Challenges in Nanomaterial Characterization – From Definition to Analysis

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Abstract

Nanomaterials have outstanding properties and have several applications, ranging from foods, cosmetics, pharmaceuticals to energy, construction, etc. As with all novel products, the benefits of nanomaterials use must be weighed against its health and environmental impact. They have different origins, natural, incidental, or engineered, they are widespread, and they need to be classified and characterized for various purposes, including nanotoxicology studies and risk assessment, workplaces and environment safety evaluation, consumer products evaluation, as well as manufacturing process control. To properly characterize nanomaterials, a consensual definition of nanomaterial is needed, and several analyses using the available characterization techniques must be performed. Various properties are relevant in the characterization process and many of them, namely size, are still a challenge that the research community is facing. The measurement of physical and chemical properties is very important in the case of nanomaterials. In view of this, in this chapter, available analytical techniques are reviewed based on nanomaterials classification, regulatory demands and toxicology assessment. Additionally, some of the current major challenges and gaps in nanomaterials characterization are identified and listed.

1.1 Introduction

Nanotechnology has been a relevant topic in the scientific community due to the unique properties of materials in the nanoscale, and it has become an enabling technology for numerous applications. Produced science on new nanomaterials (NMs), and their characteristics and applications, has been identified as a key enabling technology and keeps stimulating industrial growth, innovation and development, in the most diverse fields such as medicine, food, cosmetics, electronics, automotive, energy, construction, and other areas. Consequently, studying the exposure to nanomaterials is a critical aspect when assessing their safety and risks, particularly in three scenarios: environment, consumer products and working places [49]. Results from these studies

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will contribute to overcome uncertainties about NMs safety for human health and the environment which are still hampering a more widespread exploration of its potentials. Several authorities and official organisms are therefore defining actions for the implementation of a safe, integrated, and responsible approach for nanoscience and nanotechnologies. As an important step in that direction, definitions of nanomaterials have been proposed and are being implemented for regulatory and policy purposes in order to ensure harmonized terminology and definitions across different pieces of documentations and legislation. The difficulty in comparing the toxicity results for available nanomaterials comes from the wide variety of production processes and also from some lack of systematic work regarding physical and chemical characterization of NMs [9]. Measuring nanomaterials properties should be done by using the most appropriate technique(s) and results should be confirmed against a control or reference material, using an orthogonal analytical approach since no single technique is capable of fully characterize a nanomaterial [49]. Additional difficulties may come from the need to characterize NMs in complex and polydisperse media (e.g. biological) as well as from the fact that many times their concentration in these types of media may be quite low [1].

The toxicity of NMs for living organisms is probably the main factor hampering their use and application. To proper balance between the positiveness of their use and their toxicity is mandatory, and it should be based on adequate experimental models which ultimately depend on the adequate physical and chemical characterization of NMs [50]. However, the reliable detection, characterization, and quantification of nanomaterials is still quite challenging, particularly in complex media, and work has to be done to overcome difficulties inherent to nanoscale materials, to the difficult access to all the available techniques, and to the lack of harmonized procedures and interlaboratory studies [40].

1.2   Types of Nanomaterials

1.2.1   Classification

Nanomaterials (NMs) are basically materials that have one dimension between 1 nm to 999 nm. This would be the expected classification when simply considering the word itself. However, that is not the case and there have been several approaches to group and classify nanomaterials. Initially, in 2007, ISO/TR 27628 appears with the classification of a nanoparticle as a particle with a nominal diameter smaller than 100 nm [17]. One year later, ISO/TS 27687 proposes terminology and definitions for nanoobjects which include nanoparticle, nanofiber and nanoplate [19]. A classification tree is proposed for nanoobjects (see Fig. 1.1). In 2010, ISO/TS 80004 harmonizes terminology and definitions, and nanomaterial is defined as a material with any external dimension in the nanoscale or having an internal structure or surface structure in the nanoscale, ranging the nanoscale approximately from 1 nm to 100 nm [21]. At the same time ISO/TR 11360:2010 [20] provided a basic classification system for different types of nanomaterials which accounts for their different
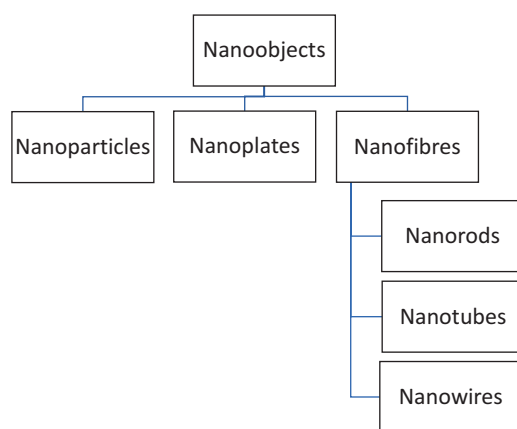

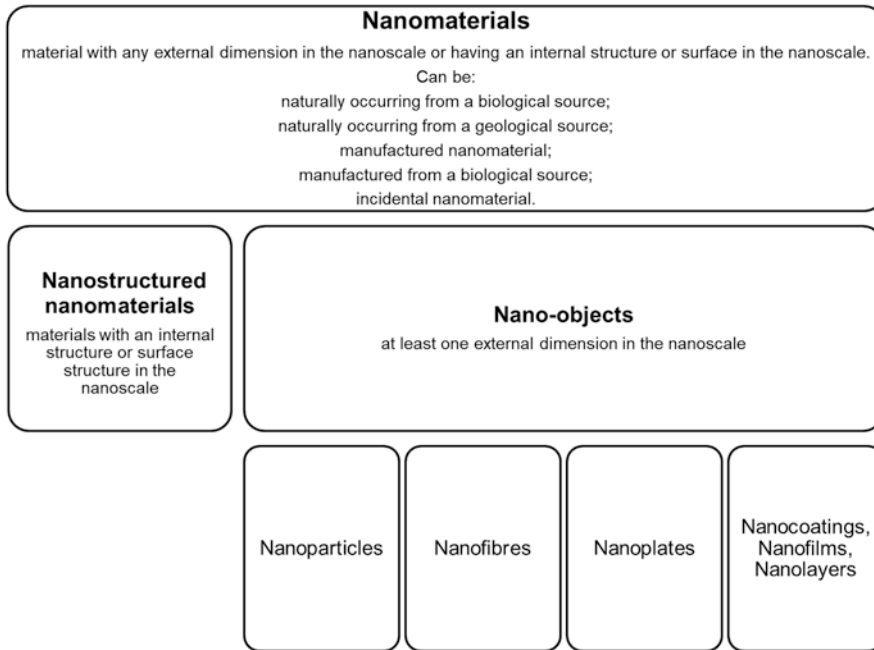**Fig. 1.1** Nanoobjects classification according to ISO/TS 27687:2008

Fig. 1.2 Classification of nanomaterials according to ISO/TR 18401:2017

properties. Following classifications trees, nano-materials are grouped according to their dimensions, structure, chemical and physical properties and functional behavior.

ISO/TR 18401:2017 [26] revisited the vocabulary and defined a nanoparticle as a nano-object with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly. These are broad spectrum definitions in which several types of materials can be included and grouped under two major categories: nano-objects and nanostructured materials (see Fig. 1.2). Regarding nanostructured nanomaterials, Hansen et al. [13] have proposed a classification quite relevant since it considers the matrix where nanoparticles may be embedded (Fig. 1.3). This classification helps to predict how challenging must be to separate and characterize nanoparticles depending on where they must be isolated from.
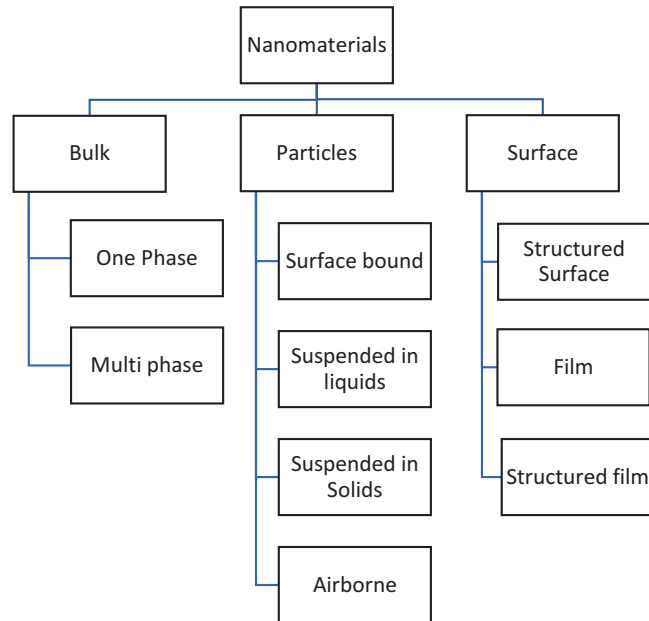
1.2.2 Properties

A systematic approach of classification and categorization of nanomaterials just based on dimension is quite limited. Other properties than size must be considered to properly define a logical hierarchy of classification.

ISO/TR 11360:2010 [20] describes a classifying system, termed "nano-tree" which aims to provide a structured view of nanotechnology and facilitates a common understanding of nanotechnology concepts.

The nano-tree depicts the understanding of the structure and relationships of nanomaterials providing means to classify them. It uses dimension and key functional properties to distinguish nanomaterials from one another and to show its relationships. By this way, nanomaterials are grouped according to their dimensions (1D, 2D and 3D), their chemical nature (eg. ceramic,

Fig. 1.3 The categorization framework for nanomaterials. The nanomaterials are categorized according to the location of the nanostructure in the material. (Adapted from [13])

metallic, organic, inorganic), their physical properties (eg. optical, magnetic, acoustic), their mechanical properties (eg. elastic, plastic), their chemical properties and their biological properties, as well as some combined properties (eg electro-optical or thermoelectric). This ISO standard, together with other proposals for the classification of nanomaterials, makes possible to harmonize their classification in a rational and systematic manner. However, all these proposals would be cross-cutting if we do not consider the size. In fact, size is the critical property that makes it possible to classify nanomaterials as such.

1.3   Risk Assessment and Regulatory Perspective

Since the begin of the study of nanomaterials, the impact of their use in the various possible areas of application has always been the main concern. In other words, because nano-scale materials have unique properties, there has always been a need to study them in isolation and to understand their impact on ecosystems, and on life in general, and human life in particular. Nanomaterials have therefore been studied from the perspective

of the risk their use poses to human life and the environment in general.

As size of nanoparticles (NPs) gets smaller, the surface area increases exponentially, which make these particles more reactive and potentially more toxic. Also, with decrease in size, their ability to penetrate plant and animal tissues increases, and at such small sizes, even well-known substances behave in uncommon ways. Penetration of NPs through the different barriers of the cells is largely dependent on size. It is a "rule of thumb" that particles with a size less than 100 nm can enter cells by crossing cell membrane. When the size becomes smaller than 40 nm they can enter into nuclei of cells while those with less than 35 nm can penetrate into blood–brain barrier [10, 42]. Larger particles (200–500 nm) can also enter cells [16] but with less probability which makes size specification of 100 nm an acceptable reference value for regulatory purposes.

Given the relevance of size for crossing barriers, more definitions began to emerge where the cut-off value was 100 nm. This was intended to create a well-defined criterion, based on which a certain group of materials was classified as 'nanomaterial' and which might deserve specific

considerations in a regulatory context. Nanomaterials are not necessarily hazardous, and size-based definitions are not specifically based on hazard or risk assessment. However, the fact that particles larger than 100 nm cannot easily cross membranes, implies that the risk of use may be much lower. In those circumstances, their risk can be addressed according to their many other properties and in the same way as materials/substances already known before the introduction of the "nanoscale". This applies when they are used both at a molecular level (liquids, solutions, gas) or as larger particles (solids, suspensions, aerosols).

In 2011, the European Commission proposed a definition for nanomaterials, based on ISO definition [6]. This definition recommendation, that has been revisited but not reviewed so far [47, 48], considers a nanomaterial defined as "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm….". The European Commission Nano Material (EC NM) definition further specifies that "fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials" and that "'particle', 'agglomerate' and 'aggregate' are defined as follows: (a) 'particle' means a minute piece of matter with defined physical boundaries; (b) 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components; (c) 'aggregate' means a particle comprising of strongly bound or fused particles" [6].

Surface area is directly correlated with particle size and with the reactivity/toxicity of nanomaterials. For that reason, the EC NM defines that "Where technically feasible and requested in specific legislation, compliance with the definition …. may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition …. where the specific surface area by volume of the material is greater than 60 m^2/cm^3. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition … even if the material has a specific surface area lower than 60 m^2/cm^3." [6]. The number size distribution should cover for the fact that nanomaterials most typically consist of many particles present in different sizes and in a particular size distribution. Without specifying the number size distribution, it would be difficult to determine if a specific material complies with the definition where some particles are below 100 nm while others are not. [47, 48].

Identification of a material as a nanomaterial according to the EC NM definition is not determined by a certain (chemical) composition, a certain structure, novel properties that are attributable to the particles' external dimensions, or by the application of the material in a specific field. Exceptions are fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm, which are explicitly considered as nanomaterials. Regarding particles origin, NMs and its possible risks do not depend on whether its particles are natural, produced incidentally, or the result of an engineering process with or without the explicit intention to manufacture a nanomaterial. In that respect, natural materials can exhibit the same properties as those that are manufactured and vice versa. Therefore, EC NM definition does not exclude certain types of materials just because of their origin. However, there is an exception to individual proteins, polymers and macromolecules; which are excluded from the scope of the EC NM definition as they are considered single molecules. Even so, if these macromolecules are assembled into solid objects with clearly defined and stable external boundaries, and if they are stable enough to retain their shape over a longer period and to allow the measurement of their external dimensions, these objects should be considered as particles.

In the same way of the European Commission (EC), many other official organizations across the world have adopted similar definitions. As EC, all of them defined 100 nm as the reference

top range size for the classification, and most of them refer to a characterization based on a number distribution. [3].

Similarly, sector organizations like the Food and Drug Administration (FDA) or the World Health Organization (WHO) have created guidelines with a nanomaterial definition. In fact, FDA has not established regulatory definitions of "nanotechnology," "nanomaterial," "nanoscale," or other related terms. Since they are already commonly used, FDA simply adopted the definition of nanomaterial as a material that have at least one dimension in the size range of approximately 1 nm to 100 nm [12]. Bigger materials in the nanoscale (here defined as sizes up to 1000 nm) were also considered to address products resulting from nanotechnology, but they were grouped in a different category. This decision reflects the fact that materials or end products can be engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the typical nanoscale range (1 to 100 nm), up to one micrometer (1000 nm).

In the same way, WHO also adopted the common definition of nanomaterial as materials that have at least one dimension (height, width or length) that is smaller than 100 nm. A nanoparticle is defined as a nano-object with all three external dimensions in the nanoscale (<100 nm diameter) and manufactured nanomaterials are defined as solid, particulate substances intentionally manufactured at the nanoscale, consisting of nano-objects with at least one dimension between 1 and 100 nm, and their aggregates and agglomerates. [51].

The definitions referred above were all very similar and were presented in a context of risk assessment of nanomaterials (NMs) exposure and therefore its toxicological impact. The toxicity of NMs may largely depend on numerous physicochemical properties, including size, shape (i.e. external size in a particular dimension), composition, surface characteristics, charge and rate of dissolution. The introduction of NMs in many different types of products and into the environment, and the human exposure to both, has raised additional needs in NM characterization in order to understand which core properties besides dimension may be relevant in risk assessment and toxicological impact on humans [28]. A new discipline arose – nanotoxicology, to characterize and categorize the adverse effects induced by NMs and to determine relationships of structure and function between nanoparticles and toxicity [4, 11].

Based on biodegradability and on the fact that biodegradable material in the human body has *a priori* a lower toxicity risk, a nanotoxicological classification system (NCS) has been proposed [30]. This simple system considers size and biodegradability and classifies nanomaterials in four main categories (I to IV) from low/no risk to high risk. However, specific methods to evaluate bioavailability, pharmacokinetic pathways, persistency, degradation by-products, cell uptake, intercellular fate, cell interaction were yet to consider at the time. Regulatory and standardization measures to characterize different nanomaterials like chemicals, biocides, consumer products and food, and to characterize human exposure, biokinetics and toxicity, require appropriate analytical development and capabilities. [32].

Risk assessment and toxicological evaluation of NMs has been always quite challenging since there is an enormous amount of different materials in different contexts of use, with different properties, and different behavior. Considering that, an approach for toxicity prediction without testing every single material and fully characterize it, is to use *in silico* methods such as the (quantitative) structure–activity relationship ((Q)SAR) [8]. To properly apply this approach, a high amount of high-quality experimental data needs to be assessed. The establishment of standard protocols (or operation procedures) is paramount for enabling the generation of this data by means of accurate measurement of the physicochemical and biological properties of ENMs [44]. Also, the set of properties to be characterized (core properties) needs to be defined in advance according to its relevance for the toxicological assessment. The Organization for Economic Cooperation and Development (OECD) Working Group on Manufactured Nanomaterials, the

Table 1.1 List of physicochemical properties that might be relevant to address for nanomaterials characterization [43]

Characterization (as on the shelf)		Characterization (in respective media)
– Appearance – Melting point – Density – Size, size distribution – N-octanol–water partition coefficient – Water solubility/dispersibility, hydrophilicity – Solubility/dispersibility in organic solvents, oleophilicity – Auto flammability – Stability in solvents and identity of relevant degradation products – Oxidation reduction potential – Storage stability and reactivity towards container material – Stability towards thermal, sunlight, metals – Catalytic activity – Radical formation potential – Flammability	– Dissociation constant – pH – Agglomeration or aggregation – Crystalline phase – Crystallite and grain size – Aspect ratio, shape – Specific surface area – Zeta potential – Surface chemistry – Stability and homogeneity (on the shelf, in water and organic solvents) – Dustiness – Porosity, pore and pour density – Photocatalytic activity – Explosiveness – Oxidizing properties	– Composition/purity – Size, size distribution – Agglomeration/aggregation – Zeta-potential – Biophysical properties (protein binding/corona characterization, residence times, adsorption enthalpy, conformation changes on binding) – Test item preparation protocol, conditioning, homogeneity and short term stability

OECD WPMN, prepared one of the most comprehensive lists of the important physicochemical characteristics for toxicological studies (see Table 1.1). In the same document, also a list of "endpoints" is presented where stability; biodegradation and toxicity, among others, are suggested [43].

The set of listed properties allows to properly characterize nanomaterials, but not all of them are equally relevant to predict toxicity [44]. In that context, size and size distribution are the most relevant since they allow to classify the material as nano in first place. As previously written in this chapter, size is also related with the ability of NMs to cross cell membranes, penetrate the nucleus or pass through blood barrier membrane. The interaction of NMs with living systems and the uptake and deposition of NMs within the human body are therefore affected by particle size. Also, the surface area increases with decreasing particle size, affecting surface energy and hence the reactivity of the material.

Before addressing toxicological tests, it is needed to understand and identify the most relevant physicochemical properties of NMs. Other properties such as particle shape (external dimensions), crystal structure, surface chemistry, sur-

face charge, and aggregation state have been identified to be paramount to correlate nanomaterials exposure with toxicity [22, 44].

The shape of NMs, and indirectly their external dimensions, is an important property that influences the biological activities of the particles [5, 34, 46]. Several nondimensional shape indexes can be used to quantify the shape characteristics of particles, such as sphericity/circularity, aspect ratio/elongation, convexity, and fractal dimensions [18]. Using quantitative descriptors for external dimensions and shape, rather than the usual qualitative ones (e.g. rod, sphere) highly potentiates the possibility of establishing better correlations between toxicity and shape, meaning a better QSAR.

Nanomaterials with the same chemical composition may affect differently the integrity and fluidity of membranes depending on the crystalline phase. The toxicity mechanism of NPs with different structure or different surface coatings will have different toxicological effects depending on their phase/crystallinity. [41, 53].

Surface chemistry plays an important role in the interaction of NMs with biological systems and subsequently, their toxicity. On the other hand, it is also relevant for the characterization of

NPs since it defines the potential surface interactions and aggregation behavior of NPs in liquid media [15, 50].

Surface charge, and consequently zeta potential is another important characteristic that may affect the toxicity of NMs. The biological interactions of NMs, their fate and hence their biological activities, are highly surface-charge dependent. On the other hand, the electrostatic stability of particles in liquid medium and, in practical terms, the influence it has on the formation of agglomerates may also affect toxicity [33].

Some NPs tend to form large agglomerates either in the dry form or in suspension. When this happens, NPs may behave like larger particles because of their increased global size. Increasing the size of NMs through aggregation may change its nanotoxicity in comparison to the exposure of isolated nanoparticles of the same material. [2, 31].

1.4 Available Techniques to Characterize Nanoparticles Size, Size Distribution and Shape

According to the common classifications in use, a nanomaterial is classified as such when that at least 50% of the particles have one or more external dimensions ('size') between 1 nm and 100 nm. This definition has already been adopted by several European countries as well as by the European Food Safety Authority [7], along with other world countries and international organisms (eg. WHO, FDA and OECD).

It is consensual from all the available definitions of nanomaterial that size, size distribution by number and shape (external dimensions) in the range of 1–100 nm and over, are critical properties to classify a material as nano.

1.4.1 Size

Regardless of the difference in scope and implementation, all definitions of the term "nanomaterial" have a common characteristic as the basic defining element: particle size. Therefore, in any case of deciding whether a material is a nanomaterial, its particle size distribution must always be determined. This involves particle size measurement from a few nanometers to a few microns. Although the particle size can be determined by a variety of analytical techniques, each technique has its scope of application in terms of material type, material properties, and achievable size range, as well as the medium in which the particles are dispersed and are to be isolated from and measured.

The European Commission through the Joint Research Center (JRC) recognizes about thirteen possible techniques and grouped them according to their working range (1–100 nm and 100 nm–100 μm) and the type of measurement signal weighting regime (light intensity or extinction, particle mass and particle number) [47, 48].

In the past few years, the measurement performance and quality assurance level of common particle size measurement techniques have improved, but these techniques still cannot measure NMs within the entire size range related to their definitions, that is, from 1 nm well into the micrometer region. Among the techniques with a wider analytical range is Analytical Ultra Centrifugation (AUC). This technique is able to separate and measure particles from some nanometers to about 30 μm. However, particles need to have an optical property distinguishable from other solution components and a density compatible with a reasonable sedimentation rate within the experimental gravitational field [52]. In comparison to other available techniques, AUC is time consuming and therefore with a low throughput [45]. Similar to AUC, is Centrifugal Liquid Sedimentation (CLS), in which particle size is determined by means of centrifugal sedimentation in a liquid and its concentration by means of the transmission of a light beam. The method is applicable to powders that can be dispersed in liquids. In it, all particles are assumed to have the same density

and comparable shapes, and its density must be different from the density of the dispersing liquid. Typical particle size range for analysis is from about 5 nm to more than 10 μm [52].

Laser diffraction (LD) spectroscopy comprises angular light scattering techniques, which are primarily designed to resolve the scattering pattern at small scattering angles. Although its typical analytical range can start at values lower than 100 nm, it is a technique usually devoted to microparticles rather than NPs. LD is prone to underestimate the amount of NPs and, thus, to overestimate the number-weighted sample median. Additionally, the sample typically needs to be diluted and it measures an equivalent sphere diameter not resolving different particle shapes. [27, 35].

Small-angle X-ray scattering (SAXS) is a technique that allows to obtain size information of nanomaterials and it is based on the interaction between X-rays and matter. Its working range is one of the narrower (1 nm to 100 nm) although under certain conditions (narrow size distributions, appropriate instrumental configuration, and idealized shape) this limit of 100 nm could be extended. This characteristic leads to underestimation of the median particle size for broad size distributions that goes beyond 100 nm, which results in a misclassification of NMs. [14].

Most of the techniques are able to measure both size distribution of equivalent spherical particles but a few can distinguish individual particles from aggregates/agglomerates [35]. Another relevant point is that, except for atomic force microscopy (AFM), all the purposed techniques are already supported by an ISO standard. This allows for the different users of each technique to access each technique with a higher level of harmonization, and better compare results obtained from different samples, in different instruments by different operators.

The results of different particle size measurement techniques are usually not in complete agreement because the measurement principles and working range behind each method are different. Accordingly, particle size results should always be reported along with the size range in which the size was measured, as well as the instrumental technique and analytical method.

A descriptor commonly used for evaluating particle size distributions is the x50.0 value, also called the median (the size at which 50% of the analyzed particles of a sample have a property of less than this value and for the other 50% of the particles the same property is higher). However, due to the differences in the measuring technique principles, the median can relate to different properties like light intensity, volume, number, mass, or some external dimension. The knowledge of material properties allows the original size distribution to be converted to other distributions, however trueness can be compromised depending on the accuracy of the material information used to do it.

1.4.2   Number-Based Distribution and Shape

Most definitions of nanomaterials refer to the distribution in number. That is, a material will be considered as nano if a part (usually 50%) of its particles (number-based distribution) is below 100 nm. Thus, any technique used has to be able to measure particles with sizes under and above 100 nm, of different shapes and chemical nature, in their isolated state or in aggregates/agglomerates and still be able to count each particle for the number-based distribution. As with any method, it is desired that these measurements are feasible in laboratory reality, *i.e.* fast, accurate and precise, robust and accessible. Nowadays there is no method or technique capable of fulfilling all these objectives, however electronic microscopy techniques have some advantages:

They operate in the desired measurement range.
They distinguish particles from aggregate/ agglomerates.
Allow number-based distribution.
Allow analysis of particles of any chemical nature.
Allow the measurement of external dimensions and shape
They are supported by standard procedures (ISOs)
They may be able to do chemical discrimination

Despite their enormous capabilities, electron microscopy techniques have certain limitations and weaknesses:

The sample preparation can be difficult and is material dependent.

Not applicable to liquid dispersions.

Among electron microscopy techniques, scanning electron microscopy (SEM) is easier to use on a routine basis (less sample preparation, less consumables, lower cost per analysis) but is limited in size range. The best instrument configuration in the best-case scenario does not perform well for sizes lower than 10 nm.

A very good automation is required in terms of image acquisition hardware and image processing software.

To obtain a representative result with statistical relevance, at least 10,000 particles need to be measured [23] which makes it time-consuming.

It is high cost.

There are other techniques available that can measure size and make the distribution in number:

Atomic force microscopy (AFM)

Particle tracking analysis (PTA)

Tunable resistive pulse sensing (TRPS) / electrical sensing zone (ESZ) / nano Coulter counter

Single particle inductively coupled plasma - mass spectrometry (spICP-MS)

Differential electrical mobility analysis (DEMA)

AFM share most of the advantages and disadvantages with electron microscopy. However, it is strongly dependent on sample preparation (immobilized particles on substrate need to be representative of the material), working range is more limited and it is not supported by an ISO standard.

All the other proposed techniques lack the ability to distinguish single particles from aggregates/agglomerates and to provide any shape information or particular external dimension. TRPS does not perform well for sizes under

30 nm and DEMA for sizes above 1 micron. DEMA only analyses samples in the form of aerosols and ICP-MS is not able to detect particles with no metals in their composition (carbon based, organic or biological).

Particle tracking analysis (PTA) is an alternative since it is supported by an ISO standard [24], it can measure particles from about 10 nm (material dependent), is quite simple to use and comparably affordable. Depending on the instrument configuration and material chemistry, it may be able to do chemical discrimination.

The interplay between sample preparation, identification of constituent particles and the accurate determination of the external dimensions of these constituent particles requires complex and often time- and resource-intensive measurement techniques. However, even when it is not possible to determine the exact number-based distribution as such, a decision whether a material needs to be classified as nanomaterial or not is still relevant.

In this context, dynamic light scattering (DLS) is a very good first choice [39, 40]. Although it is a technique with several limitations and that needs extra care and knowledge to interpret the results [25], it has several advantages that can be listed below:

– It is applicable to broad size distributions from 1 nm to more than1 μm,
– it allows a relatively inexpensive and fast assessment of particle size,
– it is fast and robust,
– it is applicable to any type of material (carbon based, organic, inorganic, biological or metallic), as long as particles remain in a stable suspension and undergoing Brownian motion.
– a minimum amount of information about the sample is needed to run the analysis
– It is non-destructive (sample may be recovered)
– small amount of sample is needed to run a test

The methods and measurement techniques that can be used to measure particle size distributions and shape can be based on very different

Table 1.2 Comparative evaluation of selected methods for size, shape and size distribution characterization

Measuring principle	Technique	Size range	Liquid dispersion	Solid/powder	Shape	Polydispersity	Size distribution of raw data	Measures constituent particles in aggregated / agglomerated samples
Microscopy	**SEM**	10 nm to >10 mm	No	Yes	Yes	Yes	Number-based	Yes
	TEM	1 nm to >10 mm	No	Yes	Yes	Yes	Number-based	Yes
	AFM	1 nm to 10 mm	No	Yes	Yes	Yes	Number-based	Yes
Separation	**FFF**	1 nm to 10 mm	Yes	No	No	Yes	Scattered light intensity based	No
	AC	1 nm to 10 mm	Yes	No	No	Yes	Light extinction-based	No
	DEMA	2 nm to 1 mm	No (only aerosolized NPs)	No (only aerosolized NPs)	No	Yes	Electrical mobility based	No
Scattering	**PTA**	10 nm to 1 mm	Yes	No	No	Yes	Number-based	No
	DLS	1 nm to 10 mm	Yes	No	No	No	Scattered light intensity based	No
Other	**ICP-MS**	10 nm to 1 mm	Yes	Yes	No	N/A	Number-based	No
	SAXS	1 nm to 100 nm	Yes	Yes	No	No	Mass based	No

Source: Adapted from [7, 35]

measurement principles, and the level of detail that they provide, and their working range can also differ considerably. For many materials, available methods already allow their classification as nanomaterials according to the accepted definitions, however, there is still a path to make to overcome the cases for which that is still too challenging (Table 1.2).

1.5 Major Challenges in Characterization

In the last two decades a huge effort, work, and investment has been put in place to improve NMs characterization in all perspectives (legal, regulatory, technical, and scientific).

So far, all relevant definitions apply to nanomaterials from 1 to 100 nm and recommend a number-based distribution approach.

Recently, the NanoDefine Methods Manual, parts 1[36], 2[35] and 3[37] have been published. With these manuals, general recommendations have been produced to support the user in the decision whether a material is a nanomaterial according to the EC Recommendation on the Definition of Nanomaterial. Those recommendations refer to the available measurement techniques, which are candidates for performing a reliable analysis of the number-based size distribution of a particulate material; as well as to Standard Operation Procedures (SOPs) for the sample preparation.

Analyzing nanomaterials remains a challenge and the main difficulties have been thoroughly identified and discussed [29, 38].

In general, one may distribute the main difficulties into two main categories: those related exclusively to size characterization and those that apply to all characterization techniques of all physical and chemical properties of nanomaterials.

In the first case one can identify:

– The size range. Many available techniques are not capable of detect and analyze particles

smaller than 50 nm, and many others have a narrow working range.
– The differentiation between isolated particles, aggregates, and agglomerates
– The ability to measure the external dimensions of particles and consequently determine their shape
– The ability to count particles individually with the aim of obtaining a number-based distribution.

The biggest challenges that can be identified for most of the available techniques are also related to the nano scale since the measurement of common properties becomes more difficult within this size range. They can be identified as:

– The preparation of the sample. The sample must be representative and have statistical significance.
– The isolation of nanomaterials included in complex matrices. Media such as soils, food, cosmetics, or pharmaceuticals still represent a major challenge in terms of separation of nanomaterials prior to analysis.
– How to report the results is critical. Linking a number to the technique, analytical method and method of sample preparation is critical.
– International Standardization. It is probably one of the biggest challenges in characterization. Ensuring that the entire community uses the same techniques in the same way allows for a better evolution of knowledge regarding the properties of nanomaterials.
– The establishment of interlaboratory tests that allows a greater degree of confidence in the results obtained in regulatory or quality control laboratories.

The more progress is made in minimizing the difficulties identified, the more and better results will be produced that will enable even better correlations to be established between a given physical-chemical property and the behavior of nanomaterials in a variety of environments, and consequently its toxicological potential. The development of methods that are both more effective and cost-efficient will help in the NMs

safety evaluation through the improvement of the existing regulatory guidelines.

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