems to have little impact on the importance of this physical process, given all types of NM are subject to the phenomenon. Sedimentation, according to Hartmann et al.[7], occurs primarily in water and to some extent in air. The end result of sedimentation can be to transfer NM from one matrix to another, such as from air or water to sediment or soil. Thus, the impact on quantitation is high for NMs in water, and medium for NMs in air. As with aggregation and agglomeration, the nature of the NM has little impact on the extent of sedimentation, which is most influenced by particle or agglomerate size. Adsorption onto other particles or NOM, like agglomeration, is dependent on surface properties, which in turn can be influenced by the ionic character of the matrix (pH, etc.). Adsorption not only impacts the concentration of NM in soil and sediment, but also in water. The nature of the NM seems to have little impact on the adsorption, as is the case for agglomeration.

Biodegradation of so-called organic compounds, which are based on carbon chemistry, frequently plays a role when substances enter the environment and often leads to lowering of the concentration of the substance, whereas metals and metal oxides do not biodegrade. Hartmann et al.[7] reported that biodegradation is not likely to have a great impact on NM, with the exception of carbon-based NM, such as CNTs. However, biomodification of the surface of NM can occur, which can lead to dissolution, complexation, or agglomeration. Similar processes are described by Sharma et al.[5] for naturally occurring nano-objects. As a result, transformations of the manufactured NM should be considered and the final concentrations of NM should be adjusted to reflect masking by transformed particles.

Table 1 — Predicted environmental transformation processes. Adapted from Hartmann et al.[7]

Process		**Impact on particle concentration or likelihood of occurrence**	**Matrix**			
			Air	**Water**	**Sediment**	**Soil**
Reaction	Photochemical oxidation, etc.	H	sMe, sMeO	sMe, sMeO		
		M				
		L				

Impact: H = high, M = medium, L = Low

s = some of the NM in this class

Me = metal such as silver or iron

MeO = metal oxide such as ZnO, TiO_2, or CeO_2

All = generally applies to all NM.

NOTE A ‘high’ impact is critical to accurately determine or model the fate and behaviour, and thus the concentration, of the NM. A ‘low’ significance is considered to have a low impact on the fate modelling or measurement of that specific NM and omitting the process will therefore not result in a large error.

Table 1 *(continued)*

Process		Impact on particle concentration or likelihood of occurrence	Matrix			
			Air	Water	Sediment	Soil
Physical	Dissolution	H		Me, MeO	Me, MeO	
		M				Me, MeO
		L				
	Aggregation	H		All		
		M	All		All	All
		L				
	Sedimentation	H		All		
		M	All		All	All
		L				
	Adsorption	H			All	All
		M		All		
		L				
Biological	Biomodification	H				
		M		All	All	All
		L				

Impact: H = high, M = medium, L = Low

s = some of the NM in this class

Me = metal such as silver or iron

MeO = metal oxide such as ZnO, TiO_2, or CeO_2

All = generally applies to all NM.

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5.5 Real-time measurements versus integrated versus spot sampling

The general aspects of sampling and their applicability to water and sediment are described in ISO 5667-1:2006[8], which describes the differences between spot samples (grab samples), periodic samples, continuous samples, series samples, composite samples, and large volume samples. In addition, benefits of each approach for sampling water are described. For sampling of air, real-time measurements provide instantaneous information on concentrations of particles but cannot identify the substance or even particle size distribution. ISO/TR 18196 provides a matrix of measurement techniques that can be used to quantify or characterize NOAA; the reader can review the document on the Online Browsing Platform (www.iso.org/obp). To provide information about the identity or size of the particle of interest, real-time measurements of ambient air can include several instruments that are employed simultaneously, especially for air samples. Alternatively, a sample could be “grabbed” from the environment. As a result, the value of ‘real-time’ measurements can be limited without the subsequent additional measurement of ‘grab samples’. The greatest disadvantage to ‘real-time’ measurements is that many instruments lack sufficient sensitivity to detect environmentally relevant concentrations of NOAA, which are often low.

Integrated sampling (i.e. collecting a sample over a long period of time) can be used to detect low concentrations of the NM of interest but the method requires large volumes of environmental media (i.e. air or water) to pass through the collection device. Thus, collection periods can extend over long periods of time (e.g. 24 h for air sampling), and the concentrations determined represent time-weighted average concentrations of the NOAA in the environment. Peak concentrations cannot be determined. However, the value of integrated sampling is that the sample can be prepared in a way that enhances the sensitivity for quantitation; for example, concentrating the NM of interest and separating materials and substances that can interfere with analysis.

5.6 Preparing samples for analysis

In general, samples collected from any environmental matrix need to be prepared for analysis to separate extraneous material and to concentrate the NOAA to improve quantitation. The following table provides typical preparation methods and subsequent instrumentation for metallic NM. Many of these techniques are applicable to other NM. Furthermore, NOAA such as those associated with soil particles or NOM might need to be extracted from matrix components, and the concentration methods may impact the aggregation state or surface properties of the NM. These procedures are described in the sections pertaining to those matrices.

Table 2 — Common methods for separation and segregation of metallic NOAA. Modified from daSilva et al.[9]

Method	Procedure	Size Range
Ultracentrifugation	Acceleration up to 10^6 xg	100 Da to 10 GDa
Filtration	Size fractionation	Down to 1 kDa
Nano-filtration	Size-exclusion membrane	0,5 nm to 1 nm
Size-exclusion chromatography	Packed porous beads as stationary phase	0,5 nm to 40 nm
Hydrodynamic chromatography	Physical separation in a narrow conduit	2,0 nm to 200 nm
Field-flow fractionation	Physical separation in an open tube based on applied field	1 nm to 1 000 nm
Electrophoretic mobility	Charge-size distribution along a gradient	3 nm to 1 000 nm
Micro-filtration	Size-exclusion membrane	100 nm to 1 000 nm

xg = times gravity
Da = Daltons
GDa = giga Daltons
kDa = kilo Daltons

5.7 Characterization and quantitation of NOAA

Selecting the most appropriate measurement method is not within the scope of this Report. However, ISO/TR 18196:2016[10] provides a list of analytical techniques that have been used to quantify and characterize NM, advantages and disadvantages, measurand, limitations, and relevant standards of each technique. A condensed list is provided in Annex A.

6 Considerations for sampling and analysing NOAA in air

6.1 General considerations

Ostraat et al.[11] reviewed sampling strategies and analytical methods for measuring airborne NM. Among the issues that were identified as relevant to the strategies were suitable dose metrics because instruments that can measure surface area (SA) will be quite different from those that measure particle number or mass. In addition, Solomon et al. of the US Environmental Protection Agency reviewed different samplers to collect and quantify airborne contaminants contained in particulate matter of diameter 2,5 micrometres or smaller (PM2.5)[12]. Although the information is specific to collection of PM2.5 particulates and quantifying their concentrations, there are lessons that can be learned regarding selection of filters depending on the substance of interest. For example, Polytetrafluoroethylene (PTFE) filters are not acceptable if the composition of the carbon-based NM is to be determined because carbon content cannot be distinguished from the Polytetrafluoroethylene (PTFE).

6.2 Transformations and dispersion in the environment

Large particles can adsorb smaller particles and act as reservoirs. Catinon et al.[13] showed that large particles (25 µm to 1 000 µm diameter) of sand, clay, pollen, and organomineral particles contained measurable levels of As, Ba, Cd, Co, Cu, Fe, Ni, Pb and Zn. These background levels of elements can impact the analyses of NOAA of interest.

6.3 Sampling considerations

Strategies used for air quality determinations can have utility for measurement of NM. Solomon et al.[12] describe multi-city sampling of ambient air for PM2.5 determinations. Their strategy adjusted for nitrate and sulfate levels known to exist in the air of each city. Samples were collected over 20 d during a specific season, and for 24 h per sample period. It is important to include "field blanks" to account for interaction of the filter medium with subsequent analyses.

Sampling over long time periods provides more data to generate robust statistical inferences about background NM parameters. Furthermore, seasonal variations need to be taken into account; for examples, Daher et al.[6] and McGinnis et al.[14] describe the seasonal and spatial variations of airborne ultrafine particles in a large metropolitan area. These variations are likely to exist in other areas and for other environmental media. McGinnis et al. also describe how collection points can be spatially separated in such a way to identify if a source is regional or local. Furthermore, McGinnis et al. indicate that the Chemical Speciation Network can provide data on annual trends of PM2.5 levels for specific urban locations.

Chemical and/or morphology (i.e. filter sampling coupled with electron microscopy) characterization can be combined with other techniques to provide temporal information about airborne NM characteristics. This strategy assumes the physico-chemical characteristics of the NOAA are sufficiently different from background NM to allow their differentiation.

What type of filters should and/or can be used depends on the particle of interest[12]. As mentioned above, Polytetrafluoroethylene (PTFE) filters cannot be used if the particles are carbon-based because Polytetrafluoroethylene (PTFE) interferes with the analysis. If the collected air is from urban settings, it is likely to contain high levels of nitrates or sulfates. To minimize artefacts for the collection of aerosol nitrate, Solomon et al. recommend that samples be collected using a denuder (coated with MgO or Na_2CO_3) followed by a single filter. According to Solomon et al., measuring nitrate on a quartz-fibre filter prepared for carbon analysis can result in a significant positive artefact for aerosol nitrate. Also, the Polytetrafluoroethylene (PTFE) filter used for mass and X-ray fluorescence (XRF) analyses should not be used for ion analysis, particularly nitrate and ammonium ions, as these species are lost during XRF analysis.

6.4 Preparation for analysis

No preparation is anticipated for real-time measurements. However, for grab sampling onto filter media, equilibration of the filter is a long-standing practice where gravimetric measurements are used. The extent to which the filter is equilibrated depends on the filter medium (see Annex). In addition, if particles captured onto the filter are analysed for composition, the extraction solvent depends on the filter medium. Again, Solomon et al.[12] provide a table with examples of extraction methods for different filter media.

6.5 Detection and quantitation

Key attributes for instruments suitable for field studies include the following: low cost; limited size resolution with 2 to 5 distinct size bins < 100 nm; simple to operate, including minimal training to collect and interpret data as well as minimal maintenance and calibration; and robust and reliable operation in a wide variety of conditions, including high and low airborne particle concentrations and broad particle chemistry sensitivity[15]. In addition, sizes > 100 nm are captured by most of these instruments so agglomerates can also be measured. The disadvantage of these instruments is that they cannot characterize the particle or determine the chemical nature of the substance. Thus, real-time measurements should be supplemented with grab sample analyses.

ISO/TR 18196:2016[10] provides a list of analytical techniques that have been used to quantify and characterize NM, advantages and disadvantages, measurand, limitations, and relevant standards of each technique. Categories and examples of real-time instruments that detect and measure airborne particles are listed in Annex A.

7 Considerations for sampling and analysing NOAA in surface water

7.1 General

As previously mentioned, detection of NOAA in surface water or aqueous waste streams is complicated by the presence of NOM, which is mainly in the form of humic substances, electrolytes, bacteria, natural colloids, suspended materials and other constituents[16][17] that can interfere with detection of NOAA, adsorb NOAA, or obfuscate background levels of naturally occurring substances. Separation of NOAA from these other constituents, concentration of NOAA to improve detection, and preparation of samples for analysis are critical steps in the process prior to detection. The procedures described in ISO/TR 16196 and ISO 5667-1, ISO 5667-4, and ISO 5667-6 provide guidance on techniques that can be used to improve detection. ISO/TR 16196 describes sample preparation methods of nanomaterials prior to treatment of biological systems; however, there may be insights into preparation methods prior to analysis. ISO 5667-1, ISO 5667-4, and ISO 5667-6 describe procedures and equipment for sampling of natural water (ISO 5667-1), lakes (ISO 5667-4), and rivers (ISO 5667-6). These standards may be previewed on the ISO Online Browsing Platform (www.iso.org/obp).

7.2 Transformations and dispersion in the environment

All substances in surface water can interact with NOM and NM are no exception. Adsorption of ions and substances onto NOM is not uncommon[18], but Wang et al.[19] demonstrated that higher NOM concentrations, in addition to pH, ionic strength, and dissolved oxygen, enhance dissolution of Cu from nano-Cu. Similarly, Zhang et al.[20] discuss how Ag undergoes several transformations to AgCl, Ag_2S, or Ag_2O in water depending on the concentration of organic matter, dissolved oxygen, pH, and intensity of sunlight.

In addition to interacting with NOM, it is also possible that NM of different composition can interact with each other in the aqueous environment. Tong et al.[21] demonstrated that Zn that had dissociated from nanoscale ZnO could adsorb onto nanoscale TiO_2 in Lake Michigan water. Measureable dissolved Zn decreased as the concentration of TiO_2 increased, and the adsorbed Zn-TiO_2 was stable for more than 24 h. Zhang et al.[22] demonstrated that chloride anions in surface and sea water can etch silver triangular nanoplates so that the shape is changed over a period of 50 d. Conway et al.[23] demonstrated that nano-Cu and nano-CuO aggregate in aqueous media and that the extent of aggregation and stability of the aggregates are dependent on the ionic strength. Thus, there are substantial differences in the behaviour of nano-Cu or nano-CuO in sea water, coastal lagoon, 50 % sea water, groundwater, storm runoff, and freshwater.

7.3 Sampling considerations

Sampling procedures and strategies for surface water are described in ISO 5667-4:2016[24] and ISO 5667-6:2014[25] and should be applicable to sampling for NOAA. In general, those ISO procedures suggest that sampling should be avoided near the surface, bank, or stagnant pool and at least 30 cm above the bottom to reduce the risk of disturbing the sediment. Collection of surface water should follow the same principles as those outlined for air sampling; that is, sampling over extended periods of time to ascertain background levels of naturally occurring nano-objects that can interfere with the analysis of manufactured NOAA, and sampling during different seasons to account for variations in natural components such as NOM and naturally occurring nano-sized particles. Once background levels are established, grab samples can be more useful than composite samples to determine water quality.

Sampling near the surface differs from sampling well below the surface. For lakes, there might be differences in the concentrations of NOAA or other contaminants at the surface versus deeper in the lake, e.g. below the euphotic zone. Thus, sampling of surface water involves submerging the container under the surface, washing the container several times with the surface water, then collecting the sample. For deeper surface water, samples from different depths can be valuable because the microenvironment (dissolved materials, etc.) may be different at various depths. Several horizontal and vertical collection devices are described in ISO 5667-4:2016[24] along with the advantages and disadvantages of each device. Studies in the literature seem to adhere to these recommendations. Zhou et al.[26] describe collecting lake water in glass bottles that had been rinsed three times with the water to completely coat the surface. A similar sampling protocol was described by Metreveli et al.[27] when collecting samples from the Rhine river at least 2 m from the river bank.

7.4 Preparation for analysis

Following collection, samples should be filtered to separate large particulates. Zhou et al.[26] filtered samples through a 0,45-µm filter to remove large particles. Metreveli and coworkers[27] used a 0,45-µm polyethersulfone filter followed by 0,1-µm cellulose nitrate and 50-kDa polyethersulfone membrane filters. What impact this practice might have on aggregates and agglomerates is unclear, although the NM of interest was silver. Use of filters should consider the potential adsorption of the NOAA of interest onto the filters. There are NM-specific approaches that can be used: for example for silver NM, a filter impregnated or treated with copper can provide a full saturation of the potential absorbing loci, thereby decreasing the adsorption of silver to the filter. However, adsorption of NOAA onto the surface of the sampling container is a separate issue that might need to be addressed[28].

Separation and concentration of NOAA might involve centrifugation or other mechanical techniques that do not alter the NM characteristics. Techniques such as field-flow fractionation, hydrodynamic chromatography (HDC), electrophoresis, centrifugation, filtration, and dialysis have been used[28]. Centrifugation at 21 000 xg for 1 h was used by Hou et al.[29] to separate soluble silver from silver NOAA. Once separated, samples should be concentrated to meet the detection limit of instruments. Farre et al.[28] and Liu et al.[30] reported on separation and extraction methods for metals (gold, silver, copper), metal oxides (titanium oxide, silicon oxide), quantum dots, and carbonaceous particles (carbon black, fullerenes, carbon nanotubes or CNTs) in aquatic and marine environments. These methods include size-exclusion chromatography, capillary electrophoresis, hydrodynamic chromatography, cloud-point extraction using different solvents, and field-flow fractionation. The method of cloud-point extraction that was reviewed by Liu et al.[30] uses non-ionic surfactants to form micelles. These micelles are then modified by temperature or pH or other external condition, and the layers are separated. In this way, NOAA of interest such as silver, gold, C60, TiO_2 and SWCNT can be extracted and concentrated.

7.5 Detection and quantitation

The document ISO/TR 18196:2016[10] provides a list of techniques that can be used to characterize and quantify NOAA. This list is summarized in Table A.2 in Annex A.2. The Technical Report does not identify which techniques may be appropriate for specific NM. The following methods described in the literature have been used to characterize and quantify NOAA specifically from an aqueous matrix.

7.5.1 Metal NM

Several studies have identified and quantified metals such as silver, gold, and iron[9][30][31][32] using UV-vis spectroscopy, ICP spectroscopy, or sp ICP-MS (single particle inductively coupled plasma mass spectrometry). In addition, electron microscopy (TEM. TEM-EDX) has been used to identify and characterize metal NOAA in water[31]. Furthermore, TEM-EDX, X-ray fluorescence, X-ray diffraction, and electron energy loss spectrometry have been used to measure elemental compositions of NM[30][31][32]. The magnetic properties of metal nano-scale zero-valent iron also have been used to determine its concentration in aquifers[33].

7.5.2 Metal oxide NM

Although there are few studies that measured metal oxide NOAA in water, there are reports of measuring NOAA in waste-water treatment facilities[34] and in the rain-water runoff from walls painted with TiO_2-based pigments[35]. After the sample was separated, elemental analysis was carried out by inductively coupled plasma optical emission spectrometry and/or ICP-MS. Electron microscopy was used to visualize the particles.

7.5.3 Carbon-based NM

There are currently no established analytical methods that are specific to quantify all carbon-based NOAA in environmental matrices. Use of non-specific methods such as electron microscopy or HPLC have been reported. In addition, Schierz et al.[36] describe near-infrared spectroscopy to quantify SWCNT. It is not clear if this approach is applicable to multi-walled CNT or graphene. Adsorption of carbonaceous NOAA onto NOM or pollutants can occur readily[37]; removal of adsorbed organic matter or inorganic substances might be necessary prior to analysis.

8 Considerations for sampling and analysing NOAA in sea water

8.1 Transformations and interaction with materials in the environment

The interaction between NOAA and sea water can be the opposite of those between NOAA and surface water depending on the ionic strength and the amount of NOM present. Increasing ionic strength can result in a destabilization of suspended or adsorbed NOAA, which results in a release of the nano-objects[5] or greater precipitation into the sediment[38]. On the other hand, algae and other organisms that live in sea water can help stabilize nano-objects[39].

8.2 Sampling considerations

Sampling procedures, equipment, and strategies for sea water are described in ISO 5667-9:1992[40]. Unlike sampling of surface water, sampling sea water requires recognition that the micro-environment changes as one probes deeper below the surface and away from sources of influent. However, sampling for contamination from rivers that flow into the sea are better conducted in tidal waters or coastal regions, compared with sampling of deep water from boats and ships. Given the likelihood that manufactured NM enter the sea via rivers[2], sampling of deep water might not be valuable.

The composition of tidal water and coastal region is influenced by seasonal weather conditions and tides. These factors can influence sampling timing and strategies to account for such variations. Sampling of tidal waters can be accomplished from piers or jetties, whereas sampling of coastal regions should be from a boat but within 5 km of the coast. The selection of the area to be sampled can dictate the type of sample to be collected; that is, a depth profile sample of various depths, or an area profile sample of the same depth but broad area. Furthermore, different types of samplers such as closed-pipe devices are better suited to depth profile sampling compared with open samplers that are used for surface or subsurface sampling.

Bruland et al.[41] describe sampling of sea water and suggest sampling at least 200 m from any ship or vessel, and simultaneously collecting up to 18 samples at different depths. This approach is consistent with the recommendations in ISO 5667-4[24] and ISO 5667-9[40] to include vertical stratification of NOAA concentrations. In their report, Bruland et al. described the use polyethylene bottles with ball valves that opened once the proper depth was achieved; the first sample was taken at a depth of 10 m.

By comparison, Strickland and Parsons[42] describe collecting samples from the euphotic zone of sea water for analysis of particulates (not necessarily NOAA). Particulates were collected onto glass-fibre filters or Millipore AA membranes. Whether these filter media are suitable for NOAA is not certain, and can depend on the substance to be analysed. The Solomon reference[12] can provide appropriate recommendations for filters that are compatible with specific NOAA.

8.3 Preparation for analysis

Preparation for analysis typically includes separation of solids from soluble components. The same considerations used for surface water are likely to be applicable to sea water. Strickland and Parsons[42] discuss filtration of seawater to remove particulates. The first step is sieving through 150-µm mesh to remove large particles and biota is followed by filtration through either glass-fibre (Whatman GF/A) or membrane (Millipore AA) filters. The pore size of the filter used will be an important determinant of the size of the particles retained in the filter. Additional filtration of the filtrate through smaller pore size filters will help establish the efficiency of retention of the smaller particle sizes) in the initial filtration. Strickland and Parsons note that some small particles can pass through the glass-fibre filter but adsorb onto the membrane filter. The addition of magnesium carbonate allows for collection of particles trapped on the membrane filter.

8.4 Detection and quantitation

The same considerations used for surface water are likely to be applicable to sea water, although a few examples of quantifying NOAA in sea water are available. Grab samples of iron NOAA in artificial sea water were characterized by Demir et al.[43] using ICP-MS, Fourier transform infrared spectroscopy and TEM. Silver and gold NOAA in surface water were quantified by Adegboyega et al.[44] and Hou et al.[29] using UV spectroscopy.

9 Considerations for sampling and analysing NOAA in sediment

9.1 Transformations and interaction with materials in the environment

As NOAA become destabilized in sea water, they more readily settle and become part of the estuarine sediment. Furthermore, NM can adsorb onto NOM in surface water and become part of the sediment of lakes and rivers. Chemical transformation can also occur; for example, Ag ion can react with dissolved sulfide in sediment to precipitate Ag_2S[20].

9.2 Sampling considerations

Sampling considerations and equipment are presented in ISO 5667-12:2017[45]. ISO 5667-12:2017 describes using polyethylene, polypropylene, polycarbonate, or glass containers to collect samples. Polyethylene is preferred to collect "those elements that are major constituents of glass", whereas glass is preferred for organic measurands. Furthermore, ISO 5667-12:2017 describes various types of "grab" samplers, the type of sediment for which the sampler is most suited, the accuracy of the sample, and nautical conditions best suited to that type of sampler. It is not necessary to review these considerations here; the reader is directed to ISO 5667-12:2017 for that information. Similar methods for collection of samples from marine sediment were described by Loring and Rantala[46]. These methods include descriptions of three types of sampling apparatus: grab samplers, such as the Van Veen grab sampler, the barrel core sub-surface sampler, and the box core sediment sampler. Each method and apparatus has its advantages and limitations.

Several studies in the literature have described collecting samples of sediment for analysis of NOAA. Antizar-Ladislao et al.[47] and Civeira et al.[48] describe collecting sediment samples from various locations along the Hugli River estuary or Tubarão River, respectively. Samples were collected in triplicate from within the top 10 cm of sediment using acid-washed PVC spatulas, and samples were pooled prior to analysis. The collection process was performed in such a way as to not disturb the surface water. Ramskov et al.[49] collected samples from Isefjorden, Denmark, and Bao et al.[50] collected samples 0 cm to 11 cm from Donghu Lake and Lushui Lake in China, using a Petersen grab sampler for determination of silver NOAA.

Samples of river sediment have also been collected by freezing the sediment using the so-called freeze-core method. Carling and Reader[51] described freezing the sediment to a copper pipe that could be cooled using liquid nitrogen, carbon dioxide, or a mixture of acetone and water. The tube is inserted into the sediment and then cooled to allow the particles to adhere to the copper. According to the authors, small and large particles will be collected up to a depth of 10 cm. Zimmerman et al.[52] compared bulk sampling to freeze-core sampling techniques of sediment and found that although repeated bulk sampling was less variable for particles > 1 mm compared with freeze-core sampling, the two methods were comparable for repeatedly capturing of particles < 1 mm. Thus, freeze-core sampling might be satisfactory for collecting NOAA in sediment. However, Zimmerman et al. point out that particles of that size range make up a small fraction of the total amount of material sampled and loss (i.e. inability to retain the particles on the copper rod) of particles < 2 mm could be substantial (~38 %).

9.3 Preparation for analysis

Following collection of the samples, the NOAA should be separated and extracted from the sediment. Loring and Rantala[46] describe a series of procedures to prepare sediment for "geochemical" analysis for metals. These procedures include drying the sediment followed by separation through sieves, centrifugation to collect the small particles, and finally crushing the samples prior to extraction.

For analysis of silver, Antizar-Ladislao et al.[47] describe drying sediment samples in an oven at 50 °C (time unspecified), gentle disaggregation of particles, and passing the particles through a 63-µm sieve prior to digestion with concentrated nitric acid. Ramskov et al.[49] separated large particles using a 120-µm sieve (wet-sieved under distilled water), then lyophilized the samples at −56 °C prior to digestion in nitric acid for determination of silver. Civeira at al.[48] air-dried the samples, separated small particles through a 80-mesh (177 µm) sieve, pulverized the particles, and then separated the particles using a 150-mesh sieve (~100 µm) prior to analysis for minerals.

A variety of methods are described by Isaacson et al.[53] that can be used to separate fullerenes from sediment. The initial step is extraction from the environmental medium. Isaacson et al. describe extraction with toluene in a Soxhlet extraction, whereas Carboni et al.[54] used a similar approach of toluene extraction for 2 h. These extraction methods are appropriate for unmodified fullerenes. For water-stable fullerenes, sodium chloride is added to the toluene (final concentration of 20 %). The different species of fullerene can then be separated using UHPLC.

9.4 Detection and quantitation

Isaacson et al.[53] provide a good overview of the limitations and processes required to quantify fullerenes from sediment that involve ionizing the carbonaceous material for mass spectroscopy. In contrast, Carboni et al.[54] use UHPLC and HRMS. For metals, ICP-MS and AAS have been described.

10 Considerations for sampling and analysing NOAA in soil

10.1 Transformations and interaction with materials in the environment

Adsorption of NOAA onto soil is expected, but some NOAA can associate with pore water rather than soil[55]. The extent to which NOAA adsorb onto soil particles is dependent on several factors, such as pore size, ionic strength, and pH of the soil, the presence of NOM, and the rate of water flow through the soil[56]. In addition, the characteristics (e.g. the hydrophobicity) of the NM play a major role. Lecoanet and coworkers[55] evaluated the mobility of NM in an artificial porous medium of glass beads that were 300 nm to 425 nm in diameter. Particles of SiO_2 (57 nm and 135 nm), TiO_2 (198 nm), ferroxane (303 nm), alumoxane (74 nm), fullerol (1,2 nm), clusters of C60 (168 nm), and SWCNT (0,7 − 1,1 × 80 nm to 200 nm) were passed through the medium and the concentration in the influent and effluent were compared. Adsorption was greater for the ferroxane, TiO_2, SiO_2 (135 nm), and nC60 particles compared with alumoxane, SiO_2 (57 nm), fullerol, and SWCNT particles. Saturation of the artificial soil was evident and occurred quickly for the hydrophobic substances compared with the others. Thus, analysis of pore water alone for NOAA can underestimate actual environmental concentrations[57]. In the case of silver, the extent of adsorption is dependent on the pH and composition of the soil (e.g. more silver might be bound to sandy soil compared with clay). Quantum dots also adsorb tightly to soil but can be degraded by soil microbes[58]. Avanasi et al.[59] investigated the adsorption and degradation of fullerenes in soils of different composition. Adsorption was greater for loam than for sandy soil; likewise, degradation might be much slower in silt loam compared with sandy loam over 2 y.

10.2 Sampling considerations

Descriptions in the literature of sample collection are much the same for soil as for sediment. Tavares et al.[60] collected soil up to 10 cm in depth using a “plastic” spade, whereas Antisani et al.[61] used a plexiglass cylinder that was inserted into the soil to collect the top 12 cm. Other sampling methods are described in ISO 5667-12:2017[45] for sediment which can be applicable to soil, and in ISO 18400-104 on soil quality.

10.3 Preparation for analysis

Procedures for preparing samples for analysis among studies that quantified and characterized NOAA in soil are consistent. First, water is removed either by air-drying[60] or lyophilisation[54]. When dry, small particles are separated from larger ones by sieving with a 2 mm sieve[58][60]. Whether the soil needs to be milled is not clear; Carboni et al.[54] milled the soil prior to sieving, presumably to increase the extraction of NOAA, which is the next step in preparation for analysis. The selection of extracting solvents depends on the NM of interest: toluene is used primarily for hydrophobic NM such as CNTs and fullerenes[54][58], whereas deionized water is used for hydrophilic NM or their dissolved components[57][62]. Some hydrophobic NOAA can be transformed in the soil to change their hydrophobicity. To account for this possibility, Navarro et al.[58] describe sequential partitioning using deionized water followed by methanol and finally toluene. In this way, different species of C60 can be captured and quantified separately. Of interest is the effect of centrifugation compared with filtration on the extraction efficiency. Hedberg et al.[57] compared centrifugation at 16 500 × g with microfiltration to separate extracted silver from the soil. These researchers found that centrifugation was more efficient at separating the dissolved silver from the soil than was filtration; however, the opposite was true for silver NOAA that were coated. This result was presumably related to the ability of uncoated silver NOAA to dissolve compared with coated silver NOAA.

10.4 Detection and quantitation

Isaacson et al.[53] and Carboni et al.[54] described the impact of naturally occurring carbonaceous materials on the detection of manufactured CNTs and C60. Presumably, this impact would be the same for quantifying carbon-based NOAA in other media and stresses the importance of identifying NOM from man-made NOAA.

Analysis of extracts for quantitation of NOAA depends largely on the NM of interest. ICP-MS and AAS have been used for quantification of silver[62][63], whereas UHPLC-HRMS was used by Carboni et al.[54] for quantification of fullerenes. In either case, subsequent analysis would include characterization and visualization of the particles, as well as quantitation.

Annex A
(informative)

Instrumentation/techniques used to quantify and characterize NOAA

A.1 Common analytical techniques to measure airborne NOAA (adapted from ISO TS/12901-1:2012)

Table A.1 — Devices for direct measurement of number, mass and surface area concentration

Measurand	Device	Remarks
Measured particle number concentration	Condensation particle counter (CPC)	CPCs provide real-time number concentration measurements between their particle diameter detection limits. They operate by condensing vapour onto sampled particles and detecting-counting the droplet formed. Typically used with a 1 000 nm size selective inlet and able to detect down to around 10 nm.
	Differential mobility particle sizer	Real-time size-selective (mobility diameter) detection of number concentration, giving number-based size distribution.
	Electron microscopy: SEM, TEM	Off-line analysis of electron microscope samples can provide information on size-specific aerosol number concentration.
Measured mass concentration	Size-selective static sampler	Assessment of the mass of nano-objects can be achieved using a size-selective personal sampler with a cut-off point of approximately 100 nm and the sample analysed by gravimetric weighing or by chemical analysis. Although there are no commercial devices of this type currently available, some cascade impactors (Berner-type low pressure impactors or Micro-orifice impactors) have selection points around 100 nm and can be used in this way.
	Tapered element oscillating microbalance (TEOM)	Sensitive real-time monitors, such as TEOM, can be used to measure nano-aerosol mass concentration online, with a suitable size-selective inlet.

Table A.1 *(continued)*

Measurand	Device	Remarks
Measured particle surface area concentration	Diffusion charger	Real-time measurement of aerosol active surface area. Note that active surface area does not scale directly with geometric surface area above 100 nm. Not all commercially available diffusion chargers have a response that scales with particle active surface area below 100 nm. Diffusion chargers are only specific to nano-objects if used with an appropriate inlet pre-separator.
	Electrostatic low pressure impactor	Real-time size-selective (aerodynamic diameter) detection of active surface area concentration. Note that active surface area does not scale directly with geometric surface area above 100 nm.
	Electron microscopy: SEM, TEM	Off-line analysis of electron microscope samples can provide information on particle surface area with respect to size. TEM analysis provides direct information on the projected area of collected particles, which could be related to geometric area for some particle shapes.

A.2 Common analytical techniques to measure the concentration and composition of NOAA (adapted from ISO/TR 18196:2016[10])

Table A.2 — Techniques to measure composition or concentration of bulk NOAA

Technique	Acronym	Chemical Composition	Concentration	Advantages	Limitations
Acoustic spectroscopy			Yes	Does not require sample dilution; applicable for concentrated samples. Method is suitable for characterizing aggregated and structured systems. Measurement is not affected by stirring and/or pumping of the sample. Absolute method; does not require size calibration. Instrument verification can be conducted by measurement of water with known acoustic properties. Solvent-based samples (non-aqueous) can be analysed	Applicable only to liquid-borne particles. Lower size limit ≈10 nm; upper size limit ≈3 mm. Lower volume fraction limit is ≈0,1 %; upper volume fraction limit is ≈50 %.

Table A.2 *(continued)*

Technique	Acronym	Chemical Composition	Concentration	Advantages	Limitations
Auger electron spectroscopy (scanning)	AES	Yes	Yes	High spatial (<1 µm) and surface (≈0,1 nm) resolution [spatial in this context refers to horizontal plane of analysis (*x-y* direction) and surface refers to depth]. Elemental mapping possible when employing a scanning electron beam. Depth profiling capability when combined with ion sputtering. High detection sensitivity; capable of analysing a fraction of a surface monolayer.	Analysis of non-conducting samples can be problematic due to surface charging effect. Surface contamination can complicate data analysis.
Differential scanning calorimetry	DSC	Yes		Minimal sample preparation required. Fast scanning allows suppression of material decomposition at higher temperatures (scan at 400 °C/min and faster). Wide temperature ranges. Measurements can be conducted in different atmospheres. Can characterize qualitative bond strength between a nanomaterial and epoxy in nanocomposites (strong or weak) and examines rigid amorphous fraction (RAF) of nanocomposite system.	Many DSC instruments cannot scan at 400 °C/min or collect data at those speeds
Electron energy loss spectroscopy	EELS	Yes		Spatial resolution of the order of the electron beam size. Any solid can be analysed. Quantitative analysis is possible. Signal includes chemical information. Direct information can be obtained on the structure of solids and oxidative state of the elements.	Need very thin specimens, < 30 nm. Intensity weak for energy losses > 300 eV. Can only be done using transmission electron microscopy (TEM).
EM based X-ray spectroscopy	EDX/EDS/ WDS	Yes		EDS is capable of semiquantitative or with the appropriate controls and sample preparation quantitative analysis. EDS can be used to generate complex multi-dimensional elemental composition maps. WDS is more sensitive to low atomic number elements than EDS and has the capacity for quantitative analysis.	Samples should be well-polished and representative of the bulk material. It may be necessary to coat non-conductive samples with a thin layer of carbon, gold, or platinum. Standards might be required for high-accuracy quantification.
Field flow fractionation	FFF	Yes	Yes	Can fractionate highly polydisperse samples into individual populations. Can employ a variety of detectors, e.g. differential refractometers, UV/Vis absorbance, fluorescence, dymanic light scattering, multi-angle static light scattering, or an inductively coupled plasma-mass spectrometer.	Lower size limit depends on particle density and the FFF method used but is typically 2 nm to 5 nm. Size can only be determined accurately by calibration, retention time, or by secondary detection. Distributions that range from the nm scale to greater than 1 µm will not be properly separated, as the elution order inverts above roughly 1 µm. Sample required to be dispersed in liquid.
Fluorescence spectroscopy	FL		Yes	Sensitive technique, down to single fluorophore level. Rapid technique for qualitative measurements. Compatible with various sample formats. Sensitive to changes in nano-object size and shape.	Interference from background fluorescence for some samples. Quantitative measurements are time-consuming and require calibration standards. Corrections for scattering are frequently required for quantitative measurements for nano-objects.

Table A.2 *(continued)*

Technique	Acronym	Chemical Composition	Concentration	Advantages	Limitations
Fourier transform infrared spectroscopy/ imaging	FTIR	Yes		Can determine chemical structure and compositional analysis of chemical mixtures in solid or gas phase. High throughput, high signal-to-noise ratio and high wavelength accuracy. Samples can be run under either ambient temperature (in purged air) or extreme environmental conditions such as ultra-high vacuum, cryo-temperature, high pressure, high temperature. Typically non-destructive and requires little sample preparation. Insensitive to stray light. Large penetration depth; can acquire spectra through low bandgap semiconductors.	Not suitable for aqueous-phased characterizations due to its sensitivity to water. Sensitive to CO_2 and requires the instrumentation to be purged to remove both H_2O and CO_2. Spectral complexity requires experienced users or access to spectral library for proper band assignment.
Inductively coupled plasma-mas spectrometry and single particle ICP-MS	ICP-MS	Yes	Yes	High sensitivity analysis — detection limits of most elements are on the order of ng/l (ng/kg) or lower. Multi-element analysis possible. Can determine element identity and quantity, typically in less than one minute. Wide linear dynamic range with eight orders of magnitude (in conventional mode). Isotopic information possible. Isotope dilution calibration possible. Can differentiate between dissolved and particulate analytes.	Spectral interference occurs when the instrument cannot distinguish the spectrum of an analyte ion from the spectrum of a concomitant having the same nominal mass-to-charge ratio. Molecular ion interferences can often be mitigated using collision/reaction cells; elemental isobars should be avoided. Contamination in blanks (acids, water, etc.) can sometimes limit detection and quantification. The use of high purity reagents and a clean laboratory environment is necessary, if ultra-trace levels are required. ICP-MS in single particle mode might not be an adequate technique to determine particle number and mass concentration in the event that the ENM size distribution extends below 10 nm for monoisotopic metal nanoparticles. Coupling ICP-MS with field flow fractionation (FFF) or another separation technique will resolve this limitation. Sample required to be dispersed in liquid. Particles with sizes smaller than the particle size detection limit will be quantified as ionic. Multiple analyses are required if no preliminary information on the particle size and number concentration in a sample is available.
Liquid chromatography-mass spectroscopy	LC-MS	Yes	Yes	Commonly used in many laboratories for a wide variety of applications. There are multiple MS detectors available depending on application needs.	Will only detect organic materials. Liquids/ dispersions only; no solid or gas analysis. Requires optimization for best separation.

Table A.2 *(continued)*

Technique	Acronym	Chemical Composition	Concentration	Advantages	Limitations
Optical absorption spectroscopy	UV/Vis/NIR	Yes	Yes	UV/Vis analysis requires only a few seconds to provide a response. Wide concentration range of analysis by varying sample path length. Quantification is available with higher end instruments. Little to no sample preparation required. Little maintenance required. Wide range of measurement accessories available to measure characteristics in both liquid and solid form including angular dependent scattered measurements. Absorption properties for some nano-objects provide information on sizes.	Corrections for scattering might be required. Solid samples require reflectance accessory. There is a wide array of accessories available to allow multiple modes of reflectance measurements to be made. These include relative, absolute and diffuse reflectance. The actual mode of reflectance required is to be determined by the specific analysis.
Raman spectroscopy/ imaging		Yes		Capable of chemical analysis of complex mixture. Insensitive to water, thus suitable for the characterization of aqueous-phased nano-objects. Suitable for *in situ* measurements in liquid, vapour and solid state and under both ambient or extreme environmental conditions such as ultra-high vacuum, cryo-temperature, high pressure, high temperature or under electrical bias, in a high magnetic field. Typically non-destructive and requires little sample preparation. Small foot-print fibre-optics-based field portable instrumentations are commercially available. Spectra can be collected from very small volume, e.g. in ≈1 µm 3 in confocal Raman microscope.	Raman scattering is very weak and can be swamped by fluorescence from trace impurities in the sample. Raman spectral complexity often requires even experienced users to access a spectral library for proper band assignment.

Table A.2 *(continued)*

Technique	Acronym	Chemical Composition	Concentration	Advantages	Limitations
Scanning electron microscopy	SEM	Yes		Wide range of magnification. Large depth of field with best resolution under 1 nm. Parfocal, i.e. the focus does not change with changing magnification. Two- and three-dimensional and topographical imaging with rich details. Fast, completing imaging and analysis often possible within a few minutes. Most samples require no or minimal preparation. Low landing energy operation or low pressure gas around a specimen allows for imaging and measurement of electrically non-conductive specimens. Low pressure gas around a specimen allows for nanometre-scale etching and deposition of materials. With specially equipped instruments hydrated, even live specimens can be examined in pressure greater than 600 Pa. The sample temperature can vary from 70 K to above 1 000 K and specimen nano-manipulation is possible. Different imaging modes giving different information, e.g. backscattered electron imaging sensitive to composition, secondary electron imaging sensitive to topography. Commercial calibration standards available for size in gold and silver. When using electron back-scatter diffraction, it is highly sensitive to orientation. When using electron back-scatter diffraction, phase identification is through crystal type.	It may be necessary to coat non-conductive samples with a thin layer of carbon before examination. The instrument may be large in size and required to be housed in an area free of significant electro-magnetic or mechanical interference and might require circulation of cooling water. Special training is required to operate the microscope and to learn the processes of sample preparation, and to recognize and minimize preparation-related artefacts. Works in vacuum, which requires solid samples small enough to fit inside the vacuum chamber. Electron irradiation can alter the sample. Measurements on small particles might have high measurement uncertainties. Distinguishing between agglomerated and aggregated particles might be difficult. When using electron back-scatter diffraction, only polished surfaces can be examined. When using electron back-scatter diffraction, crystallinity determination of phases with resolution is down to 10 nm size (when polished, cross-section of sample including embedded nano-objects is examined). For particle size distribution, a relatively large number of independent images should be obtained in order to have a statistically relevant sample representation.
Secondary ion mass spectroscopy	SIMS		Yes	Identification of trace elements up to 2 000 Da with transmission > 10 %, with better than 1 parts per million sensitivity. 3D elemental distribution with 10 nm depth resolution in depth profiling mode. Detailed chemical isotope ratio information, used for exact quantification [spot to spot reproducibility (1 s): 0,4 per mille; mean internal error (1 s): 0,3 per mille]. Chemical imaging of surfaces with ≈500 nm spatial resolution. Depth profiling of inorganic with ≈10 nm depth resolution. NanoSIMS is a term used to denote the use of an ion beam with an extremely small spot size (down to 50 nm) to provide high spatial resolution analysis.	Samples analysed under vacuum. Sample preparation (embedding in resins and polishing). Sample preparation is not necessarily required when running TOF-SIMS. Number of simultaneous monitored elements can be limited but a TOF analyser is capable of monitoring ions of m/e into the hundreds and thousands. Not considered a bulk analysis technique. Homogeneous material is needed to obtain nano-shape information. Extreme care should be taken during sample handling to avoid contamination.