Integrated Analytical Strategies and Multimodal Characterization Techniques for Nanoparticles: Comparative Insights

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Abstract

The special physico-chemical properties of nanoparticles, related to their quantum size effect and high surface-to-volume ratio makes imperative the use of special characterization methods to measure agglomeration and dispersion. The review further explores extensive experimental techniques for size and morphology determination as well as crystallinity and the surface chemistry and magnetic properties and thermal behavior of nanoparticles as well as their responses to optical. Techniques are classified based on a systematic examination of the underlying physical principles and the conveyed information. Instruments for example Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and Electron Backscatter Diffraction (EBSD) allow direct observation and microstructural characterization at nanoscale. Spectroscopic and scattering techniques such as X-ray diffraction (XRD), X-ray absorption spectroscopy (XAS), UV–Visible spectroscopy, Raman spectroscopy and photoluminescence allow researchers accurate characterisation of structural/composition and optical properties. The surface sensitive methods XPS, FTIR, NMR, and ToF-SIMS provide information regarding the interaction of ligands and their chemical state. Analysis performed using particle dispersion techniques: DLS, NTA and DCS, as well as thermal and surface area analysis: TGA and BET, enable to comprehensively review the system.

This features reviews not only of techniques such as SQUID, VSM, Mössbauer spectroscopy, and XMCD, along with Magnetic Force Microscopy (MFM) for studying magnetic nanoparticles but also of more advanced techniques such as EELS, SANS, XCT, and RIXS which play a more specialized role in investigating the electronic structures and architectural aspects of the particles and stress that the future for accurate and trustworthy results that remain valid as nanomaterial complexity continues to increase across the sciences will come from multimodal property-focused characterisation strategies.

Keywords: Nanoparticle Characterization, Analytical Methods, Multimodal Analysis Structural and Surface Properties Magnetic and Optical Properties, Nanoscale Imaging

1. Introduction

They differ significantly from the same material at the bulk level the most important material. The high surface-to-volume ratio endows them with the unique feature of greatly enhancing the reactivity of the molecule. It, therefore, changes the characteristics of the substances, such as electronic properties as well as optical behavior and chemical behavior. once reduced to the nanoscale, the mechanical properties also change [1]. This makes nanoparticles become commonplace in scientific exploration and technological leaps since, with the amazing features of matter, they have become crucial in such fields as catalysis, medicine, and energy. The broad development of synthesis methods, from the mechanical to the chemical routes, has allowed for the mass production of a diverse plethora of nanostructures, which previously seemed unthinkable on such a scale [2]. The rapid production of nanoparticles surpasses the capacity of the available characterization techniques. Since most theories are practically untested, they create their external methodologically, and low production levels in laboratories make it difficult to assess most nanoparticle characteristics. The nature of nanoscience as a fusion of many scientists makes it difficult to obtain all the necessary tools needed for such a complex instrument. Coordinating the highly skilled nanotechnology team requires not only technical expertise but also logistical infrastructure [3]. Researchers need to comprehend that the strengths and weaknesses of any single method are so severe that no single method can handle all types of analysis. Although other more sophisticated analysis techniques can still be highly accurate, two or more techniques working parallel analyze aspects of nanoparticle parameters, such as size and shape, and surface chemistry and crystallinity, which are often more stable.

This article reviews the main techniques used in the characterization of nanoparticles comparing their characteristics, explaining the reasons why some of them are better for some aspects of nanoparticle characterization, in the context of the growing role of nanotechnology across disciplines. Scientists classify these methods based on whether they take an image or a spectrum, what kind of information they measure (such as size or elemental composition), or what kind of materials are necessary. It also describes the underlying principles and capabilities of each methodology and presents examples of how researchers are using these techniques to predict nanoparticle properties. The authors are provided with detailed assistance in selecting suitable characterization techniques that meet the specific needs of their research.

2. X-ray-based Techniques

Analysis of the structure and chemical composition of nanoparticles is strongly supported by X-ray-based techniques. When used in combination with X-ray Diffraction (XRD), X-ray Absorption Spectroscopy (XAS) (standard, EXAFS and XANES) and Small-Angle X-ray Scattering (SAXS) these methods provide complementary information to complete the characterization of the nanoparticles.

2.1 The X-ray Diffraction (XRD)

X-ray Diffraction (XRD) procedure is still popular technique concerning the crystal structure, phase composition, lattice parameters and crystallite size. The Scherrer equation is used by researchers to determine crystallite sizes by analyzing peak broading in diffaction pat terns. The XRD method gives statistically correct result through volume average in case of the study of nanopowders. In order to interpret crystallographic information, researchers can compare the diffraction pattern with reference data stored in databases, which can include the International Centre for Diffraction Data (ICDD) [5].

Even though XRD is very useful, but it also has its own limitation. Amorphous materials may not be detected by XRD upon broadening of the diffraction peaks beyond 3 nm particle sizes, thus the structural analysis would be untrustful. The particle sizes obtained from TEM analysis are usually contradictory with those from XRD. These deviations are due to both the internal lattice strain of the pozollanic dust and the existence of various crystalline domains, and the XRD detecting limits the large surface grain boundary of these particles [6]. Change at the highest intensities shows the preferential orientation or anisotropic crystal growth of nanocrystals. The relative intensity of diffraction peaks depends on the particle shape, so it is used to obtain information about the sample morphology [7].

2.2 XAS (X-ray Absorption Spectroscopy)

X-ray Absorption Spectroscopy (XAS) including EXAFS and XANES provide element-specific information about atomic chemical and structural environment. The methods provide strong tools for the determination of the oxidation state, coordination environment, interatomic distances and bond disorder of significant importance when analyzing disordered or noncrystalline nanomaterials [8]. Through EXAFS, scientists can determine tail numbers and bond lengths during evaluation x-ray absorption near-edge structure (XANES) is the source of accurate information on the electronic structure (oxidation states and symmetry) of the absorbing atom and its neighbors. The requirement for synchrotron radiation makes these measurements a non-routine use, but they are highly precise and sensitive [9].

The millisecond time resolution, and hence high time-resolution, ED EXAFS measurement to capture short-range structural changes, is of particular interest when observing rapid reactions and thermal processes inaccessible to other experimental techniques with slower time resolutions or temperature limits. The method is crucial for the investigation of structural changes taking place during synthesis or catalytic processes [10]. The XAS techniques are uniquely positioned to study the interactions of nanoparticles with ligands and surfactants which are molecules that attach to surfaces. Surface modifications of nanoparticle alter bond length, form lattice wrinkles at their interfaces, and subsequently alter growth dynamics and lead to structural outcome [11]. EXAFS is also sensitive to lattice distortions that arise from steric effect and the chemical interaction of the organic capping agents[12]. The multiple cation distributions in mixed-metal oxide nanoparticles can be distinguished using EXAFS and structural parameters such as coordination symmetry and interatomic distances as well as bond disorder can be obtained. EXAFS data analysis is now also applied with the Wavelet Transform (WT), in addition to the conventional Fourier Transform (FT); with these the signal resolution on top of overlapping structures is enhanced and the complex spectral interpretation becomes clearer [13].

The XAS methods, such as EXAFS, are powerful tools for comparing the structures of the nanoparticles and their bulks. They are able to probe the local environment around essential elements induced by nanoscale confinement or doping to elucidate how nanomaterials differ from their bulk counterparts [14].

2.3 The SAXS side (Small Angles X ray Scattering, SAXS)

Small-Angle X-ray Scattering (SAXS) offers a nondestructive and statistically relevant method for nanoparticle size, shape and aggregation status assessment in both liquid suspensions and solid matrices. This technique is sensitive to changes in electron density and operates at a length scale where XRD is sensitive to long range crystalline orders. X-ray diffraction gives us an indication of the crystallite size and small angle X-ray scattering of the overall particle size including amorphous layers or coatings [15]. SAXS is often employed for in situ observation of nanoparticles nucleation and growth. This combination of SAXS and UV-Vis + WAXS allows for a direct monitoring of the time-evolution of the particle formation process in different chemical and thermal environments. Small angle neutron scattering (SANS) is complementary to the SAXS due to contrast advantages for lighter atoms and it enables to study the isotope labelling in soft or in hybrid material [16].

The use of the SAXS technique exhibits resolution limitations, thus limiting its applicability in the analysis of large aggregates or systems with large polydispersity. To verify the accuracy of the particle size and distribution data in such cases, scientists may often integrate TEM or DLS with other methods [17]. Interpretation of the abovementioned SAXS data of core-shell nanoparticles requires careful modeling to attribute the contribution of each layer [18].

X-ray-based approaches provide a versatile and powerful toolbox for scientific research. XRD characterizes crystallinity and long-range order, XAS characterizes local chemistry and oxidation state, and SAXS provides the structural information about size, morphology, and level of aggregation in colloidal or bulk systems. The complementary use of these methods represents the core of comprehensive NMs characterization strategy [19].

3. Structure, Composition, and Major Characteristics

The nanoparticulate features are characterized beyond structural crystallinity by surface chemistry, organic functionalization, particle dispersion, ligand binding and optical properties. A variety of analytical techniques based on certain physical principles are performed individually or simultaneously in combination to achieve this goal. These methods study the different nanoparticle properties and are also very important for a detailed characterization.

3.1 FTIR (Fourier Transform Infrared Spectroscopy)

Fourier Transform Infrared Spectroscopy (FTIR) is a widely used vibrational spectroscopy method that quantifies the absorption of infrared radiation by molecular bonds in a sample. The method functions based on identification of IR frequencies that covalent bonds normally absorb in relation to specific vibration modes. Fourier Transform Infrared Spectroscopy (FTIR) offers the critical information regarding the surface functional groups, ligands, or functional groupings (carboxyl, amine, thiol, or hydroxyl) and serves to study the interaction of the ligands with NPs. FTIR examination can distinguish between the two bonding environments and also follow the changes introduced by the different surface functionalization methods. FTIR analysis is fast and non-destructive, yet is limited in terms of sensitivity to low concentration samples, in which overlapping spectral peaks and water and CO_2absorption may impose difficulties in interpretation [20].

3.2 NMR spectroscopy

The method of NMR spectroscopy gives insights into the chemical environments of individual atomic nuclei those of hydrogen, carbon, phosphorus, and fluorine, in particular. NMR in nanoparticle research has been used on the dynamics and binding modes of ligands, the spatial distribution of organic shells and their interaction with molecules adsorbed on the surface. Solution NMR provides information on ligand mobility and surface coverage at high surface ligand concentrations, while solid-state NMR can be used for samples that cannot be dissolved or are incorporated in a matrix. This method provides strong specificity and non-destructive sample analysis but suffers from a low signal-to-noise ratio, especially in heavily diluted samples, and also from a high relaxation rate, in the case of the paramagnetic materials, and consequently requires either a relatively high amount of sample or a long measurement time [21].

3.3 Brunauer–Emmett–Teller (BET)

The specific surface area of the nanoparticle powders is measured by the Brunauer–Emmett–Teller (BET) method using gas adsorption. The method determines surface area by physical adsorption of inert gases, usually nitrogen, at cryogenic temperatures from adsorption isotherms. Nonetheless BET analysis is an ideal technique for porous materials with large surface areas; BET analysis favors catalysis and adsorption studies. The technique assumes monolayer coverage and erroneously measures the surface area of non-porous materials and nanosized particles exhibiting large agglomeration. The surface area and pore size values obtained via the BET method lack information on pore distribution, and the shape and the chemical characteristics of the pore surface [22].

3.4 TGA Thermogravimetric analysis (TGA)

TGA (Thermogravimetric Analysis) is a thermal analysis technique where mass of a sample under controlled heating in an atmosphere is monitored. It is a widely used methodology for determination of organic ligands and surfactants, polymer coatings, nanomaterial moisture content. This TGA technique allows researchers to identify decomposition temperatures and assess the oxidation stability and the residues in the sample. Although the method has been found to give reproducible results by simple procedures, it does not give chemical specificity and other methods like FTIR (Fourier Transform Infrared) and mass spectrometry have to be employed for the determination of decomposition products. The interpretation of findings is complicated by the cascade of weight loss measures that occur over analysis [23].

3.5 Low-Energy Ion Scattering

LEIS offers the possibility set nanoparticles with monolayers attached to a surface and use it to determine the surface composition and the monolayer thickness as a function of size, using its surface sensitive character. Only the outermost of atomic layer of species can be resolved by LEIS due to the sub-surface penetration depth of low energy noble gas ions used for scattering from surfaces. The method is particularly suited to surfaces modified chemically and surfaces that constitute self-assembled monolayers. Although LEIS is highly sensitive, it is ultra-high vacuum sensitive, which is also difficult to achieve because of sample charging, especially in non-conducting spectra, and its difficulty to quantitate elements in complex mixtures or those with low atomic numbers (H) [24].

3.6 UV–Vis spectroscopy

Ultraviolet–Visible (UV–Vis) as an optical detection technique to observe the absorption of light within the UV/VIS range due to the electronic transitions in the nanoparticle systems. The wavelengths and strengths of LSPR peaks in such metal nanoparticles like Ag and Au are sensitive to both their sizes and shapes, as well as their aggregation and the surrounding dielectric. The best method for real-time synthesis and stability monitoring in terms of time and non-contact operation is UV–Vis spectroscopy. This method in addition to indirectly estimating the particle size and not providing chemical specificity is sensitive to polydispersity effects as well as to variations in refractive index and colloidal aggregation with references to the limitations of the methods.

3.7.1 Luminescent properties-photoluminescence (PL) spectroscopy

The photoluminescence (PL) spectroscopy is an optical technique this allows for the light emission, of excited NPs, to be quantified. When electrons absorb photons, they move to higher-energy states and emit when falling back down. The emission spectrum also exhibits the bandgap characteristics of nanoparticles as well as the defects and surface passivation effects, with particular emphasis on semiconductor materials such as quantum dots and metal oxides. PL spectroscopy provides valuable information about the quantum efficiency of devices and trap states and energy transfer processes. The accuracy of this approach depends on the surface quenching effects, sensitivity to environment and is dependent on well-controlled excitation sources as well as sample concentration measurements [26].

3.8. Dilatation Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS), also called photon correlation spectroscopy measures the hydrodynamic size of the particles that are suspended in liquid. The method makes use of Brownian motion that results in a variation of light scattering intensity over time which permits the calculation of the particle size distribution via the Stokes–Einstein equation. DLS is still widely used, because it is quick and works well with liquid samples. This method also preferentially weights larger particles since it only measures intensities in a weighted manner, and does not provide information on shape anisotropy, besides that it leads to very poor results for highly polydisperse or multimodal populations [27].

3.9.3 Nanoparticle Tracking Analysis (NTA)

Nanoparticle Tracking Analysis (NTA), an alternative approach, employs video microscopy to monitor the Brownian motion of each particle, one at a time. Analysis of particle trajectory and movement rates allows NTA to produce size distributions based on particle number, as well as estimate the concentration. NTA is suitable for the determination of polydisperse samples with low particle concentration and systems with small size discrepancy among the particles. This method can achieve better resolution than DLS, but may have the accuracy problem that depends on the operator and cannot analyze particles with diameter less than 30 nm, and it is interfered by light and have low refractive index contrast (the change of the refractive index of tissue regions around the gold particle before and after the binding of gold particles). [28]

3.10 The Difference Centrifugal Sedimentation (DCS)

The nanoparticles are separated by their sedimentation velocities in a rotating density gradient using the Differential Centrifugal Sedimentation (DCS) technique. The sedimentation rate is a function of particle size, shape and density, and this makes DCS a better tool for accurate particle sizing and analysis for bimodal or trimodal distributions. DSC Differential Centrifugal Sedimentation (DCS) is particularly effective when used with spherical nanoparticles and core-shell nanoparticles. To correctly interpret the results, one should know the particles' density and shape. DCS is essentially a destructive process where particles are dispersed throughout targeted fluids (while the shear forces during rotation traps the risk of soft or hollow particle deformation) [29].

3.11 Inductively Coupled Plasma Mass Spectrometer-MS (ICP-MS)

Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), and Matrix-Assisted Laser Desorption/Ionization (MALDI), enhanced compositional analysis becomes available. Beyond measurement of particle concentration and size estimation via single particle detection, ICP-MS can also simultaneously measure the elemental composition of the nanoparticles. The approach shows good sensitivity toward metal nanoparticles but involves sample digestion. ToF-SIMS provides high-sensitivity surface chemical mapping via sputtering away outermost layers followed by secondary ion analysis, whereas MALDI probes organic coatings (ishnanoparticle conjugates and soft materialdelwasinterfaces. Although these are very powerful techniques their diffusion is limited by the matrix effects, the necessity of an high vacuum and the difficulty in interpreting data [30].

Techniques that can be used to help assess particle concentration, surface charge, colloidal stability, molecular weight distribution of coatings, and phase transition behavior include Resistive Pulse Sensing, Electrophoretet Light Scattering tests (zeta potential), Gel Permeation Chromatography (GPC) and Differential Scanning Calorimetry (DSC). These methods are of great importance in characterizing the physicochemical and thermal properties in polymer–nanoparticle hybrids and biological nanostructures even if not universally applicable to every type of nanoparticle [31].

Together these methods form a complete characterization of nanoparticles, surface chemistry, and thermal properties as well as size, optical, and colloidal properties. The selection of proper methods has to take into account the properties of nanoparticles as well as the final property and the environmental medium. Data validity and reliability are based on the merging and cross-validating of analytical approaches towards full results.

4. Techniques for the Characterization of Magnetic Nanoparticles

Magnetic properties of nanostructures are the main focus of many studies on techniques and methods of their magnetic characterization, which are of a critical need for hard magnetic materials like iron oxide (Fe 3 O 4), cobalt (Co), alloy nanostructures. The computational methods adopted to investigate magnetic nanoparticles allow revealing their basic properties like saturation magnetization, magnetic anisotropy, blocking temperature and superparamagnetism, which are important for application in biomedicine and data storage technology as well as for catalysis or magnetic separation.

4.1 Superconducting Quantum Interference Devices (SQUID)

As one of the most sensitive approaches for magnetic nanoparticles analysis, superconducting quantum interference device (SQUID) magnetometry is notable. SQUID (Superconducting quantum interferece device) utilizes the quantum interference in superconducting loops to measure very weak magnetic signals. By scanning the magnetic moment using different temperature and magnetic field, this instrument gives a full picture for the over all magnetization saturation and coercivity remanence and blocking temperature. SQUID magnetometry is ideal for detecting weak magnetic signals in low-concentration samples and for discriminating ferromagnetic from superparamagnetic behavior. Even through SQUID systems are more sensitive, they are costly, require expensive and cryogenic temperature (usually liquid helium), and meticulous sample preparation and calibration [32].

4.2 Vibrating Sample Magnetometry (VSM)

Vibrating Sample Magnetometry (VSM) is a more readily available method. In VSM, the sample is physically perturbed (i.e., made to vibrate) in a uniform magnetic field, and the induced voltage in pickup coils surrounding it is used to measure the magnetic moment. The VSM provides comparable M–H and ZFC–FC curve data SQUID does, albeit at less sensitivity. VSM offers fast magnetic characterization at a wide temperature and without need to cool down to very low temperature. The VSM is not sensitive to very weak magnetism, and also is unable to achieve the low-temperature transition and the weak superparamagnetism [33].

4.3 Mössbauer spectroscopy

Mössbauer spectroscopy is a powerful tool for the investigation of iron-based nanoparticles. This method is based on the observation of solid-state nuclei which absorb resonantly gamma rays delivering detailed information on the oxidation state, in combination with magnetic order and site symmetry, and electric-field gradients. Mössbauer spectroscopy is useful to determine the different iron oxidation states in magnetic NPs, as well as provide information about the surface/core spins and the magnetic anisotropy energy. It can successfully distinguish phases like hematite, magnetite and maghemite when structure based methods are unable to differentiate. The latter approach is applicable only to certain, like the ^{57}Fe, isotopes and requires a careful modelling of spectral line profiles to interpret [34].

4.4 Ferromagnetic Resonance (FMR)

Ferromagnetic Resonance (FMR) makes it possible to measure the dynamic magnetic response of nanoparticles by detecting the absorption of microwave radiation resulting from a static magnetic field. FMR also provides information about the NP size distribution as well as the shape anisotropy distribution, magnetic anisotropy constants and crystallographic defects and demagnetizing fields. This approach is especially suitable for recognition of fine particles as well as detection of structural defects coupled with a characterization of the particle-particle interaction. The FMR method requires noninteracting spherical particles to make reasonable models and the broadening of the resonance lines complicates the interpretation in complex systems [36].

4.5 XMCD

XMCD is a synchrotron based technique that combines X-ray absorption spectroscopy with circularly polarized light to determine magnetic properties at the atomic level. In the XMCD method element-specific information (spin and orbital magnetic moments and oxidation states including the coordination sphere) introduces an additional advantage in the analysis of the multicomponent or core–shell magnetic NPs. The XMCD method enables the separation of ferromagnetic and ferrimagnetic contributions and the magnetic properties at the atomic sites. The use of this method is restricted to synchrotron facilities and it is a very demanding method regarding the alignment of the sample, which must be stable during exposure [37].

4.6 Susceptibility meters

AC or DC meters of susceptibility are designed to measure how strongly a substance will become magnetized when subjected to an external magnetic field. They enable one to measure the blocking temperature, to identify the super to ferromagnetic transition, and to detect interparticle magnetic interactions. AC susceptibility enables to explore relaxation mechanisms in frequency-dependent mode whereas DC measurements show magnetization curves at different temperatures. Though these methods are simple, they are not capable to treat multiphase systems and over lapping relaxation phenomena [38].

4.7 Magnetophoretic mobility measurements

The magnetophoresis mobility analysis is an applicable method to investigate the motion of magnetic particles in the solutions. The method measures the velocity of particle drift in the presence of an external magnetic field gradient, providing information on particle size and magnetic core content and magnetization. The method is especially significant for biotechnological areas such as magnetic targeting, drug delivering, and magnetic separation of cells. Quantitative analysis requires careful calibration as measurements are sensitive to fluid viscosity properties, particle coatings, and aggregate [39].

4.8 Superparamagnetic Relaxometry (SPMR)

The diagnostic method, known as Superparamagnetic Relaxometry (SPMR), tracks how long magnetic nanoparticles retain a magnetic signature after a magnetic field is turned off. This technique has proven valuable for detection and localization of superparamagnetic particles in imaging and biosensing applications. The analysis of the decay of the magnetic response over time is the basis for obtaining particle size, magnetic core volume, and hydrodynamic radius indirectly. SPMR appears to perform well in biological and clinical settings but is not capable of detailed compositional or structural analysis, and is hence used complementary to other characterization approaches [40].

These magnetic methods together provide a complete toolbox for the characterization of static and dynamic properties of magnetic nanoparticles by researchers. On the other hand, only SQUID and VSM are able to provide information about the magnetization on a large scale, whereas Mössbauer spectroscopy and XMCD obtain information on atomic scale. By sedimentation velocity and size-dependence of the FMR spectra, it is possible to learn how the particles interact and move in the energy landscape. To obtain a complete picture of the low-temperature magnetic properties of nanoparticles under various field strengths and ambient conditions multi-modal studies using these analytical probes are necessary.

5. Microscopy Approaches

Characterization of nanoparticles in terms of physical parameters such as morphology, size, surface texture, internal structure, agglomeration is much important and still needs a microscope based technique for visualization. Microscopy provides direct observation in terms of the high resolution images that reveal vital nanostructural features in real space rather than indirectly. There are several specialized microscopy techniques in use in nanoscience – each can provide unique information based on its physical process.

5.1.Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is one of these promising imaging methods when it comes to nanoscale imaging, as it is one of the most widely used imaging methods. The approach involves shooting a high-energy electron beam through an ultra-thin sample and recording how the electrons scatter as they pass through. Using this technique, atomic resolution images of the internal structure, like lattice fringes and grain boundaries, as well as defects can be obtained. TEM is often used for the determination of particle size distribution and crystal morphology studies in scientific investigation. HRTEM provides atomic plane visualization and shows crystalline and amorphous domains while SAED patterns provide diffraction based phase identification and orientation information. Although TEM has an excellent resolution, it has some limitations: The thickness of the sample that can be observed under a TEM is the one that must be thinner than 100 nm; however, it also requires extensive preparation including drying and dispersion. TEM shows 2D views of 3D structures, making interpretation largely incomplete without the support of additional tomographic approaches [41].

5.2 SEM (Scanning Electron Microscopy)

In the Scanning Electron Microscope (SEM), a focused electron beam is directed onto a specimen to detect secondary or backscattered electrons emitted therefrom. It forms detailed images, showing the surface structure (morphology) and texture based on the materials. Scanning Electron Microscopy performs even better for the imaging of particles larger than 10 nm and aggregates, agglomerates and agglomerates for which it is spread on surface or embedded in matrices. Working of SEM is easier than TEM and sample preparation of SEM is simpler as compared to TEM waterproof non-conductive needs only form of conductive coating. The spatial resolution of SEM is lower than that of TEM as it is unable to visualize the internal structures of specimens. Furthermore, charging effects that are particularly prominent for organic or insulating materials lead to imaging artefacts [42].

5.3 Scanning Transmission Electron Microscopy (STEM)

Scanning transmission electron microscopy (STEM) Fusing the concepts of TEM and SEM, STEM is a hybrid type of microscopy. In STEM mode, the focused electron probe is scanned across the sample, and the transmitted electrons form high-resolution images. The method allows for the harmonic generation process to be used for both imaging and analysis e.g. the image can be used for real-space elemental mappings e.g. via EDS and EELS. It reaches high-contrast imaging of heavy and light elements (contributing to its unique ability in studying core–shell structures, doped nanoparticles and atomic interfaces). STEM brings serious challenges because it requires an high cost equipment and complex data analysis, and very thin samples should be stable under the electron beam [43].

5.4 Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) operations are based on the interplay of a sharp cantilever probe with the sample surface. The scanning tip detects deflections generated by van der Waals, electrostatic and contact forces and translates them into topographic maps. AFM imaging of soft biological samples and insulating nanoparticles is nondestructive and requires no conductive coatings or vacuum, and so can be performed in atmospheric and aqueous environments, in contrast to electron microscopyh[3]. The system generates 3D surface profiles in combinations with roughness data and mechanical properties like adhesion strength, along with stiffness/elastic modulus. The AFM technique is limited by both the size scan range but also its slow imaging speed and by virtue of tip–sample convolution to give incorrect size readings making measurements particularly difficult for nanomaterials that are small and/or not regular in shape. Reproducibility is affected when AFM tips are worn out or contaminated [44].

5.5 Field emission scanning electron microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) is a more sophisticated version of SEM in which a field emission gun gives rise to a more coherent and smaller electron beam compared to the standard tungsten filament SEM. With the capability to visualize particles less than 10 nm FESEM, and a stable electron beam with exceptional performance for the surface-sensitive imaging purposes. SEM stands as one of the best techniques to analyze well-made particle structures and nanoparticles. FESEM encounters existed surface charging for non-conductive samples like conventional SEM and cannot inspect the internal structures [45].

5.6 STEM mode HAADF

This STEM HAADF imaging contrast is due to atomic number contrast, which creates images based on changes in atomic number (Z). The increased scattering power of the heavier elements leads to brighter contrast, which offers the potential for high-quality HAADF images for the determination of elemental distributions in core–shell particles and heterostructures. When combined with EDS and EELS, HAADF allows structure–composition mapping at the nanometre and sub-nanometre scale. HAADF contrast suffers from the effects of atomic number contrast but requires sample preparation that must be very thin and accurately aligned, making them susceptible to beam damage [46].

5.7 Electron Tomography (ET)

ET addresses the problem of 2D projections in electron microscopy by reconstructing 3D images from multiple TEM or STEM images taken at different tilts. The approach works in a way to study the shape anisotropy and reveal internal voids and intricate structures of NPs. Non-spherical particles, hollow structures, and biological nanoconjugates are the particles for which researchers find this approach technically advantageous. ER requires precise alignment and higher level software for image reconstruction, in addition to high computational resources. In the process of tilt series acquisition both sample drift and beam damage may affect the ultimate resolution [47].

5.8 Environmental Scanning Electron Microscopy (ESEM)

Combination of ESEM with Cryo-TEM provides unique imaging solutions for nanoparticles in hydrated and biological or volatile conditions. ESEM is a technique that is capable for imaging of samples in low vacuum or during wet conditions to decrease charge effects and to image that which is soft or wet. Sample is preserved and a dose of electron beam is dramatically reduced by rapid freezing of sample in cryo-TEM. The proper use of these methods is indispensable for biomedical experiments and the characterisation of interactions between nanoparticles with proteins or lipids. These methods require special equipment, as well as sample holders and cryogenic sample manipulation capability [48].

Microscopy provides fine spatial images but typically probes only a small subsample, which may not be representative for the overall sample. When processing microscopic images to determine the splash systems' size distribution, aspect ratio, or surface area, attention has to be paid to ensure accurate image processing, statistical and representative sampling together with care not to bias due to agglomeration or non-representative sampling areas. The best results from microscopy are obtained, when researchers employ these techniques in combination with spectroscopic and compositional or scattering, to establish a full picture of nanoparticle systems.

6. Other Advanced Techniques

The field of nanoparticle characterization has evolved so that contemporary metallic and semiconductor nanoparticles characterization may be achieved with the aid of several advanced and novel techniques in addition to many of the traditional structure, optical, thermal, and magnetic characterization techniques. When normal methods are not sensitive, resolving or selective enough these techniques are required. These methodologies offer unique insights into the behavior of materials on the dynamic and atomic and nanoscale interactions and compositions and thus advancing the field of nanomaterial chracterizations.

6.1 X-ray Photoelectron Spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS) studies the binding energy of core-level electrons, which are emitted from the surface of the material when being impinged by monochromatic X-rays. XPS provides accurate elemental composition as well as the oxidation state and chemical bonding environment for elements ranging from lithium to uranium. (Huang et al.) take advantage of XPS for the analysis of surface coatings and oxidation states of metal cores in addition to elemental distributions in core?shell structures in nanoparticle systems [49]. This method is non-destructive and applicable to thin films with thicknesses of several nanometer under normal process condition. The technology requires ultra-high vacuum and its sensitivity to light elements, such as hydrogen is poor and the sample in non-conjugate materials can charge requiring charge compensation (see for instance Refs. [50]).

6.2 Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS)

Magnet Target TOF-SIMS is one of the most powerful surface analysis techniques for providing molecular and elemental information from surfaces and sub-surfaces.

Time-Of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) A S)TCAN (surface analysis technique that uses a pulsed ion to impact the surface and then measures the mass of the secondary ions that are emitted. ToF-SIMS provides both elemental and molecular information at high submicron spatial resolution, which are essential to produce chemical images of surfaces and interfaces. This method delivers excellent performance in characterization of organic coatings on nanoparticles and mapping of the distribution of ligands and the interaction of biomolecular. The procedure enables trace impurities to be singled out and isotope ratios to be established. The method is semi-destructive and subject to matrix effects and ion fragmentation due to careful sample handling under vacuum [51].

The MALDI-imaging, in combination with Time-of-Flight (TOF) Mass Spectrometry (MS) is particularly prevalent among methods applied to large organic molecules: polymers, proteins, and biomolecules on its nanoparticle bearing. In this approach, the matrix absorbs the laser energy for soft ionization of the analytes. Tangent: Scientists can determine the molecular weights of ligands as well as polymer distributions and bioconjugates with MALDI. This modality is advantageous when conventional mass spectrometry results in the dissociation of fragile molecules. The method generally suffers from surface-insensitivity and low signal-to-noiseratios due to ion suppression or matrix-derived background noise [52].

6.3 SANS Small angle neutron scattering (SANS) was performed using the Andenberger solid Nebula 20 test reactor transmission small angle spectrometer; the 'wavelength'spread' (i.e. the dispersion of the neutron flight path lengths) was 0,4 A; 4.71 10 5 neutrons °A (s cm2) were incident on the sample.

Small-Angle Neutron Scattering (SANS) is the neutron scattering counterpart to SAXS, which uses neutron in contrast to X-rays to probe the internal structure and size of nanoparticles at the nanometer length scale. As a result of the unusual manner in which neutrons scatter off light elements, SANS is particularly well suited to study systems rich in hydrogen such as hydrogenous materials, polymeric coatings and soft matter-nanoparticle composites. Contrast can also be enhanced in systems where there are two or more components, by the use of deuterium labelling 13, 15, 16 or other isotopic substitution. SANS is an impressive technique but requires access to neutron sources, e.g. nuclear reactors or spallation sources andense acquiring times, and careful modeling of the data on scattering [53].

6.4 X-ray Computed Tomography (XCT)

Three-dimensional visualization of nanoparticles and nanoaggregates within materials can be obtained using X-ray Computed Tomography (XCT), which can be done without the need for sectioning using nanoscale or synchrotron-based XCT. XCT operates based on differential X-ray absorption, and is thus well suited for studying porous materials, agglomerates, and hierarchical structures. By means of high-resolution XCT, analysis of void distributions in addition to the interactions between particles distance and density gradients can be obtained. The main limitations are the long scanning time, radiation damage to the samples and lower spatial resolution compared to electron tomography at an atomic scale [54].

6.5 RIXS

RIXS serves as a third-generation synchrotron method to probe the electronic and vibrational excitations in a material by energy losses of an incident pho ton. Local electronic structure in terms of the oxidation state, in addition to information about the charge transfer process, can be obtained from this method. The RIXS method is particularly important for the investigation of nanoparticles (NPs); to elucidate the catalytic nature of NPs; metal–ligand interactions and mixed-valence states. The main drawback of this approach is its limited availability due to the synchrotron radiation as well as a complex spectral deconvolution requiring large computational resources [55].

6.6 EELS

After elastic electron scattering, energy can be transferred to the target by transferring energy to one of the atom’s bound electrons, by promoting an electron to an unoccupied state, by creating a secondary electron, yielding a characteristic core hole in the atom (ACM-Model) or by exciting an inelastically scattered electron in the continuum.

In conjunction with the STEM, Electron Energy Loss Spectroscopy (EELS) is a technique to measure energy changes of electrons passing through a sample. EELS provides elemental maps down to near-atomic resolution and can detect light elements that are invisible to X-ray techniques. EELS yields information on valence state, as well as electronic transitions and bonding. Interface composition and elemental mapping information are also obtained as part of the EELS nanoparticle analysis, and electronic structure information is also observed. EELS requires ultrathin samples and beam-induced damage and spectral noise is present when the detection area has low signal [56].

6.7 Raman Spectroscopy

Raman Spectroscopy, on the other hand, uses inelastic light scattering with monochromatic light sources such as lasers in order to observe vibrational modes in molecules as well as in crystalline materials. Raman Spectroscopy is widely used to analyze the structural "fingerprints" of nanoparticles and measure chemical bonds within particles, and to understand their phase composition. The Raman spectroscopy is an excellen tool to determine polymorphs and strain as well as the doping level or disorder of carbon based nanostructures. SERS enhances the signal so that scientists can pick up signals from low coverage adsorbed molecular species. The use of Raman spectroscopy is limited due to fluorescence interference, diminished material sensitivity, and limited depth penetration [57].

6.8 NEXAFS and EXAFS

NEXAFS and EXAFS are, however, indispensable techniques for studying electronic states and local atomic environment even for operando or in situ investigations. The second challenge is to study in situ the redox properties with respect to the coordination chemistry and the structural transformation that occurs during the chemical reaction includes the redox behavior of the materials, and on the other hand, to perform in situ research on the redox behavior, the phase transformation and the coordination chemistry with respect to the chemical reaction. There are some drawbacks to these techniques, i.e. high operational cost, a complex spectral analysis and a requirement for synchrotron access [58].

Even though these state-of-the-art approaches are only available to high-level experts with abundant of equipment base, they largely circumvent critical limitations inherent to conventional methodologies. These techniques are applied by scientists in the investigation of fine structural details as well as dynamic chemical process and of the surface interactions controlling nanoparticle properties in various real world applications including catalysis energy storage drug delivery environmental remediation [59]. Multimodal characterization methodologies which involve these emerging techniques offer sophisticated and detailed approaches to making sense of the complexity of nanoparticulate systems.

7. Conclusion

As the properties of NPs are dependent on size and complexity and are significantly different from bulk forms, multiple measurements are needed to characterize NPs. For the characterization of crystallinity and phase structures and particle sizes at both short and long ranges, X-ray diffraction (XRD), X-ray absorption spectroscopy (XAS), and small-angle X-ray scattering (SAXS) are invaluable. Analytical methodologies (FTIR, NMR, XPS, LEIS) are essential for low-dimensional compounds to determine the surface chemistry and bonding environment, as well as for the interaction with ligands. Thermal and SA measurements from TGA and BET techniques can also be performed together to gain more information about the organic content and surface reactivity. In particular, optical techniques such as ultraviolet–vis (UV–vis), photoluminescence (PL), and Raman spectroscopy are employed to study the electronic transition, quantum confinement effect, and vibrational mode but they require physical characteristics confirmation by environmental sensitivity and indirect measurement technique.

Dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and differential centrifugal sedimentation (DCS) deliver valuable information regarding dispersion and particle size in solutions but feature different levels of analytical resolution as well as sensitivity towards polydispersity. The super paramagnetic behaviour and magnetic domain interaction in magnetic nanoparticles are discussed based on SQUID, VSM, Mössbauer spectroscopy, FMR, XMCD, relaxometry studies, revealing the details of coercivity and the domain interaction. Magnetic force microscopy (MFM) is a tool that allows one to map magnetic domain structure at the nanometer scale and unites morphological information with magnetic information. Microscopy methods as TEM and HRTEM are essential for shapes and internal structure visualization and defects detection but cryo-TEM and ESEM enable imaging on hydrated specimens and biological structures. EBSD is a vital tool for the measurement of crystal orientation EBSD and phase orientation within polycrystalline aggregates has emerged. ToF-SIMS, MALDI-TOF, ICP-MS, SANS, RIXS, EELS, NEXAFS, and XCT provide the composition, surface electronic structure and isotopic distribution, and internal porosity. Although costly and with limited access, such techniques do not prevent adding great analytical power. The summary demonstrates that a single approach could not be used to comprehensively define the nature and structure of the nanoparticles. A property-biased complementary approach provides a means of obtaining a holistic and reproducible view with relevance to nanoscience.

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