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INTRODUCTION

R. Tantra, J. C. Jarman, and K. N. Robinson

Quantitative Surface Chemical Spectroscopy Group, Analytical Science, National Physical Laboratory, Teddington TW11 0LW, UK

1.1 OVERVIEW

Over the course of the past few decades, the word "nanomaterial" started to shine in reporting and publishing; nanomaterial thus became the new buzzword, giving the impression of a new type of technology. In fact, nanomaterials are not new at all and can be found in everyday lives, with most people not being aware of their existence. Nanomaterials exist in nature, for example, in volcanic ashes, sea sprays and smoke [1]. In relation to manufactured nanomaterials, they have existed as early as the 4th century. The Lycurgus Cup, a glass cup made with tiny proportions of gold and silver nanoparticles is an example of Roman era nanotechnology. The use of nanoparticles for beautiful art continued ever since, and by 1600s it is not uncommon for alchemists to create gold nanoparticles for stained glass windows. These days, there are far more uses; nanomaterials thus represent a growing class of material already introduced into multiple business sectors. For example, in early 20th century, tire companies used carbon black in car tires, primarily for physical reinforcement (e.g., abrasion resistance, tensile strength) and thermal conductivity to help spread heat load. Although nanomaterials have been around for a long time, it was only the invention of the scanning tunneling microscope in 1981 and the discovery of fullerenes in 1986 that really marked the beginning of the current nanoscience revolution. This led nanoscientists to conduct research, to study their behavior, so as to control their properties and harness their power.

Nanomaterial Characterization: An Introduction, First Edition. Edited by Ratna Tantra.

Over the past few decades, research activity on nanomaterial has gained considerable press coverage. The use of nanomaterials has meant that consumer products can be made lighter, stronger, more aesthetically pleasing, and less expensive. The huge impact of nanomaterials to improve quality of life is clear, resulting in faster computers, cleaner energy production, target driven pharmaceuticals, and better construction materials [2, 3]. In particular, carbon nanotubes (CNTs) have been hailed as the wonder nanomaterial of the 21st century. CNTs are composed entirely of carbon and classed as high-aspect-ratio nanomaterial. They can be visualized as a single layer of carbon atoms in a hexagonal lattice called graphene and subsequently rolled to form a seamless cylinder/s. CNTs are classed as either single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs). As the name suggests, the former are in the form of a single tube, whereas the latter consist of multiple rolled layer or concentric tubes. CNTs typically have a diameter of 1–20 nm and a length that can be many millions of times longer. MWCNTs are normally thicker than SWCNTs, with a maximum diameter exceeding 100 nm.

According to the National Science Foundation's National Nanotechnology Initiative (NNI), the global nanotechnology market could be worth $1.2 trillion by 2020 [4]. There is huge demand for CNTs alone, with a worldwide commercial interest being reflected in its production capacity, estimated in 2011 to be 4.5 kt/year [5]. This represents a huge growth from the production of around 0.25 kt/year in 2006. Bulk, purified MWCNTs sell for approximately $1 per gram, between 1 and 10 times as expensive as carbon fibers. SWCNTs, in contrast, are currently several orders of magnitude more expensive than MWCNTs [5].

Most commercial applications of CNTs involved incorporating the powders to produce composite material with special properties, for example, electrically conductive plastics and lithium-ion batteries in laptops. A more recent exploitation of CNTs is when they are used as materials for sporting equipment. For example, CNT-based frame was used in a bicycle that won the Tour de France in 2005. The incorporation of CNTs into the material improved stiffness and fracture toughness without compromising other properties. The result is a bicycle that features minimal weight and maximal strength.

Although it is clear that nanomaterial holds great potential to form the basis of new products with novel or improved properties, concerns surrounding their potential harmful effects on health and the environment have been the topic of much debate. In over a decade, a scientific discipline called nanotoxicology [6] has emerged, which aims at understanding potential hazards posed by nanomaterials and subsequent risk implications, should, for example, they enter the human body through inhalation, ingestion, skin uptake, or injection. The field is thus interdisciplinary in nature and at the interface of biology, chemistry, and material science.

Undoubtedly, nanomaterial research spans across different disciplines, from material science to nanotoxicology. Common to all of these disciplines, however, is the need to measure physicochemical properties of nanomaterials. As mentioned in the Preface section, the goal of the book is to lay a common foundation, giving an introduction to nanomaterial characterization, thus allowing the reader to build background knowledge on this topic. This chapter gives an overview and focuses on generic topics/issues of relevance to nanomaterial characterization. It is sub-divided into four parts. The first part discusses why nanomaterials are unique in relation to their physicochemical properties. The second part presents the relevant terminology, such as the definition on what constitute a nanomaterial and what the different properties actually mean. Terminology is important as it avoids misunderstandings and ensures that the correct term is being used among stakeholders such as researchers, manufacturers, and regulators. The third part of this chapter focuses on good measurement practices; like any other research there is a need to generate reliable and robust data. In order to promote an integrated approach to quality assurance in the data being generated, topics such as method validation and standardization are covered. The last part of the chapter presents some of the common practices that are carried out in nanomaterial research, such as sub-sampling and dispersion. Although this chapter is intended to give a general overview for readers coming from different disciplines, many of the specific examples presented are of relevance to nanotoxicology.

1.2 PROPERTIES UNIQUE TO NANOMATERIALS

Undoubtedly, nanomaterials can exhibit unique physical and chemical properties not seen in their bulk counterparts. An important characteristic that distinguishes nanomaterial from bulk is associated with reduction of scale, which results in materials having unique properties arising from their nanoscale dimensions.

The most obvious effect associated with reduction of scale is the much larger specific surface area or surface area per unit mass [7]. An increase in surface area implies the existence of more surface atoms. As surface atoms have fewer neighbors than atoms in bulk, an increase in surface area will result in more atoms having lower coordination and unsatisfied bonds. Such surface atoms are overall less stable than bulk atoms, which means that the surface of nanomaterials is more reactive than their bulk counterparts [8].

Note that increase in specific surface area due to a reduction in size is an example of what is termed as scalable property. Scalable properties are those that can change continuously and smoothly with size, with no size limit associated with a sudden change in the properties. In addition to scalable properties, nanomaterials can also exhibit non-scalable properties; by this we refer to those properties that can change drastically when a certain size limit is reached. In this respect, nanomaterials cannot be simply thought of as another step in miniaturization. An example of non-scalable property is quantum confinement effects [9], which can be exemplified by some nanomaterials such as quantum dots. Quantum dots are semiconducting nanoparticles, for example, PbSe, CdSe, and CdS, with particle sizes usually smaller than ~10 nm [10]. Similar to all semiconductors, quantum dots possess a band gap; a band gap is an energy gap between valence and conduction bands in which electrons cannot occupy. In the corresponding bulk material and when at room temperature, electronic transitions across the band gap are the main mechanism by which semiconducting materials absorb or emit photons. These transitions are excited by photons of specific wavelengths, which correspond to the energy of the band gap and generate an excited electron in the conduction band and a hole in the valence band. Photons can be emitted by the recombination of these electron–hole pairs across the band gap, in which the wavelength and hence color of the emitted light will depend on the size of the gap. If not recombined, the electron–hole pairs exist in a bound state, forming quasiparticles called excitons. In quantum dots, the particle size is usually 2–10 nm, thus approaching Bohr exciton radius. The reduction in size thus results in the quantum confinement effect, in which the edges of the nanoparticle confine the excitons in three dimensions. This has the effect of increasing band-gap energy as the particle is made smaller, causing the previously continuous valence and conduction bands to split into a set of discrete energy levels, similar to those present in atomic orbitals. This is why quantum dots are sometimes called “artificial atoms.” Hence, in quantum dots, band-gap energy can be tuned simply by changing the particle size. The color of the absorbed and emitted light can thus also be varied by altering the size of quantum dots. With such special properties, it is not surprising that quantum dots have applications in LEDs, solar cells, medical imaging and many other fields [11].

Another interesting nonscalable property that can be associated with nanoparticles is localized surface plasmon resonance (LSPR). This can be observed, for example, if we decrease the size of gold [12], small enough to result in a color change from gold color (as in bulk) to a variety of colors. In the bulk form, gold is shiny and reflects yellow light, whereas at 10 nm, gold absorbs green light and appears red. As the particle size increases, red light is absorbed and blue light transmitted, resulting in a pale blue or purple color. This phenomenon can be explained by the fact that the mechanism for generating color is quite different between bulk and nanoscale gold. In bulk, an electronic transition between atomic orbitals (5d and 6s) absorbs blue light, giving gold its yellow color, while the reflectivity is due to the presence of free electrons in the conduction band of the metal. If the size of the gold nanoparticles is reduced, it can restrict the motion of these free electrons, as they will be confined to a smaller region of space, that is, to the nanoparticle. If the particles are small enough, all of the free electrons can oscillate together. When resonance occurs, this leads to a strong absorption of certain frequencies of light that corresponds to the resonant frequency of the electron oscillation. This resonant frequency is highly dependent on the particle size, shape and the medium it is suspended in, for example, 50-nm spherical gold nanoparticles in water gives the suspension a cherry-red color due to the strong absorption of green–blue light. Overall, the LSPR is a phenomenon that occurs due to the collective oscillation of surface electrons with incident light at a specific wavelength. It is worth mentioning that the LSPR phenomenon is different from the quantum mechanical effect as observed in quantum dots, as the mechanism of producing color in metal nanoparticles is different from that in semiconducting ones.

1.3 TERMINOLOGY

1.3.1 Nanomaterials

The term “nano” has long been used as a prefix, as exemplified by nanoliter, nanomanufacturing, nanolithography, nanosystems, and so on. In science and engineering, "nano" refers to one billionth (10^{-9}) of a unit and thus a nanometer being defined as 1 billionth of a meter.

Historically, the word nanomaterial has been used to refer to products derived from nanotechnology. The term nanotechnology itself has been defined as far back as 1974 by Professor Norio Taniguchi, to mean a direct extension of silicon machining down into the regions of smaller than 1 μm [13]. In recent years, several definitions of the term nanomaterial have been proposed by various international organizations and committees (as summarized in Table 1.1), to include International Organization for Standardization (ISO), Comité Européen de Normalisation (CEN), that is, the European Committee for Standardisation, Organisation for Economic Cooperation and Development (OECD), EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), EU Scientific Committee on Consumer Products (SCCP), and American Chemistry Council (ACC) and European Commission (EC).

In addition to those listed in Table 1.1, national authorities and organizations from other countries such as Australia have also provided their own definitions. Although our findings seem to indicate that there are variations in the definition of what constitute a nanomaterial, all definitions have indicated so far an upper dimension limit of 100 nm. However, this is not always the case. The Soil Association, for example, sets this upper limit to be 200 nm, whereas the limit is 300 nm with Friends of the Earth. Unless stated otherwise and to avoid confusion, the book will adopt the

TABLE 1.1 Nanomaterial as Defined by Different Organizations

Source	Definition [14]
ISO TS 80004-1 CEN ISO/TS 27687	"Material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale". Nanoscale here has been defined as "size range from approximately 1 nm to 100 nm".
OECD	"Material which is either a nano-object or is nanostructured." Here, nanoobject is a "material confined in one, two, or three dimensions at the nanoscale. Nanostructured here is defined as having an internal or surface structure at the nanoscale; nanoscale is defined as size range typically between 1 nm and 100 nm".
EU SCENIHR	"Any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less".
EU SCCP	"Material with one or more external dimensions, or an internal structure, on the nanoscale, which could exhibit novel characteristics compared to the same material without nanoscale features. Here nanoscale means having one or more dimensions of the order of 100 nm or less."

(*continued*)

TABLE 1.1 (*Continued*)

Source	Definition [14]
EC: Cosmetic Products Regulation	"An insoluble or biopersistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm."
ACC	"An Engineered Nanomaterial is any intentionally produced material that has a size in 1, 2, or 3- dimensions of typically between 1 – 100 nanometres. It is noted that neither 1 nm nor 100 nm is a 'bright line' and data available for materials outside of this range may be valuable. Buckyballs are also included even though they have a size <1 nm." However, the following are excluded: 1. "Materials that do not have properties that are novel/unique/new compared to the non-nanoscale form of a material of the same composition 2. Materials that is soluble in water or in biologically relevant solvents. Solubility occurs when the material is surrounded by solvent at the molecular level. The rate of dissolution is sufficiently fast that size is not a factor in determining a toxicological endpoint. 3. For those particles that have a particle distribution such that exceeds the 1 – 100 nm range (e.g. 50 – 500 nm) if less than 10% of the distribution falls between 1 – 100 nm it may be considered as non Engineered Nanomaterial. The 10% level may be on a mass or surface area basis, whoever is more inclusive. 4. Micelles and single polymer molecules."
EC	"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. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %. By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials."
EC : for novel foods (amending Regulation (EC) No 258/97), under discussion	"Any intentionally produced material that has one or more dimensions of the order of 100 nm or less or is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic to the nanoscale."

ISO definition as in Table 1.1. ISO has been especially chosen as it operates on an international level and most recognized globally.

In addition to the definition of nanomaterial, there is also a need to differentiate some other similar terms. In particular, nanomaterials and nanoparticles are often used interchangeably, but they are clear differences. According to the ISO definition, nanoparticle is a “nano-object with all three external dimensions in the nanoscale”; nano-object here is a “material with one, two or three external dimensions in the nanoscale.” Nanomaterial, however, is a *material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale*. In both cases, the nanoscale is referred to as a *size range from approximately 1–100 nm* [15]. In this book, the terms nanomaterial and nanoparticle will be differentiated accordingly, in accordance to ISO definitions.

1.3.2 Physicochemical Properties

An important part of nanomaterial research is to identify what the relevant physicochemical properties that one should measure and define the corresponding measurands, that is, the quantity intended to be measured. However, this depends on the scientific field and nanospecific application. In some cases, these have already been defined by the relevant scientific community and are published in standard documents. Let’s consider the field of nanotoxicology. In this community, physicochemical properties of relevance have already been defined, in accordance to published ISO standard document and OECD guidelines [16, 17]. Having two separate guidelines can cause some confusion, and it is wise to read both and make comparison. There are several things worth highlighting when comparing the two:

a) OECD refers to “endpoints,” as opposed to ISO’s “properties.”
b) OECD also has a much longer list of endpoints, that is, 16, compared to ISO’s 8.
c) Some of the OECD’s endpoints can be categorized under the same umbrella as an ISO property. For example, the OECD *particle size distribution – dry and in relevant media* and *representative TEM images*, is similar to ISO *particle size and particle size distribution*. In addition, the ISO *surface chemistry* can potentially encompasses quite a number of OECD endpoints: *surface chemistry* (*where appropriate*), *redox potential*, *radical formation potential*, *photocatalytic activity*, *octanol-water partition coefficient*.
d) Some OECD endpoints have not been taken into account within the ISO document. *Dustiness* and *pour density*, for example, cannot be categorized under any of the ISO properties, even though they are highly relevant in nanotoxicology. In nanotoxicology, the property of *dustiness* is important as it relates to the properties of airborne nanomaterial and thus of relevance in workplace hazard/risk scenarios.
e) The OECD endpoint *representative TEM images* is unusual as this is specific to an analytical technique rather than a physicochemical property. This endpoint can be incorporated under various ISO properties, such as *particle size/size distribution* and *shape*.

Table 1.2 aims to summarize and integrate the information from ISO and OECD guidelines. A limitation of the OECD guideline is that the measurand is less well defined. As a result, the measurands (apart from dustiness and pour density) in Table 1.2 are those that have been defined by ISO [16].

1.4 MEASUREMENT OF GOOD PRACTICE

There is a network of organizations in Europe called Eurachem, whose main mission is to promote best practice in analytical measurement. According to Eurachem, "analytical measurements should be made to satisfy an agreed requirement, that is, to a defined objective and should be made using methods and equipment which have been tested to ensure that they are fit for purpose" [18]. To achieve this, there is a need to understand several key terms such as method validation and standard documents.

1.4.1 Method Validation

The term "*fit for purpose*" implies that the method must be sufficiently reliable and robust [19, 20]. To ensure that a method is fit for purpose, a validation process must take place.

The process of validation may not be straightforward as it is hard to tell when method development ends and validation begins. The two processes can be considered as an interactive process and will not be differentiated here. The first step in method validation is to be clear on the stated objectives for carrying out the analysis and subsequently to establish what the analytical requirements are. The analytical requirements are often related to factors such as specificity, selectivity, accuracy, repeatability/reproducibility, robustness (e.g., not sensitive to operator and day-to-day variability), and analysis time. Other practical issues may also be taken into account such as speed of analysis, costs, technical skill requirements, availability, and laboratory safety. A method can then be developed by choosing the best analytical technique in which parameters such as sample type (matrix) and size, data requirements, for example, qualitative or quantitative, expected level of analytes, and likely interferences, will be taken into account.

As part of the method development step, it is necessary to conduct a literature research to check if suitable methods already exist as existing methods can potentially be used and modified, if necessary. Once a method is developed, it must be refined to demonstrate that it is fit for purpose. Hence, as part of the validation process, an assessment has to be made in order to verify whether the method fulfils the analytical requirements being set, in which round robin studies [21–24] are often carried out. Method validation is not trivial, and sometimes it may be necessary to conduct a prevalidation step to identify any necessary refinements that can be made to the method. Prevalidation study can be conducted among a few established/competent laboratories, preferably with registered/recognized validation authority (RVA), for example, European Centre for the Validation of Alternative Methods (ECVAM). The

TABLE 1.2 Physicochemical Properties of Relevance to Nanotoxicology Community, as Defined by ISO and OECD Guidelines

ISO Properties	Corresponding OECD "end-points"	Measurand; from ISO, Unless Stated Otherwise
Particle size and particle size distribution	Particle size distribution – dry and in relevant media Representative TEM images	"The physical dimensions of a particle and, for collections of particles, the distribution of the sizes of the particles determined by specified measurement conditions" "Equivalent spherical diameter, for particles displaying a regular geometry (unit m); the length of one or several specific aspects of the particle geometry, (unit m) the particle size distribution, the number of peaks and their width are a set of values, often displayed as a histogram, which for each of a number of defined size classes which shows the quantity of particles, being either the number of particles, or the cumulative length, area, or volume of these particles or the signal intensity they produce"
Aggregation/ Agglomeration state in relevant media	Agglomeration/ Aggregation Representative TEM images	Aggregate is "strongly bonded or fused particles where the resulting external specific surface area might be significantly smaller than the sum of known specific surface areas of primary particles". Agglomerate is "collection of weakly or loosely bound particles or aggregates or mixtures of the two in which the resulting external specific surface area is similar to the sum of the specific surface areas of the individual components" "Particle size (unit, m); number of aggregate (or agglomerate) particles in comparison to the total number of primary particles, unit (number/number); number of primary particles in the aggregate (or agglomerate), unit (number/number); distribution of number of primary particles per aggregate (or agglomerate)."

(*continued*)

TABLE 1.2 (*Continued*)

ISO Properties	Corresponding OECD "end-points"	Measurand; from ISO, Unless Stated Otherwise
Shape	Representative TEM images	"A description of the contour or outline of the surface of the nano-objects or collection of nano-objects, aggregates, agglomerates, that make up the material under investigation" "Size-independent descriptors of shape (examples are ratios of extensions in a different direction such as aspect ratio, unit (m/m) or fractal dimension); distribution of values of the size-independent shape descriptors"
Surface area/ mass-specific surface area/ volume-specific surface area	Specific surface area Porosity	This is the "quantity of accessible surface of a sample when exposed to either gaseous or liquid adsorbate phase. Surface area is conventionally expressed as a mass specific surface area or as volume specific area where the total quantity of area has been normalised either to the sample's mass or volume" "Specific surface area is defined as surface area of a substance divided by its mass, unit [m^2/g]; or surface area of a substance divided by its volume, unit [m^2/cm^3]. The research should also consider reporting results in both m^2/g and m^2/cm^3."
Composition	Crystallite size. Crystalline phase.	"Chemical information and crystal structure of the entire sample of nano-objects including: a) composition b) crystalline structure including lattice parameters and space group, and c) impurities, if any" "The number and identity of elements alone or in molecules (can be expressed as a chemical formula with a specific stoichiometry; crystalline state; crystallographic structure; chemical state of atoms/elements; molecular structure-conformation including dextrorotatory and levorotatory (handedness); spatial distribution of the above items."

(*continued*)

TABLE 1.2 (*Continued*)

ISO Properties	Corresponding OECD "end-points"	Measurand; from ISO, Unless Stated Otherwise
Surface chemistry	Surface chemistry, where appropriate Redox potential Radical formation potential Photocatalytic activity Octonal–water partition coefficient, that is, to what degree colloidal suspended particle in the aqueous phase can also be suspended in a nonaqueous phase (such as octanol)	"Chemical nature, including composition, of the outermost layers of the nano-objects and their aggregates and agglomerates greater than 100 nm" "Elemental and molecular abundance unit [mole/mole], including thickness for fixed layers or [number of molecules/surface area] or [number of molecules bound /theoretical number of molecules bound with perfect reaction or perfect packing] for chemically reacted species that do not form a distinct phase; reactivity: standard chemical reaction rate concepts [mole/ (dm^3/s)] preferably of a species of toxicological interest or its surrogate. Measurement of reactivity is very specific to the measurement of the species to which it is reactive (such as reactive to water) and typically involves measuring products or by-products of that reaction."
Surface charge	Zeta-potential (surface charge)	"Electrical charge on a surface in contact with a continuous phase" "Net number of positive and negative charges per unit particle surface area, unit [Coloumb/m^2]; zeta potential, unit [V]"
Solubility/ dispersibility	Water solubility/ Dispersibility	"Solubility is the degree to which a material (the solute) can be dissolved in another material (the solvent) so that a single homogeneous phase results. Dispersibility is the degree to which a particulate material (the dispersed phase) can be uniformly distributed in another material (the dispersing medium or continuous phase) and resulting dispersion remains stable (for example one hour or one minute)"

(*continued*)

TABLE 1.2 (*Continued*)

ISO Properties	Corresponding OECD "end-points"	Measurand; from ISO, Unless Stated Otherwise
		For solubility this is the "maximum mass or concentration of the solute that can be dissolved in a unit mass or volume of the solvent at specified (or standard) temperature and pressure, unit [kg/kg] or [kg/m^3] or mole/mole]". For dispersibility, this is "the maximum mass or concentration of the dispersed phase present in a unit mass of the dispersing medium (solvent) or in a unit volume of the dispersion (solvent plus dispersed phase) at specified (or standard) temperature and pressure, units [kg/kg], [kg/m^3], or [mole/mole].
N/A	Dustiness	OECD definition: This is defined as the "propensity of a material to generate airborne dust during its handling, and provides a basis for estimating the potential health risk due to inhalation exposure". [17] "The measurand of interest is the degree to which a given nanomaterial can remain in the air column before settling. This would require investigation and characterisation of interactions of nanomaterials with other common airborne particulate matter." [17]
N/A	Pour density	OECD definition: This is the "apparent density of a bed of material formed in a container of standard dimensions when a specified amount of the material is introduced without settling". "Determination of bulk density." [17]

purpose of the prevalidation is to assess protocol performance and carry out any subsequent actions needed to refine the protocol. After prevalidation, a formal validation trial with other RVAs or other appropriate sponsors can be carried out.

In nanomaterial research, every effort should be made towards method validation, as only when the conditions of method validation are met, only then a higher metrological standard of measurement, that is, making traceable measurements, can be considered. According to Eurachem/CITAC [21], traceability is *property of the result of a measurement or the value of a standard whereby it can be related to stated references*, *usually national or international standards*, *through an unbroken chain of comparisons*, *all have stated uncertainties*. The traceability framework thus focuses on two main activities: calibration and development of an uncertainty budget. Calibration is defined as the *comparison of an instrument against a reference or standard, to find any errors in the values indicated by the instrument* [25], whereas uncertainty of measurement *is the quantified doubt about the result of a measurement*, which can be established by evaluating the uncertainty budget. This chapter will not delve into the details on how to perform uncertainty budget analysis as this can be found elsewhere [18, 26]. In brief, in order to establish an uncertainty budget, major components contributing to the measurement uncertainty has to be identified and quantified as standard deviations (uncertainties). The contribution of each major component is then statistically combined and the combined uncertainty computed.

In metrology, the ability to make traceable measurements, ideally to the SI units of measurements, is always desirable. However, in some instances, it has to be appreciated that making traceable measurement is difficult and unachievable. In nanotoxicology research, for example, an incomplete traceability chain is likely as calibration is often being carried out under conditions too different from the application.

1.4.2 Standard Documents

A standard document provides “requirements, specifications, guidelines or characteristics that can be used consistently to ensure that materials, products, processes and services are fit for their purpose”[27]. According to BSI 0:2011, standards can aid in “a) facilitating trade, particularly in reducing technical barriers and artificial obstacles to international trade b) providing framework for achieving economies, efficiencies and interoperability c) enhancing consumer protection and confidence and; d) supporting public policy objectives and, where appropriate, offering effective alternatives to regulation”[28]. As such it is not surprising that standard documents on measurement and test methods, specifications, terminology, management, and management systems [29] exist.

So, what can be classified as standard documents?

Standard documents generally fall into one of the following two categories: formal and informal standards. Formal standards are made by official standard organizations, proceeding through government recognized National Standard Bodies (NSBs) at a national, regional or international level. NSBs include British Standards Institute (BSI, founded in 1901), Deutsches Institut fur Normung (DIN, 1917), Schweizerische Normen-Vereinigung (SNV in 1919), Standardiseringen I Sverige (SIS in 1922), Norges Standariseringsforbund (NSF in 1923), Den Danske Standardiserings Kommission (DS in 1926), L’Association francaise de normalisation (AFNOR in 1926), and so on. By the end of the 20th century, the work on regional and international standards became more prominent. In some cases, this had meant that standardization work previously carried out at a national level was transferred to regional, for example, European Committee for Standardization (CEN) or international working groups (WGs) in, for example, ISO [29].

Unlike formal standards, informal standards are published by Standards Development Organizations (SDOs), with some being well known and highly respected, for example, ASTM International (previously the American society for Testing

Materials), IEEE (previously the Institute of Electrical and Electronic Engineers), SAE (Society of Automotive Engineers), SEMI (Semiconductor Equipment and Materials International), TAPPI (formerly the Technical Association of the Pulp and Paper Industry), and OECD. The process to develop an informal standard is the same as those used for formal standards, the only difference being that development and approval is undertaken by members of the SDO rather than through a network of NSBs [30]. Although not technically categorized as "standard" in the real sense of the word, there also exists "private standards," which are developed for internal use, for example, used in companies. Obviously, such standards have less impact and global recognition and often not considered as a viable route.

In addition to the classification of formal versus informal standards, a further sub-classification can be made, on the basis whether the document is considered to be a normative or informative document. Normative documents are "those documents that contain requirements which must be met in order for claims of compliance with the standard to be certified." Informative documents on the other hand, are those that "do not contain any requirements and it therefore not possible for compliance claims to be certified" [30]. Normative means that it's an official formal part of the specification, whereas informative means that it's there to be helpful, for example, aid understanding but cannot be used in formal circumstance, such as appeal to it in a court of law or as part of an audit process.

So, what process is involved in developing a standard document?

In general, the development and publication of a formal document standard is often a long process. The first step involves identification of new work and begins with a proposer, which might be a corporate, public organization, individual, or a consortium [28]. The proposer must then decide if the standard should exist at a national, for example, British, regional, for example, European or at an international level, for example, ISO. The proposer then must demonstrate the need for the standard, that it will be widely/actively supported, that there are enough resources to complete the project in a reasonable time and there no conflict that exists with existing standards [28]. The work of drafting a standard can then be undertaken under by, for example, a suitable Working Group (WG). The members in the WG will then draft document and build a consensus before releasing the draft for comments. The final draft will then be put to a voting period, thus rely on consensus, that is, an agreement between people and organizations that will be affected. After successfully going through a voting period, a final document can then be published as a standard document. As a ball park figure, a national standard can take between 1 and 3 years to produce, whereas international standards usually require the consensus of a more number of participants and therefore usually take longer to publish [28]. Figure 1.1 schematically shows the different stages of standard development/publication and the corresponding relative level of impact associated with the different stages.

Before embarking on any standard development work, it is important that the proposer undertakes a review of existing standards applicable and to identify relevant technical committees and working groups. In relation to nanomaterial characterization, ISO is active in producing standards under Technical Committee on Nanotechnologies, TC229. This committee consists of four working groups and is actively

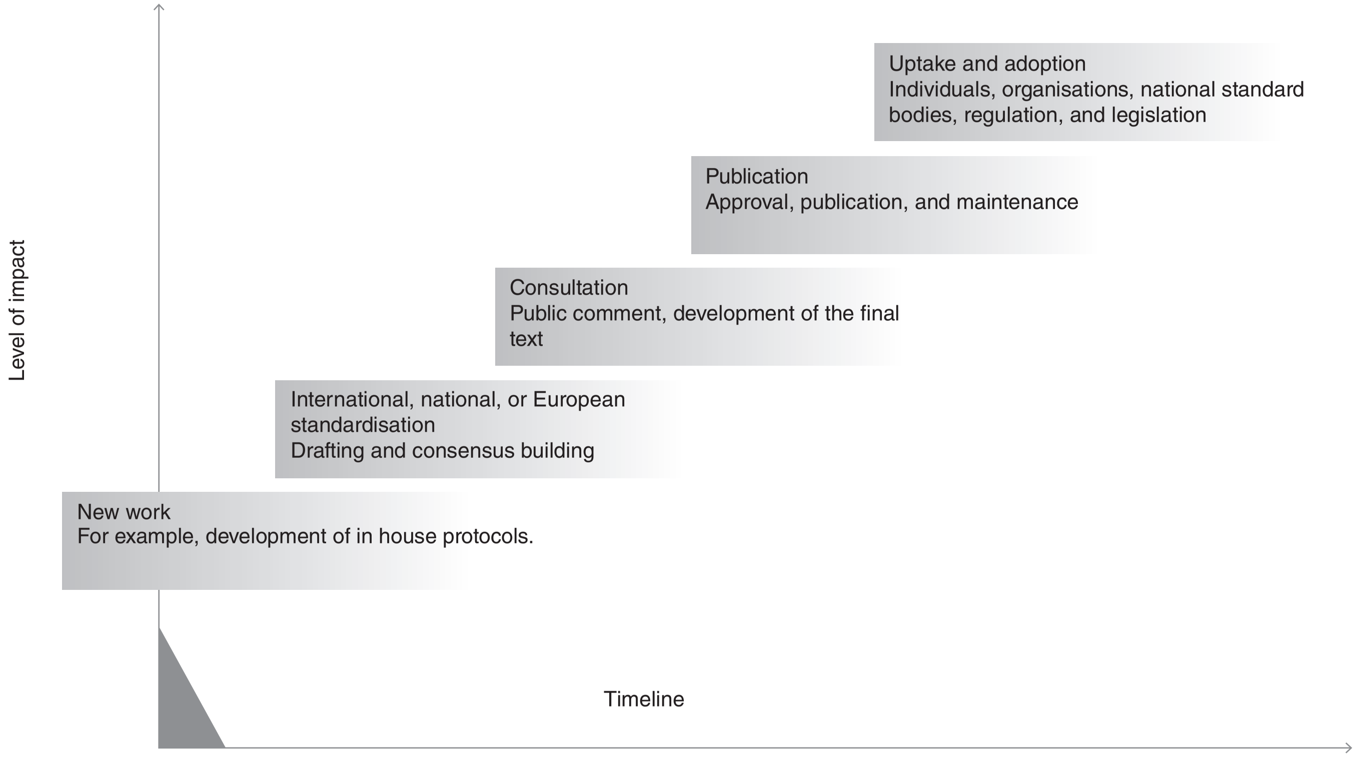

Figure 1.1 Schematic illustration of the process in standards development, publication, and uptake.

publishing on (i) terminology and nomenclature, (ii) measurement and characterization, (iii) health, safety, and the environmental, and (iv) material specifications. To date, the total number of published ISO standards related to this TC is 42. Further details of ISO standards can be found elsewhere [31].

1.5 TYPICAL METHODS

The following sections list two of the most common methods that can be associated with nanomaterial characterization, namely, sampling and dispersion.

1.5.1 Sampling

Sampling is defined as “a procedure whereby a part of a substance, material or product is taken to provide for testing or calibration a representative sample of the whole” [32]. The main purpose of sampling is to collect the entire sample and reduce its size for subsequent analysis. If a representative sample is not obtained through sampling, then this step in an experimental investigation can be a major source of data variation. Errors incurred through the sampling stage can be minimized if proper sampling methods are carried out. There are several ways to minimize sampling errors. First, there is a need for suitable mixing to ensure homogeneity prior to removal of sample aliquots. Second, there is a need to increase the sample size, by taking measurements from large number of sample increments.

When nanomaterial is in the form of dispersion, achieving reliable sampling is potentially straightforward. For example, the sampling step may involve sufficient agitation of the dispersion before an aliquot is extracted by using a pipette. In relation to powder material, the process of sampling is more challenging, as sampling will be more prone to segregation error. This can arise when particles are exposed to gravitational, rotational, vibratory/aeration operations, or other types of mechanical motion, resulting in fine particles to migrate to the bottom and larger particles being concentrated at the top [32]. Segregation error is more problematic with free or easily flowing powders and those having a significant range of particle size distribution.

In relation to powder sampling, there is a need to carefully consider the different options of sampling. The five common sampling methods are scoop sampler, cone and quartering, table sampler, chute riffler, and spinning riffler (also called rotary sample divider) [33]. Table 1.3 gives an overview of the sampling methods, along with their inherent limitations.

Allen and Khan [32] have evaluated the different sampling methods shown in Table 1.3, in which the performance of each method was assessed by sampling a known particle size distribution of sand mixtures. Their findings show that out of all the methods listed in Table 1.3, the spinning riffler is the most reliable method for sampling, as this method incorporates little operator bias. In addition to reliability, the spinning riffler is also most practical, especially when dealing with kilograms of materials. However, there is one prerequisite that must be fulfilled when using the riffler, in that powder must be free-flowing in nature.

TABLE 1.3 A Comparison of Powder Sample Reduction Methods

Sampling Device	Description	Limitations
Scoop sampler	This method is the simplest for sample division and involves an operator, using a scoop, to extract laboratory samples from some portion of the bulk sample. This method is reliable for samples that are homogeneous and exhibit poor flow characteristics	All of the bulk material do not go through sampling process. Operator dependent, for example, operator decides where to scoop and what quantity to extract. Sampling more likely to be atypical due to segregation of the material. Not suitable for heterogeneous sample
Cone and quartering	This technique involves placing the sample on a flat surface in the form of a conical heap. The heap is then spread out and flattened into a circular cake, which is then divided into approximately equal quarters. One pair of opposite quarters is removed, combined and formed into a new cone for the process to be repeated (with the other two quarters discarded). The process is repeated as many times as is necessary to obtain a sample of the required size. Good for powders with poor flow characteristics and minimal segregation	Operator dependent; errors can occur due to differences in the manner the heap is formed and sub-divided

(*continued*)

TABLE 1.3 (*Continued*)

Sampling Device	Description	Limitations
Table sampler	This utilizes tilted surface (in which there is a series of holes and splitting prisms) over which a powder sample is allowed to slide. The prism breaks the stream into fractions and some of the powder will fall through the holes and then discarded. Ultimately, at the bottom of the plane, a decreased quantity of sample is collected. The method has the ability to separate large quantity of material	It is necessary that the incoming powder is uniform and consistent. Hence, the method is dependent on the initial feed being uniformly distributed, with a complete mixing after each separation; this is a condition not general achieved
Chute riffler	This utilizes chutes, that is, funneling or channeling device, to divide the powder. Unlike the spinning riffler, the chute riffler has no moving parts. It has the ability to separate large quantity of material and to reduce powder samples in half after one pass	The technique is subject to error and operator bias if segregation is allowed to occur in loading the bulk-sampling through
Spinning riffler (also called rotary sample divider)	This utilizes a series of smaller receivers (or collection tubes), mounted in such a way so as to collect a flowing powder stream over a very short time period. The powder flows from a “hopper” to a “vibratory chute” and then to a “receiver” that holds the containers, which are rotating in a circular motion at a constant speed. The method is able to separate large quantity of material. Spinning rifflers are available in different sizes, in which common commercial systems can provide samples ranging from about 0.5–300 g [34]. It has the ability to do large quantity of powder efficiently	Limited to free-flowing powder only

If a spinning riffler is to be used for nanomaterials, then it must be validated for different nanomaterials in accordance to ISO 14488 guideline [35]. This ISO standard document makes recommendations on how to choose and use a riffler. It also highlights the importance of validating the instrument for each new material to be riffled. According to the document, the simplest way to validate is by mass validation, which consists of several steps. First, there is a need to measure the mass of the gross sample together with the masses of all the increments; this is to be repeated three times. Then, there is a need to calculate the mean loss of the material. If the mean loss of material is larger than 1%, then the riffler is either not working properly or that the riffler is not a suitable method.

1.5.2 Dispersion

Dispersion of powder nanomaterial into a liquid matrix is another common practice in nanomaterial research [36] and basically involves three main stages:

a) Wetting of the nanomaterial powder. The purpose of this step is to substitute solid–air interface with solid–liquid interface, such that the particles are sufficiently "wetted." The efficiency of wetting will depend on the comparative surface tension properties of the nanomaterial and the liquid media, as well as the viscosity of the resultant mix. The wetting step can easily be achieved by mixing the powder with several drops of liquid media, to form a thick paste. In the case where a powerful ultrasonic probe is used, wetting may occur simultaneously during sonication step.
b) De-agglomeration of the nanomaterial, using a de-agglomeration tool. Here, sufficient shear energy is needed to break up loosely bound agglomerates in the powdered nanomaterial. There are various de-agglomeration tools that exist on the market to include mills (ball, stirred media, centrifugal, and jet mills), stirring (magnetic or overhead stirring), high-speed homogenizer, high pressure homogenizer, ultrasound sonicating bath, and ultrasound probe sonication or ultrasonic disruptor [37].
c) Stabilization of the dispersion. Stability can be impacted by the choice of the liquid dispersant and things added to the dispersant, for example, surfactant that can lead to marked changes in its interfacial properties [38].

To date, no standard guidelines exist that details how to disperse powdered nanomaterials into a liquid matrix. In some science areas such as nanotoxicology, the need to produce stable and reproducible dispersions is important for the purpose of data reliability and comparability. Recent works by Tantra et al. [39] have shown how huge data variability can arise from using different dispersion protocols. Their findings highlighted the importance of controlling the dispersion step, as factors such as particle concentration, sonicating time, can all influence dispersion quality.

1.6 POTENTIAL ERRORS DUE TO CHOSEN METHODS

Characterizing the property of nanomaterial (with current instrumentations) may not be straightforward. With some nanomaterial samples, getting reliable data is not easy to achieve and can lead to a situation in which experimental data can get reported without proper understanding of the associated errors and propagation of such errors. Sources of experimental errors may arise from a number of factors, to include polydispersity of the nanomaterial and the difficulty to measure such highly polydisperse samples.

Baalousha and Lead [40] have highlighted that most of the nanomaterials tested in nanotoxicology are far too polydisperse and that materials close to monodispersity are needed. The main issue with having a highly polydisperse sample is the lack of analytical techniques that can measure the corresponding properties accurately. Anderson et al. [41] show most routine methods can characterize particle size distributions of monomodal distribution. However, if the particle distribution is away from the ideal, then errors can be incurred during the measurements. A typical example to highlight this point is light-scattering-based methods such as Particle Tracking Analysis and Dynamic Light Scattering. Both techniques have been shown to mainly reliably detect a single population of particles corresponding either the largest or smallest particles in a multimodal sample. Clearly, the inadequacy of the instrumental methods to characterize highly polydisperse nanomaterials can pose a barrier to reliable measurement [42].

In addition to issues associated with polydispersity, nanomaterials dispersed in complex, for example, biological, environmental matrix, which contains other interferents, can also pose problems where measurement is concerned. Hence, an instrument with high selectivity and sensitivity may be needed. Apart from the presence of interferents, nanomaterial–media interactions can be dynamic in nature and in the example of nanotoxicology research, the physicochemical properties measured at a given time may not be directly associated with observed biological effects. Nanomaterials dispersed in complex medium may also be unstable, potentially resulting in agglomeration and sedimentation, which may pose further difficulties for the instrument to measure the sample under such conditions. Due to the analytical challenges posed in nanotoxicology, it is difficult to reliably assess the potential transformation of nanomaterials in an environmental or mammalian system [43].

1.7 SUMMARY

The potential benefits of nanomaterials to society and economy are clear and, as such, much research on nanomaterial is currently being conducted, covering a wide range of disciplines. This introductory chapter is a good starting point for readers, to get to grips with some of the key topics, to include terminology, measurement of good practice, issues/challenges, and so on. An important point to highlight is for researchers to choose the right methods and the need to validate such methods for their nanospecific applications. Researchers are encouraged to give careful thought in identifying potential sources of error associated with their measurements, which will lead to improved experimental design and methods employed during an investigation.

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