

Chapter 1

Engineered Nanomaterials: Their Physicochemical Characteristics and How to Measure Them

Rambabu Atluri and Keld Alstrup Jensen

Abstract Numerous types of engineered nanomaterials (ENMs) are commercially available and developments move towards producing more advanced nanomaterials with tailored properties. Such advanced nanomaterials may include chemically doped or modified derivatives with specific surface chemistries; also called higher generation or multiconstituent nanomaterials. To fully enjoy the benefits of nanomaterials, appropriate characterisation of ENMs is necessary for many aspects of their production, use, testing and reporting to regulatory bodies. This chapter introduces both structural and textural properties of nanomaterials with a focus on demonstrating the information that can be achieved by analysis of primary physicochemical characteristics and how such information is critical to understand or assess the possible toxicity of engineered nanomaterials. Many of characterization methods are very specific to obtain particular characteristics and therefore the most widely used techniques are explained and demonstrated.

Keywords Nanomaterials • Nanoparticles • Nanostructures • Physico-Chemical Characterization • Properties • Microscopy • Spectroscopy • Specific Surface Area • Functionalization

1.1 Introduction

In today's world, there has been a change in the comfort of human life in many ways ranging from smart and light-weight materials, technologically advanced buildings with self- or easy-to-clean coatings, small communication systems, functional foods, advanced medication system and light-weight and/or high-speed transport systems. This progress is not only linked to the development of specialty materials

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but also the methods for their characterization and analysis. Invention of new materials leads to categorize materials research into different branches such as nanoscience and nanotechnology i.e. fundamental principles of molecules, structures at 1–100 nm and their application of these structures into useful nanoscale devices and functional products.

Looking into the history of material development, nanomaterials as such are not new. They have been in use since B.C and A. D. An iconic example of it is Lycurgus cup, which exhibits the most remarkable characteristic of color diffraction between green to red under different lighting conditions [16]. This is due to the suspension of tiny gold (nano) particles within the glass matrix, whose diameters are comparable to the wavelength of visible light. The quantity of these particles was quite low and even the glass makers did not know these features. If the glass manufacturers had known the explanations for the color changes and a way to investigate the properties, nano-products we might have reached beyond the nanotechnology stage by the twenty-first Century [7]. Lack of instrumentation and methods to study the internal, external and structural properties at nanoscale levels have led a little progress until late 1930s, where commercial electron microscopes were established. At the same time spectroscopic methods became available beginning with the discovery of X-ray diffraction by Max von Laue and his colleagues in 1912 [14], which enabled determination of nano- to atomic scale structures in materials. In later years development of quantum science and computing has added speed by which material innovation may occur.

Today a large number of nanomaterials exist and are still developed as needs and ideas arise for new applications and new properties. Developments especially occur in the development of advanced nanomaterials with tailored properties. Such nanomaterials include nanoparticles which have been chemically doped or modified to have a specific tailored surface chemistry. Therefore particulate nanomaterials (nano-objects in ISO terminology) are grouped into mono and multi-constituent nanomaterials (ISO/TR 11360:2010(E)). Mono-constituent nanomaterials are also referred to as being first order nanomaterials whereas multiconstituent nanomaterials are second, third generation materials etc., which describes the presence of one or two to the n 'th additional compounds enclosed or coating the nano-object considered the core or skeleton of the nanomaterial. Clearly this plurality of possible structural and compositional combinations can require the combination of several techniques to characterize a nanomaterial.

In this chapter, we will focus on demonstrating the information that can be achieved by analysis of primary physicochemical characteristics of nanomaterials and how such information can be used to identify and understand the nanostructures within nanomaterials. A range of methods used in the analysis of nanomaterial characteristics is quite large. Many of these methods are very specific for particular characteristics and therefore most widely used techniques are explained. Compared to bulk materials, nanomaterials need one or more analysis, sometime a specific technique to clearly understand the properties and applications associated with it. Moreover, it is described how such information is key to understand or assess the possible toxicity of engineered nanomaterials.

1.2 Types of NM and Physicochemical Properties to Measure

In principle nanomaterials can be produced for all non-gaseous elements in the entire periodic system. Some nanomaterials are produced in very large amounts (e.g., carbon black, SiO₂, TiO₂, ZnO, Fe-oxide, carbon nanotubes, and nanoclays) whereas others, but still technologically important materials (e.g., Ag, Ce-oxides, quantum dots such as CdSe, and nanoporous materials) are produced in much smaller quantities. Nanomaterials, such as nano-cellulose and graphene, also have high industrial potential and currently have very high focus [10, 24–26, 47]. A recent analysis of nanomaterial producers in the world showed 22 materials produced by up to 300 manufacturers (Table 1.1; [3]). However, a world list of nanomaterials in production covers a much wider range without including considerations of chemical derivatives achieved by e.g., surface chemical modifications and also considering variations in dimensions and structural variations, which is important for e.g., carbon nanotubes. Future Markets estimates the 2010 worldwide production of nanomaterials was 21,713 tons, a tenfold increase from 2002 and is estimated to more than double to 44,267 tons by 2016, driven by demand from applications in electronics, energy, medicine, chemicals, coatings and catalysts.

As for all other particulate materials, there are a number of physico-chemical characterization end-points define the different nanomaterials. The characteristics and properties to be assessed depend strongly on chemical type and chemical-structural complexity of the nanomaterials and on the purpose. One may discriminate between the end-points required to identify the material versus the end-points required to characterize its dispersibility into a given matrix versus characterization required for chemicals registration and finally for full risk assessment [22, 49]. The characterization can be divided into characterization of primary physicochemical properties and secondary properties, which describe the state, reactivity and fate of the nanomaterial during and after release and/or exposure. The primary character-

Table 1.1 List of nanomaterials in production according to Future Markets (2014)

Carbon based	Metal oxides and Metalloid oxides	Metals, Salts	organics	Others
Single Wall CNTs	Silicon dioxide	Gold	Dendrimers	Nanoclays
Double wall CNTs	Titanium dioxide	Nickel	Nano-cellulose	Tungsten carbide
Multi Wall CNTs	Aluminium oxide	Silver	Organic dyes	
	Antimony tin oxide	Palladium	Organic pigments	
	Bismuth oxide	Quantum dots	Polymers	
	Cerium oxide			
Fullerenes	Cobalt oxide			
Graphene	Copper oxide			
Nano-carbon black	Iron oxide			
	Magnesium oxide			
	Manganese oxide			
	Yttrium oxide			
	Zinc oxide			
	Zirconium oxide			

ization end-points including average size/size-distribution including agglomeration/aggregation state, shape/morphology, surface area and porosity, atomic structure, chemical composition, surface chemistry and a long list of available analytical techniques were listed as candidates to enable such data. We refer to the chapters by Zuin et al. [49] and Jensen et al. [22] and additional references [1, 2] for more detailed information on these end-points and characterization techniques.

1.3 Physicochemical Characterization to Identify Nanomaterials

1.3.1 Chemical and Structural Properties

The chemical composition and structure of nanomaterials, similar to regular materials, is the key to identify and group the nanomaterial into its material class. A combination of chemical and structural information is particularly important if different generations of nanomaterials should be identified as proposed in Atluri and Jensen [5].

1.3.1.1 Composition

The chemical composition is the key to classifying various nanomaterials. Groups could be ceramic, metallic, semi-metallic/semi-conducting, polymers, carbon-based, and organic/inorganic as proposed in ISO/TR 11360 (2010), but more detailed classification is most likely needed for both material and regulatory purposes and already proposed for carbon allotropes (e.g., [18]) and in more detailed as exemplified for fullerenes [12].

The chemical composition of a nanomaterial refers to entities of which the material is composed. The function of nanomaterials is influenced by the chemical composition and hence different physical, chemical, mechanical and biological properties. The observed toxicity of nanomaterials often linked to their composition in the form of coating, and impurities. For example, it has been shown that CNTs show considerable toxicity especially because of catalyst metal contaminants such as the metal oxides, introduced during production and purification process. The toxicity of CNTs is due to the release of metal contaminants and their ability to cross the cell membrane [35]. Others demonstrated that CNTs containing different surface charge and modification, size, and length, could influence the potential toxicity. Especially the presence of individual separated stiff fibrils or fibers are considered one of the key characteristics leading to severe pulmonary toxicological effects [13, 23, 33]. Understanding the effects of CNTs on the biocompatibility, toxicity, and risk assessment may sometimes lead to conflicting results and hard to predict where the toxicity comes from [30]. In addition, there is no consistency of constituent species and amounts of metal impurities in CNTs made from the same

process but of different batches and are quite different in materials obtained from different vendors, which makes it more complex to generalize the toxicity of CNTs. Table 1.2 shows chemical compositions of different Multiwall-CNTs as derived by Wave-Dispersive X-Ray fluorescence spectroscopy (WDXRF). All the materials were purchased from different vendors in the form of pristine and surface functionalized CNT. Irrespective of their group, the purity of MWCTNs as pure carbon ranges between 86 and 97 % and a wide range of metal oxides as impurities.

The chemical composition can be analyzed using a range of methods. Depending on the method used, the elemental analysis will range from qualitative to quantitative analysis. The most common characterisation methods for analyzing the chemical composition of nanomaterials are X-Ray fluorescence spectroscopy (XRF), Energy Dispersive X-Ray Spectroscopy (EDS/EDS), Electron Energy Loss Spectroscopy (EELS), X-Ray photoelectron spectroscopy (XPS), Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Nuclear Magnetic Resonance (NMR), Raman Spectroscopy, and Static secondary Ion Mass Spectrometry (SIMS). However, all the methods are not equally suitable for quantification of the material chemical composition. For example variation depending on extraction procedures may be important for indirect methods such as ICP-MS.

1.3.1.2 Crystalline Phases

Identification of the atomic structure of materials has been essential for their identification for several decades and is the whole foundation for modern mineralogy and materials science. It is also evident from the current toxicological literature that the toxicological effects of engineered nanomaterials can vary considerably depending on the structural properties [27]. In particular, the biological responses of many nanoparticles largely depend on the crystal phases but of similar in their composition. For example, the composition of silica is *stoichiometrically* similar; various forms of silica differ in their physicochemical and toxicological properties. It exists in crystalline and amorphous state with long and short range order, respectively. It is well known that inhalation of crystalline silica shown to be serious adverse effects among workers in the form of increased lung cancer and has been classified as a human lung carcinogen with important differences between quartz [trigonal (α -quartz) or hexagonal (β -quartz)] and the less abundant tridymite [orthorhombic (α -tridymite) or hexagonal (β -tridymite)] polymorphs. However, synthetic amorphous silica (SAS) has so far not shown any adverse effects because of their amorphous state [29]. Similar distinctions between rutile and anatase phases of titanium oxide can be made [42].

The powder X-ray diffraction technique is a fundamental technique for the identification of crystalline phase of nanomaterials. When an incident beam of X-rays (a form of electromagnetic radiation with a wavelength of 1\AA) interacts with a target sample, the waves are scattered from lattice planes separated by an interplanar distance d . The scattered waves interfere constructively, and the path difference between two waves undergoing constructive interference is given by $2d\sin\theta$, where

Table 1.2 Chemical composition of various carbon nanotubes measured by WDXRF from [19]

MWCNT group	MWCNT code	Type	C	Al ₂ O ₃	CaO	CoO	Fe ₂ O ₃	La ₂ O ₃	MgO	MnO	NiO	P ₂ O ₅	SO ₃
Standard materials	NRCWE-026	Pristine	84.4	14.97	–	0.11	0.29	–	–	–	–	–	–
	NM-401	Pristine	99.7	–	–	–	–	–	–	–	–	0.14	–
	NM-402	Pristine	96.1	2.43	–	–	1.31	–	–	–	–	0.16	–
	NM-403	Pristine	99.1	0.24	–	0.12	–	–	0.19	0.16	–	0.14	–
	NRCWE-006	Pristine	99.6	–	–	–	–	–	–	–	–	0.14	–
Group I	NRCWE-040	Pristine	98.6	–	–	–	0.20	0.32	–	–	0.56	0.15	–
	NRCWE-041	OH	99.2	–	0.13	–	0.13	–	–	–	0.31	0.15	–
	NRCWE-042	COOH	99.2	–	0.25	–	–	–	–	–	0.21	0.14	–
Group II	NRCWE-043	Pristine	98.5	–	–	–	–	–	–	–	1.20	0.15	–
	NRCWE-044	OH	98.6	–	–	–	–	–	–	–	1.04	0.14	–
	NRCWE-045	COOH	96.3	0.52	–	0.25	1.17	–	–	–	1.34	0.16	–
Group III	NRCWE-046	Pristine	98.7	0.29	–	0.25	–	–	0.22	0.30	–	0.14	–
	NRCWE-047	OH	98.7	0.27	–	0.25	–	–	0.22	0.30	–	0.15	–
	NRCWE-048	COOH	98.8	0.26	–	0.24	–	–	0.19	0.28	–	0.14	–
Reference	NRCWE-049	NH ₂	98.8	0.26	–	0.25	–	–	0.19	0.29	–	0.15	–
	Printex 90	–	99.3	–	–	–	–	–	–	–	–	–	0.66

To ease interpretation, all data were reported in wt % of the oxides of the elements determined and their content below 0.1 % is not reported

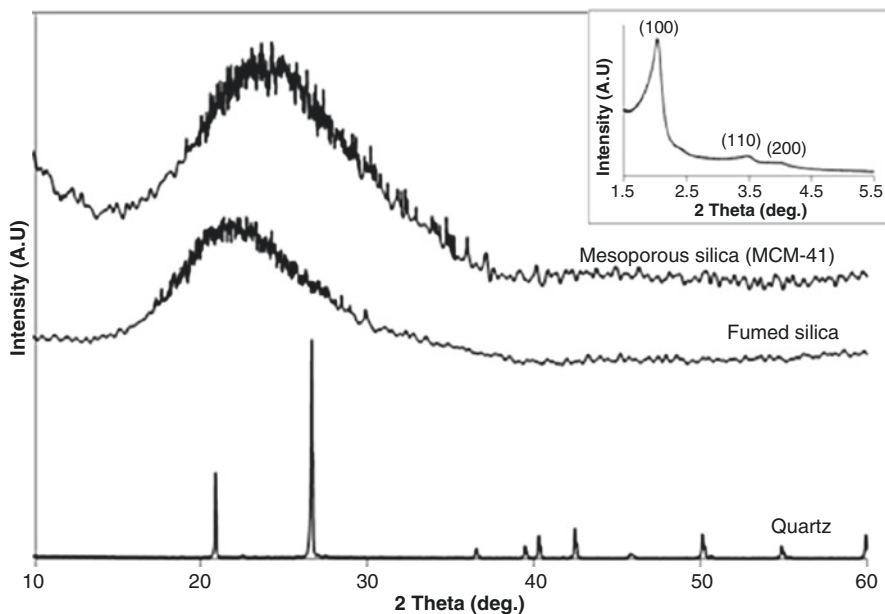


Fig. 1.1 Powder X-Ray diffraction (XRD) graphs of quartz, fumed silica, and mesoporous silica (Inset: shows low angle peaks of mesoporous silica)

θ is the scattering angle. The intensity of the scattering wave as a function of scattering angle gives a diffraction pattern. Both the positions and the relative intensities of the diffraction peaks are indicative of a particular structure, such as cubic, hexagonal and help to determine crystal symmetry to structure determination. One possible limitation of XRD while using for nanomaterials is their peak-broadening at lower scale where nanomaterials become amorphous structure, but may still be crystalline [34]. Though, the size limitation is more of a material specific and indeed depends on the accuracy of diffractometer, users must be aware of the problem while measuring nanomaterials.

Figure 1.1 shows the X-Ray diffraction patterns of different silica materials such as quartz (Sigma-Aldrich), Mesoporous Silica (MCM-41, [8]) and fumed silica (Sigma-Aldrich). Between 20 and 30° 2θ , the amorphous nature of silica is indicated by a single broad peak, for both MCM-41 and fumed silica. Due to ordered pore structure, mesoporous silica shows (Fig. 1.1 inset) three peaks (100, 110 and 200) between 2θ angles of 1.5 – 6° , which are consistent with 2 dimensional (2D) hexagonal cylindrical porous network with space group symmetry, $p6mm$ [6]. On the other hand, the crystallinity of quartz results in a range of diffraction peaks, consistent with hexagonal crystal system with a space group, $P3_121$.

Compared to laboratory X-Rays, the synchrotron radiation sources give more intense and higher energy radiation with very shorter angles of scattering <0.10 . In addition, an in-situ Small-angle X-ray scattering (SAXS) pattern can be recorded in a short time while the XRD pattern needs at least a couple of minutes for acceptable

data. The structural growth information from the nucleation stage during the nanoparticle synthesis can thus be accessible with the SAXS technique. SAXS is also an X-ray diffraction-based technique, where synchrotron radiation is used as a source, which occurs when charged particles are accelerated in a curved path or orbit. Any charged particle which moves in a curved path or is accelerated along a linear path will emit electromagnetic radiation. When the wavelength of the electromagnetic radiation is of the same order as the length of a sample particle, the particle will scatter the radiation. Detection and analysis of this scattering pattern can yield valuable information about the size, shape, and internal structure of the particle.

Both XRD and SAXS only give a diffraction pattern for further interpretation of the crystal phases of nanoparticles. However, microscopy methods such as AFM, SEM, and TEM give 2-dimensional images of the nanoparticles at the atomic scale. In particular, direct imaging by TEM images gives not only the amplitude but also the phase information of the structure factors of the crystal. The indexed Fast Fourier Transform (FFTs) of the TEM images can be further used to refine the lattice parameters and thereby the crystal symmetry. TEM information is obtained from a single crystal (~ 108 times smaller than the size of a specimen for XRD) whereas XRD data is from the bulk material. The importance of TEM imaging for structural investigation is best explained, for instance, in refining the symmetry of mesoporous silicas and their pore connectivity in the amorphous silica network [4, 6]. Typical TEM images recorded along the $[1\ 0\ 0]$, $[1\ 1\ 0]$ and $[1\ 1\ 1]$ direction and corresponding FFT-diffractograms of all the samples are shown in Fig. 1.2. Crystallographic reconstruction procedure and image processing of the images gives 3D-electrostatic potential density model as shown in Fig. 1.2d. The reflection conditions derived from the TEM images and their corresponding FFT-diffractograms, confirms the space group symmetry of mesoporous silica as $Pm\bar{3}n$. The pore structures of a sample may be visualized from the 3D-electrostatic potential maps reconstructed from the structure factors obtained from electron crystallography. The boundary between pore and the pore-wall is determined by the so-called threshold value (related to the pore volume fraction), derived from the mesopores volume and the silica wall density (2.2 g/cm^3). Overall, HRTEM combined with electron crystallography gives not only the 2D images of nanomaterials at atomic resolution but also their tomographic view at macro-scale.

This type of analysis can be used to describe the nanostructures as well as location of doped or nature of nanomaterial cores, which is important for identification of nanomaterial class and nanomaterial generations if not known beforehand.

1.3.1.3 Surface Modification/Functionalization

Surface treatment or surface modification or functionalization or doping of nanomaterials induces distinct chemical and physical properties compared to their pristine form. For example, the surface treatment of silica with methyl groups is an effective way to disperse the silica nanoparticles in a wide range of organic

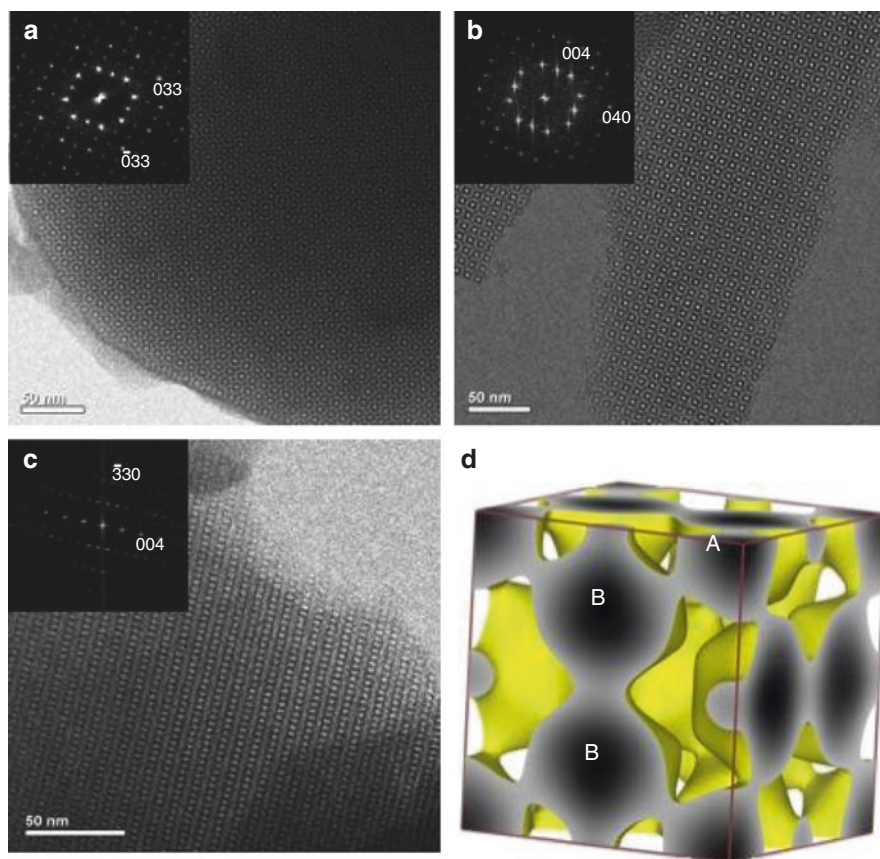


Fig. 1.2 HRTEM images of mesoporous material SBA-1 type, viewed parallel to the [100] (a), [111] (b) and [110] (c) directions. Inset shows FT diffractograms recorded from regions of the particles shown. (d) The 3D-electrostatic potential map of mesoporous silica showing two types of cavities (A, B) with open cavity-connecting windows to the neighboring cavities [6]¹

solvents. Functionalization has been used to conjugate drug molecules, polymers and organic groups to NPs. It has been demonstrated that non-covalent attachment of polyethyleneimine (PEI) polymers to the silica surface not only increases cellular uptake but also generates a cationic surface to which DNA and siRNA constructs could be attached [43]. In another case, functionalization has also been shown to protect NPs against agglomeration and render them compatible in other phases. Silica coating on semiconductor materials such as CdS nanoparticles

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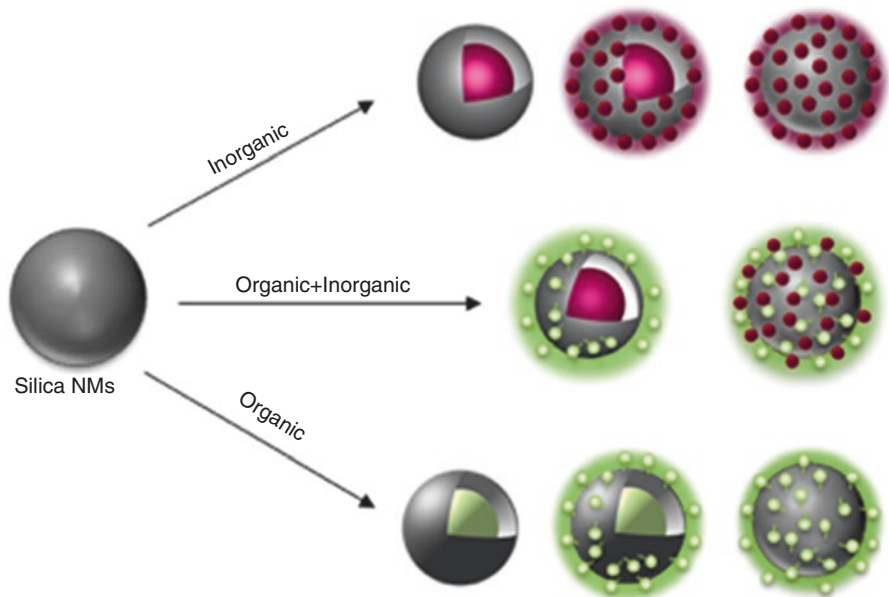


Fig. 1.3 Schematic illustration of several derivations after surface modification/functionalization of silica nanoparticles. *Green*: organic, *Plum*: Inorganic, *Light Black*: Silica

improves the stability of the particles as well as prevents coagulation during the chemical or electronic processing [20, 38, 40, 44, 45]. Therefore, surface functionalization of NPs is rather necessary to render specific functionality over the core NPs. Similar surface-chemical modification of carbon-based materials such as fullerenes and CNTs are usually considered essential for dispersion and any application in matrix nanocomposites [18].

In general, surface modification of nanoparticles can be achieved by chemical and physical modifications. Most often a combination of both methods being used for higher generation of engineered nanomaterials. The most frequently used and industrially relevant method for nanocomposites based on polymers/nanoparticles includes solution belding or melt blending [48]. Depending on the function and application, various surface treating agents such as organic and/or inorganic in the form of elements, compounds and materials can be used to modify the surfaces of nanoparticles. The complexity of the nanoparticles derived by their chemical doping and surface modifications, which depends on the extent and location of the surface treating agents. Complexity is the location of the compounds either external (E) or as a core (C) of nanoparticle matrix or combination of both, thanks to the chemistry of nanomaterials for merging many phases into a single but multifunctional system. Figure 1.3 shows possible derivations of silica nanoparticles after modification; mostly rely on chemical and physical or combinations of both the methods. Apart from organic compounds for surface treatment, various inorganic nanoparticles used to construct hybrid architectures of silica including nanoparticles assembled at the surface of a

silica particle, encapsulation of nanoparticles in a silica shell etc. Several physico-chemical characterization methods can be used to determine the chemical complexity of doping and surface chemical modification of nanomaterials both quantitatively or qualitatively, including electron microscopy, XRF, XPS, molecular spectroscopy such as FT-IR and X-Ray, Raman, and mass spectrometry (GC/LC/ICP). In addition, Thermal Gravimetric Analysis (TGA) is the most widely used and relatively easy method for quantitative analysis of surface modifications.

1.3.2 Textural Properties

1.3.2.1 Size and Size Distribution

Materials exist in different forms and size depending on the source and manufacturing process. Size and size-distribution is the key characteristic of nanomaterials and key for their identification for regulatory purposes [17, 21]. Nature has a large number of nano-sized materials with properties that are distinct and relatively comparable to man-made nanomaterials. The carbon based nanoparticles such as Buckminsterfullerene and graphene are typical examples of it.

As nanomaterials (NMs) are generally defined by having small dimensions in the nanoscale, i.e. between ca. 1 and 100 nm and have large surface to volume ratios, their physicochemical properties are different from the properties known for bulk materials [36]. As the size of a particle decreases, the proportion of atoms on the surface of the particle increase and, consequently, the physicochemical properties will be different from the properties known for non-nanomaterials.

Therefore, significant efforts have been put forward by different government bodies and policy makers to define the size limits of a nano-object. According to the EU recommendation, "*Nanomaterial' means 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 1–100 nm*".

Particle size measurements have a direct impact on the reliability of products containing nanoparticles and provide a basis for toxicological studies of nanoparticles. However, selecting right particle size methods have a great impact on the reliability of the size and size distribution. Different methods (Table 1.3) often have different bases for the particle size and size distribution, for example dry samples vs. wet sample dispersions. In addition, the principles of methods such as light diffraction or electron microscopy should also be taken into account for a correct interpretation of the measurements. The quality of measurements also depends on the different screening criteria such as size measurements for spheroidal and non-spheroidal particles, agglomerates, and aggregate particles and size measurements for environmental, health and safety evaluations. Currently, methods are rather advanced for quantitative size-distribution analysis of granular nanomaterials (see e.g., [11, 31]).

Table 1.3 Table shows different particle size methods and typical size ranges

Method	Typical measurement range	Type of size distribution	Available standards
SEM	1 nm–10 μm^{a}	Number based	ISO 16700: 2004
TEM	0.5 nm–1 μm^{a}	Number based	ISO 13322–1: 2004 ISO 13322–2: 2006
SAXS	>5 nm	Scattering intensity based	ISO/TS 13762:2001
AFM	>1 nm	Number based	ASTM E2859–11
XRD	<100 nm	Scattering intensity based	ISO 20203: 2005
Centrifugal liquid sedimentation	>20 nm	Extension Intensity based	ISO 13318–3: 2004 ISO 13318–2: 2007
DLS	1 nm – several micro meters ^b	Number and volume based	ISO 22412:2008
Particle tracking analysis	>25 nm	Number based	ISO/DIS 19430
Field flow fractionation	1 nm–200 nm	Intensity based	–

^aSome instruments have lower detection limits

^bRanges may be wider and vary considerably with instruments and software

1.3.2.2 Aggregation/Agglomeration

Due to high surface energies, nanoparticles have tendency to form agglomerates/aggregates. As summarized in Schneider and Jensen (2009) [50], the agglomeration of nano-objects in nanomaterials can be due to different phenomena, which can be grouped into: electrical properties (e.g., van der waal forces), magnetic properties (e.g., ferromagnetism), physical interlock (e.g., entanglement), and bridging (liquid film or greasy coatings). Some of these agglomeration phenomena have weak forces and require moderate energy for dispersion, whereas others have large effect or binding forces and require dedicated efforts to disperse the nano-objects. Aggregates consist of nano-objects bound together with significant interface contacts and high binding energies. Aggregates can therefore not be separated without breaking the material.

Agglomerates or aggregates make it especially difficult to explore the properties and applications of nanomaterials. In reality the nano-objects in nanomaterials rarely exist as separate units unless this is a particular target in the manufacturing process and in many cases the particles are not in uniform shape, particularly when the materials are scaled up. Particles do agglomerate and aggregate and have different size ranges depending on the use and the environment. In many synthetic processes for nanoparticles, especially surfactant-free chemical reactions, aggregation or agglomeration occurs immediately as particles are generated. The terms agglomeration and aggregation are still rather confusing for defining the particle state.

If the properties associated with the aggregation or agglomeration is not understood, the analysis may sometimes give misleading results on data such as particle size and dispersion level. In another context, increasing use of manufactured

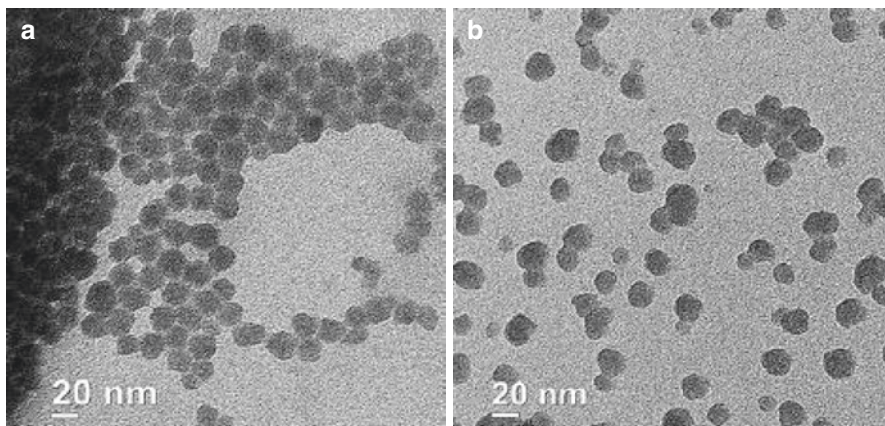


Fig. 1.4 Transmission electron microscopy images of silica nanoparticles showing their agglomeration state depending on the sample preparation methods such as (a) drop-on-grid and (b) grid-on-drop

nanoparticles ensures these materials will make their way into the environment during their lifecycle. The state of these nanoparticles in the environment and biological systems remains a question, which stress the importance of understanding the mechanisms of aggregation and agglomeration.

Methods for determining the state of aggregation and agglomeration are mainly the same techniques as used for the nanoparticle size determinations. It is highly recommended to follow a special preparation protocol before doing the measurements, to avoid misleading information on the state of particles in the dry state vs. dispersion state. In this aspect, preparation of TEM grids shows (Fig. 1.4) a nice attempt on the importance of methods such as drop-on-grid and grid-on-drop showing the dispersion of particles on the grid.

Strategies for preventing aggregation mainly come from conventional colloid science in which particles are coated with foreign capping agents and/or the surface charges are tailored to separate them via electrostatic repulsions [28]. High energy mechanical methods such as grinding, sonication have also been used as a post treatment for making the dispersions and fine particles more homogenous. ISO TR 13097 2013–06 on dispersion reports the methods that can be used to monitor the state of nanoparticles in solutions, acceleration procedures and data evaluation. The OECD is now looking to prepare a new test guideline, which can refer to existing ISO standards.

1.3.2.3 Shape

The beauty of engineered nanoparticles is their hierarchal shapes and associated physical – chemical properties. Unlike bulk materials, the thermodynamic and surface energy considerations at nanoscale are more complicated by the high surface

area to volume ratio. For a material with a perfect symmetric sphere, the total surface energy is lowered by decreasing the amount of surface area corresponding to a given volume. On the other hand, faceted nanoparticles show high number of reactive and high atom density facets and may influence the properties such as dissolution, aggregation, and reactivity. Bottom-up methods are used for producing a large variety of nanoparticles. Depending on the synthesis conditions and composition, variety of shapes such as spheres, wires, rods, plates, spheroids, tubes etc. have been developed.

The shape of nanoparticles plays an important role in understanding the properties associated at nanoscale. Particularly for nanomedicine applications, the shape of nanoparticles has recently been identified as a key factor influencing circulation time, bio-distribution, cellular uptake, as well as targeted drug delivery. The realization of shape factor came from the non-spheroidal shapes of various and bacteria, but still improving their ability to evade an immune response [41]. However, shape effect studies show considerable toxicity to human cells and question the health and environmental fate of nanoparticles. It was shown that wire-shaped silver nanoparticles induced a strong cytotoxicity to human cells (A549) than spherical silver nanoparticles. It was argued that small diameter of nanowires shown to induce cell membranes but the large length of silver wires does not allow a complete entry as compared to the spherical nanoparticles [39]. In another example, ZnO nanoparticles of different shapes also show toxicity to marine algae [32]. Therefore, shape is an important parameter when considering the fate of nanoparticles.

For shape determination of nanoparticles, electron microscopes such as SEM, TEM and SPM (Scanning Probe Microscopy) are used. However, for intrinsic properties such as crystal structure, symmetries and surface morphology, electron microscopy combined with tomography are used. Figure 1.5 shows SEM images of different zinc oxide particles, synthesized under various reaction conditions and concentrations. The morphology of zinc oxide formed by the reaction of zinc salts and hydroxide ions is very dependent not only on pH, temperature, concentration, and reaction time but also on the stirring time and water addition sequence of the reactions. By controlling these factors, flower-like, needle-like, star-like and spherical morphologies were obtained. Likewise, different metal, non-metal, and metal oxide nanoparticles including silica, TiO₂, iron oxide, gold, and, silver has been developed with various shapes and aspect ratios.

1.3.2.4 Surface Area and Porosity

A distinct characteristic of nanomaterials as compared to their bulk form is the area of accessible surface, as described by the specific surface area. Nanomaterials possess high surface area per unit mass due to a high portion of atoms at the surface relative to the atoms in the interior of the particles. Due to their large surface area to volume ratio, nanomaterials are highly attractive, and therefore, lead to a lot of new properties stemming from quantum effect and surface/interface effect. For applications requiring a large surface area per unit weight such as hydrogen storage for

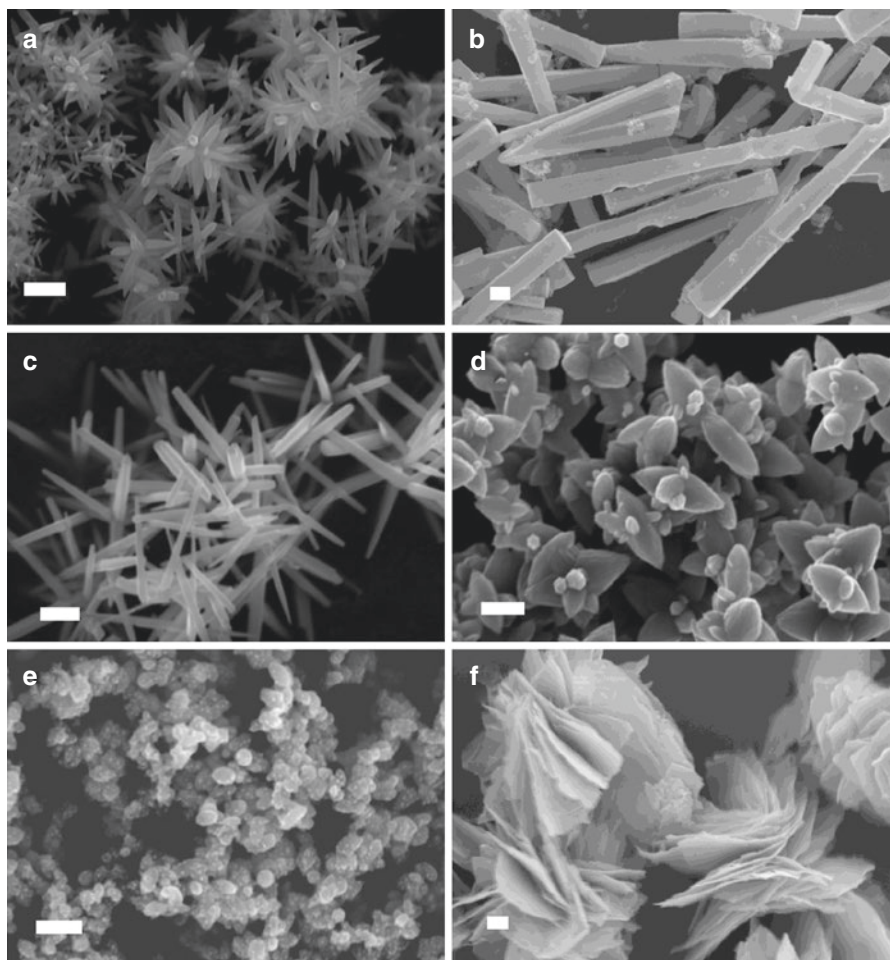
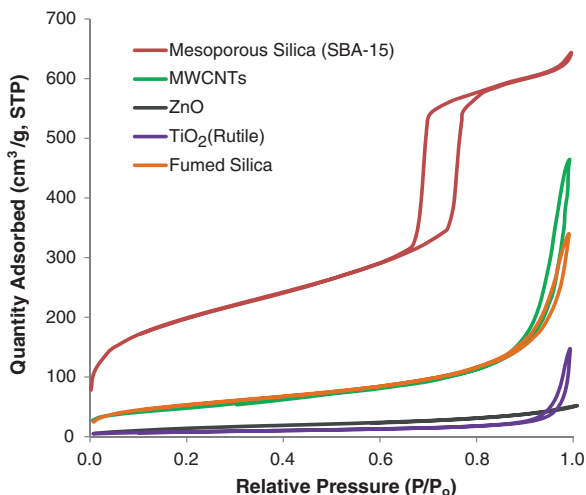


Fig. 1.5 Different shapes of ZnO particles, under various synthesis conditions such as aging time, concentration, pH (=10, 8, 7), and temperature gives (a) Flower-like, (b) Prism-like, (c) Needle-like, (d) Star-like grains, (e) Aggregated spheres, and (f) Sheet-like flakes, respectively. Scale bar is 1 μm .

vehicles, chemical sensing, light harvesting, and for catalysts, nanomaterials or nanostructured materials be promising candidates. A typical example and so far holds the highest experimental surface areas of any porous materials reported to date is metal-organic frameworks (MOFs), displaying $\sim 7000 \text{ m}^2/\text{g}$ of surface area [15]. To put it another way, just a few grams of nanoparticles offers a surface area equivalent to the size of a football stadium. On the flip side, a greater toxicity was found from nanomaterials than from their larger counterparts associated by high absorption capacity. It was shown that ultrafine titanium oxide nanoparticles are more inflammogenic and cytotoxic than when compared to the fine sized particles of lower surface area [37].

Fig. 1.6 Nitrogen adsorption-desorption isotherms of various nanoparticles



Having high surface and interface areas of nanoparticles induce a strong interaction with the surrounding liquid or gaseous adsorbents. Depending on the strength of the interaction and surface charge, the adsorption can be described as being either physical or chemisorption. Chemisorption is characterized mainly by a strong interaction between adsorbate-adsorbent while physical adsorption is due to mainly dispersion forces, i.e. weak intermolecular forces between non-polar molecules. The latter is more favored for surface area and porosity measurements because of its non-destructive nature and the ease of quantification of the adsorbate.

For powders, the specific surface area is usually determined by the nitrogen adsorption technique using the BET (Brunauer, Emmett and Teller) method [9], by which one may also measure the surface area given by nanoporosity. Though, other techniques such as SAXS (Small Angle X-Ray Scattering) developed for solutions, but the methods are not routinely usable.

Gas adsorption is a prominent technique for the comprehensive characterization of surface area and porosity measurements. The adsorption of gases such as N_2 , He, Ar (adsorbate) at various relative pressures on a solid (adsorbent) gives information of textural properties including surface area, pore volume, and pore size. The measurement is performed volumetrically; calibrated volumes of gas are added to a sample tube that is immersed in liquid nitrogen with a known amount of sample. The amount of gas adsorbed can be calculated from the measured pressure difference in the sample tube after the addition of a known volume. If the amount of adsorbed gas is plotted against the pressure, an isotherm is obtained. From this plot, surface area, pore volume, and pore size can be derived. Unfortunately, the gas adsorption method for surface area and porosity measurements is not applicable for liquid based materials.

Figure 1.6 shows nitrogen adsorption-desorption isotherms of various porous and nonporous nanoparticles. The shapes of the isotherms are different, and the adsorption volumes of nitrogen differ extensively between the nanoparticles. Mesoporous silica,

SBA-15 [46] shows high absorption capacity than the other nanoparticles, indicative of high surface area material. This is due to the ordered pore structure at mesoscale and possesses a narrow pore size distribution as evidenced from the reversible hysteresis loop (Type IV). MWCNTs and Fumed silica exhibit a moderate amount of adsorption at low relative pressures and resembles the inter-particle pore characteristics. The other nanoparticles exhibit nonporous characteristics and most of the adsorption comes from the surface of the particles and hence exhibit low surface areas. The shape of the isotherm sometimes distinguishes the porous vs nonporous materials and even on the types of various porous materials. Most of the materials exhibit six types of adsorption isotherms. For microporous materials, the pore filling occurs in a continuous way (Type I) and the majority of micropore filling occurs at relative pressures below 0.1. The Type II isotherm is typical for macroporous and non-porous solids where monolayer coverage followed by multilayers at high relative pressures. Type III and Type V isotherms are characteristic of weak adsorbate-adsorbent interactions and are most commonly associated respectively with non-porous and microporous adsorbents and microporous or mesoporous solids. Type IV isotherms are typical for mesopores where pore-filling occurs by pore condensation.

The BET equation was used to calculate the surface area (S_{BET}) from the adsorption data obtained in the relative pressure (P/P_0) range from 0.05 to 0.3. The total pore volume (V_{tot}), which is an important parameter for porous nanoparticles calculated from the amount of gas adsorbed at the highest relative pressure (P/P_0), implies how open the pore structure is. The pore size distributions (PSD) of the nanoparticles are calculated from the adsorption branch of the isotherm and fitting various pore shapes such as cylindrical, bottleneck, slit type pores.

Most widely accepted PSD models are the Barrett-Joyner-Halenda (BJH), Horvath-Kawazoe (HK), and the Non-local density functional theory (NLDFT) models. The Surface area and porosity properties derived from the nitrogen isotherms on selected nanoparticles are shown in Table 1.4. From the table, it is clear that porous nanoparticles show high porosity properties than nonporous particles and mesoporous silica (SBA-15) being the high in pore characteristics.

Finally, BET surface area has also been considered as an important parameter to identify nanomaterials. As per the EU recommendation, volume specific surface area (VSSA) proposed as a complementary definition to distinguish nanomaterials from non-nanomaterials. The recommended VSSA (i.e. greater than $60 \text{ m}^2/\text{cm}^3$) corresponds to a 100 nm sphere.

1.4 Conclusions and Closing Remarks

Engineered nanomaterials represent one of the most fascinating developments in recent years. They are increasingly used to construct products with attractive features and uses. More than 20 nanomaterials of type are already used in consumer products and estimates to demand 50,000 tons/year in near future. With industrial production specific reporting requirements also arise for chemical registration and

Table 1.4 Surface area and porosity of selected nanoparticles

Nanoparticles	BET surface area, m ² /g	Pore volume, cm ³ /g	Type of porosity
Mesoporous Silica (SBA-15)	670	0.95	Porous (meso)
MWCNTs	170	0.67	Porous (meso)
TiO ₂ (Rutile)	28	0.20	Nonporous
ZnO	51	0.09	Nonporous
Fumed Silica	190	0.44	Porous (meso)

toxicological testing. It is evident that the many different nanomaterial and derivatives result in different properties of the specific nanomaterials. Therefore, there are increasing demands for proper methods and standard operational procedures for physicochemical characterization of nanomaterials and updated systems for chemical classification. As a first step it is important to be able to identify a nanomaterial, which is a key topic in ongoing research projects.

In this chapter, we gave examples on the key physicochemical characteristics and the type of data one can achieve. A variety of techniques can be used to obtain both structural and textural properties, which are essential to identify and report uniqueness of specific nanomaterials. Electron microscopy is the most widely used instrument for chemical and structural investigation of nanomaterials at relatively high resolution and even close to atomic levels. In principle, other non-destructive methods are also applicable, such as X-ray diffraction and gas absorption isotherms. It is anticipated that this type of data will be important in the next generation of material grouping and categorization systems as well as to understand what drives particle toxicity in greater depth. Even-though, significant progress have been made over the last decade, there is still no full understanding on how the different physicochemical parameters relate to nanomaterials hazards. In fact the parameters are still only used to some extent in this types of exploration. The challenge will be even greater acknowledging that complex nanomaterials have entered industrial scale production and needs to be included in such analysis.

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