LATEST FRONTIERS FOR NANOMATERIAL CHARACTERIZATION

Abstract

Nanomaterials, with their unique and tunable properties arising from their nanoscale dimensions, have revolutionized various fields such as electronics, medicine, and environmental science. Accurate and reliable characterization of these materials is essential for understanding their behaviour and optimizing their applications. This abstract presents a comprehensive review of the current state-of-the-art techniques for characterizing nanomaterials.

The application of advanced characterization techniques in specific nanomaterial categories, including nanoparticles, nanocomposites, and nanotubes, highlights their impact in fields like drug delivery, catalysis, and energy storage. Surface analysis of nanomaterials is an important aspect and it has relevance to the properties of nanomaterials. Commonly employed techniques for the characterization of nanomaterials are Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), X-ray Diffraction (XRD), Dynamic Light Scattering (DLS), and Fourier Transform Infrared Spectroscopy (FTIR). Some cutting-edge advancements in surface-sensitive techniques like X-ray Photoelectron Spectroscopy (XPS) and Scanning Probe Microscopy (SPM) have also been used. Spectroscopic techniques, such as Raman spectroscopy and UV-Vis spectroscopy can also be used, to elucidate the electronic and optical properties of nanomaterials.

In a holistic understanding of nanomaterials, the challenges are also associated with characterizing nanomaterials, such as sample preparation, agglomeration, and data interpretation. These challenges can

Authors

Dr. Deepak Singh Rajawat
Department of Chemistry
IIS (Deemed to be University)
Jaipur, India.
drdeepaksr@gmail.com

Dr. Ruchi Singh
Department of Chemistry
IIS (Deemed to be University)
Jaipur, India.
drsinghruchi2012@gmail.com

Dr. Durga K. Mewara
Department of Chemistry
M.D.S. University
Ajmer, India.
durgamewara1@gmail.com

be overcome by the use of complementary techniques and multi-mode approaches. In conclusion, this chapter underscores the importance of rigorous nanomaterial characterization in advancing fundamental understanding and unlocking the full potential of nanotechnology. It serves as a valuable resource for researchers, engineers, and scientists seeking to delve into the intricacies of nanomaterials and make informed decisions about their applications and development.

Keywords: Nanomaterials; scanning electron microscopy; nanotechnology; environmental science; drug delivery.

I. INTRODUCTION

A rapidly developing interdisciplinary field, nanotechnology has numerous applications across all branches of science and technology. The fundamentals of nanotechnology are based on the observation that materials' characteristics change substantially as particle sizes shrink to the nanoscale range. However, determining the particle size is a difficult task that presents difficulties for the scientists working on this subject. Therefore, improved control of the size and shape of the materials in the nano range has been made possible by the discovery of numerous advanced nano characterisation techniques.

The molecular reactivity of nanoscale materials increases exponentially as a result of their high surface-to-volume ratios, which frequently show characteristics that are different from those of their bulk counterparts. These features include vastly different electrical, optical, chemical, and mechanical traits. Recent research is focused on the preparation and application of nanomaterials. These nanostructures can be made using a variety of procedures, including mechanical, chemical, and other approaches. To better understand how nanomaterials behave, it is crucial to thoroughly explore a number of their properties, including surface energy, size and shape, surface charge and surface composition. [1]

Today, consumer demand for increasingly compact and potent electronic devices has greatly accelerated the advancement of nanoscience. The phrases "nanoscience" and "nanotechnology" are now used to refer to atomic-scale manipulation of matter, nanostructured technologies, and particulate science. Scientists have given nanoscience a lot of attention, but there are currently only a few commercially viable uses. However, despite all of the work being put into producing high-performance, inexpensive materials on a wide scale, nanoparticles' distinctive features are still very attractive for commercialization. In the fields of medicine, computers, energy materials, sensing, detection, and catalysis, nanoparticles themselves are of tremendous interest. [2]

The industry's ability to comply with regulations and the use of nanomaterials in commercial applications will be significantly impacted by accurate and dependable NP measurement techniques. As the production of nanoparticles increases, more accurate measurement techniques will be required. Consequently, it is necessary to thoroughly characterise the nanomaterials made using different techniques. More and more nanomaterials are being produced now compared to ten years back, which calls for the development of more precise and trustworthy methods for characterizing them. However, occasionally, such characterisation may be deficient. This is because properly studying nanoscale materials, as opposed to bulk materials, presents intrinsic difficulties. Since nanoscience and nanotechnology are multidisciplinary, not every research team can conveniently access a comprehensive range of characterization capabilities. It is frequently required to define NPs in broader terms, demanding a comprehensive approach that incorporates complementary methods. [3,4]

II. CHARACTERIZATION OF NANOPARTICLES

Nanomaterials have optical, electrical, thermal, and structural properties that rely on their size, shape, makeup, and structure. These qualities are the primary crucial factors that determine how well a nanomaterial functions physiologically. The evaluation of

nanostructured materials requires the use of specific techniques that offer the structural and compositional research of these materials. Nanostructured materials can take many different forms, including thin films, nanoparticles, and bulk nanostructures. Characterization techniques are essential for the exploration of these novel nanomaterials.

Sophisticated characterization techniques enabled us to identify the advantages and disadvantages associated with various nanomaterials. With the help of these techniques, it becomes possible to do detailed structural analysis and study molecular interactions, surface purity and elemental identifications. Most of the characterization techniques are suited for characterising nanostructures for application in the biomedical area because most are non-destructive, direct measurement equipment with repeatable results and the highest atomic-scale spatial resolution available.

The challenges associated with the analysis of nanomaterials are a shortage of suitable reference materials for the calibration of analytical tools, difficulties associated with sample preparation and the interpretation of the data. Additional unsolved issues in NP characterisation include the determination of the concentration of nanoparticles, specifically in large-scale production, as well as their analysis in complex matrices. [5,6,7]

III. MICROSCOPY TECHNIQUES

Different methods for evaluating nanomaterials using microscopy have been introduced throughout the last few years. The wavelength or source of light used to make the image has a significant impact on the microscope's ability to resolve small details. Based on the image-producing source, microscopes can be categorized into two groups: optical or light microscopes (OM) and electron microscopes (EM).

Due to its open design and simple operation, optical microscopy is employed in the characterisation and study of nanomaterials. Classical optical microscopes have not undergone an adequate assessment so far for determining the size of nanoparticles, maybe because of perceived diffraction constraints, despite the fact that optics-based approaches have the advantage of high throughput. However, employing low partial coherence and the lowest condenser aperture, traditional bright-field optical microscopes were able to view nanoparticles as small as 3 nm in diameter, including through-focus images. Clearer imaging of nanoparticles was made possible by the condition of greater coherence. The mobility of the nanoparticles was successfully tracked using through-focus optical images under the low partial coherence condition. However, measuring the size of nanoparticles does not currently employ optical microscopes.

The operating principles of an optical microscope (OM) and an electron microscope (EM) are comparable. Fundamentally and functionally, both are the same; they both include magnification, feature a condenser lens system as a source of illumination, and involve magnification. There are several other differences. The source, i.e., the fact that OM employs a focused, accelerated electron beam whereas EM uses visible light, is the main distinction. In OM, optical lenses are employed, but in EM, electron lenses are used. While the contrast in an EM is achieved either through scattering absorption (SEM) or diffraction (TEM), it is accomplished through either absorption or reflection in an OM. Compared to an EM, the diffraction characteristics of an optical microscope severely limit its resolution. EM provides information on composition and crystallography that OM cannot. With the use of both

microscopes, surface examination of the materials can be accomplished. With EM, materials are scanned on a fine and extremely small scale by focussed, accelerated electrons that can see through the sample. It produces images with a higher magnification and better resolution as a result. The confines and restrictions of OM were the primary drivers behind the development of electron microscopes. Scanning electron microscopes (SEM) and transmission electron microscopes (TEM) are the two major forms of EM that are accessible. AFM is frequently used in place of SEM and TEM to analyze NP size, shape, and surface. [8,9,10]

Some of the instrumental techniques which can be used to determine the physicochemical properties of nanomaterials are described below.

1. **Transmission Electron Microscope (TEM):** The method that is most frequently used to describe nanomaterials is TEM. It gives direct images and chemical information about nanomaterials up to atomic dimensions of spatial resolution. The size, shape, localisation, dispersity, and aggregation of NPs in 2-dimensional pictures are all shown by TEM. An electron gun produces an electron beam of high voltage to produce a picture. In the electron cannon, the tungsten filament cathode serves as an electron source. The electron beam is subsequently accelerated by the anode and focused using electromagnetic and electrostatic lenses. After passing through the thin object, the electrons from this beam either hit a fluorescent screen at the base of the microscope or scatter. The specimen is also evacuated by the electron beam. [11] These transmitted electrons then land on the fluorescent screen at the microscope's base. This results in an image shadow, with the image's constituent components showing in varied degrees of darkness depending on their density. Following that, this image can be either captured or immediately examined using a TEM. On a fluorescent viewing screen that has been covered with phosphor or scintillator material, it is seen. Following are the components of a TEM: (i) electron source, (ii) image-producing apparatus, and (iii) image-recording apparatus. [12,13]

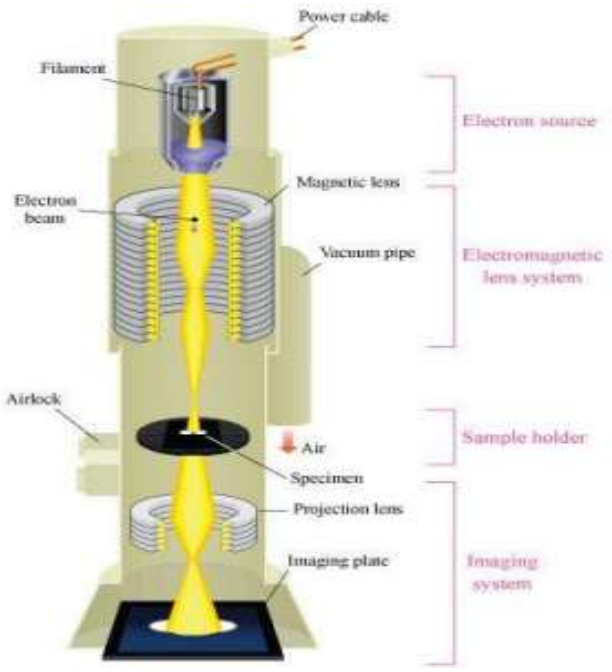

Figure 1: Layout and functioning of Transmission Electron Microscope

2. **Scanning Electron Microscope (SEM):**Scanning Electron Microscopy (SEM) has emerged as a powerful tool for the characterization of nanomaterials. Its ability to provide high-resolution imaging and surface analysis at the nanoscale has significantly contributed to the understanding of nanomaterial properties and behaviour. SEM allows researchers to visualize and study the surface morphology of nanomaterials. This is particularly important for understanding the shape, size, and distribution of nanoparticles, nanotubes, nanowires, and other nanoscale structures. By obtaining high-resolution images, researchers can observe fine details and any irregularities in nanomaterial morphology.

SEM employs a tungsten filament lamp as the source similar to the TEM. The emitted electrons are controlled by a set of lenses until they hit the sample. The interaction of the electron beam with the specimen generates signals which provide information about the surface topography and composition of the specimen. The sample is mounted and coated with a delicate layer of heavy metal elements which allows spatial scattering of electric charges on the surface of the specimen allowing better image production, with high clarity. [14] When the secondary electrons reach the detector, they strike a scintillator. It emits flashes of light which get converted into an electric current by a photomultiplier, sending a signal to the cathode ray tube. Then it produces an image. The nature of the sample has an enormous effect on the number of supplementary electrons that reach the detector. SEM offers several details about the NPs, including their size, shape, aggregation, and dispersion. Even air-dried samples can be viewed right away; SEM viewing of the specimen does not require additional handling.

Following are the components of a SEM instrument: (i) electron source, (ii) vacuum and (iii) column

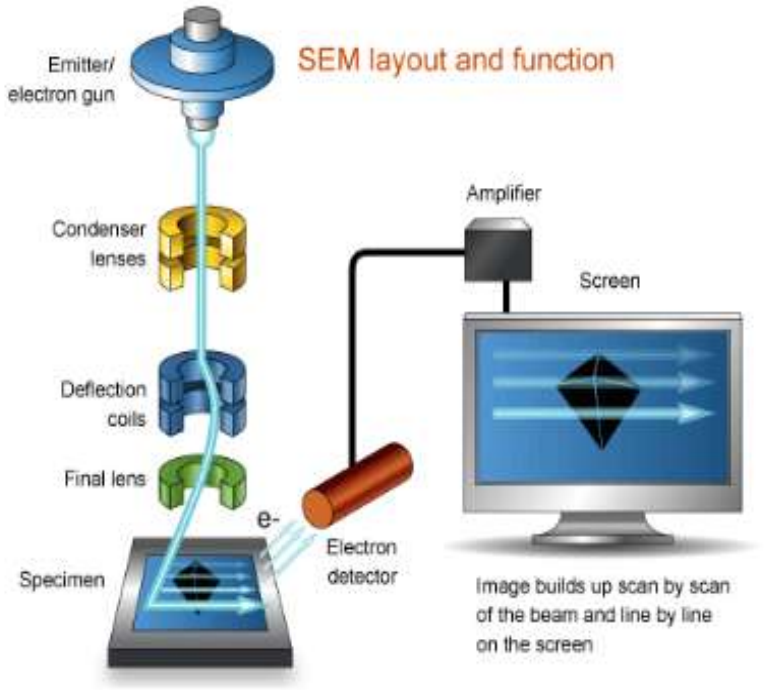

Figure 2: Layout and Functioning of Scanning Electron Microscope

Overall, the size, shape, and levels of aggregation and dispersion of nanomaterials can all be revealed by TEM and SEM. Better spatial resolution and the capacity to perform extra analytical measures are two advantages TEM has over SEM [15]. Along with TEM's benefits, there are some disadvantages. [16] A significant trade-off in TEM measurement is the need for a high vacuum and a small sample slice for electron-beam penetration.

3. **Energy Dispersive X-Ray Spectroscopy (EDX):** A scanning electron microscope (SEM) along with an EDX detector can produce more information about a sample than the SEM alone. EDX spectroscopy can be employed for the detection of the elemental composition of a substance. EDX can locate elements with atomic numbers higher than boron when they are present in concentrations of at least 0.1%. Evaluation and identification of materials, detection of contamination, analysis of spot detection zones up to 10 cm in diameter, quality control screening, and other duties are included in the use of EDX.

In a typical SEM, samples that come into contact with the electron beam interact with it and emit recognizable X-rays. It is easy to discriminate between different elements and figure out how much of each is present in the sample since no two elements have the same X-ray emission spectra. The X-ray is created when the sample atom's nucleus comes into contact with the main electron stream. When an atom is stimulated by an electron beam, it is ejected from the nucleus and leaves an electron hole. [17] An electron from the atom's outer shell, which has a higher energy, will take the place of the missing expelled electron and release the extra X-ray in the process. An X-ray continuum (produced by the slowing of electrons) and a distinctive X-ray (produced by higher shell electrons filling the electron hole in the nucleus shell) make up the X-ray that is released as shown in Fig. 3.

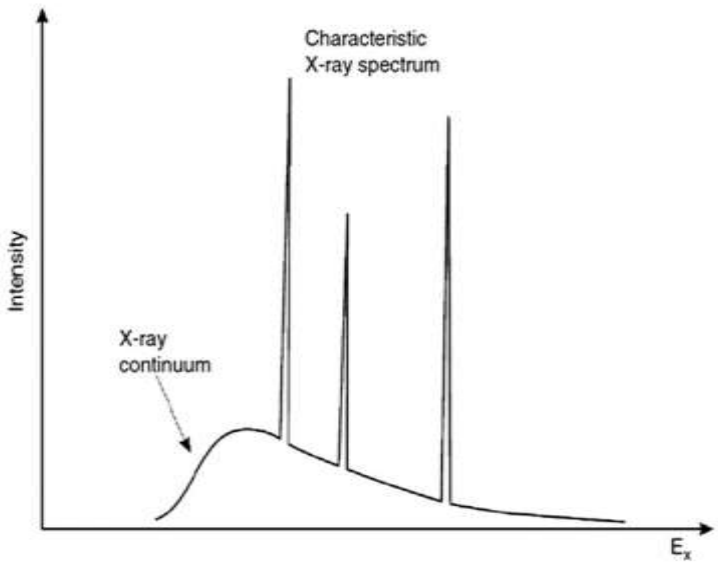

Figure 3: Sample EDX spectra

The requirement to identify the elements in the sample and differentiate them is more important than the X-ray continuum. The atomic number of the sample, the probe current, and the accelerating voltage all play a role in the X-ray continuum's intensity. On the other hand, the energy dispersive spectrometer will capture the distinctive X-ray to measure the specimen's constituent composition.

4. **Atomic Force Microscope (AFM):** Binnig, Quate, and Gerber designed the atomic force microscope (AFM), a potent high-magnification microscope, in 1986. The cantilever, an elastic probe, of the AFM gadget is linked to a very sharp, delicate tip. A force is applied to the tip once it comes into contact with the sample surface, and the strength and direction of the force depend on the proximity of the surfaces as well as the nature of the surfaces. The strength of the interaction between the tip and the surface is measured along with the relative position of the tip to build an image of interaction strength as a function of position. Depending on the specific technique used, this image may represent surface topography or chemistry. The surface points are identified as the tip sweeps the sample surface, one by one, and then they are plotted as a 3D surface on a computer screen. Atomic force microscopy involves moving a pointed physical probe quickly over a sample surface while maintaining the tip close to the sample to create high-resolution three-dimensional pictures. The tip is often built onto a cantilever beam, enabling both the system's displacement-sensing capabilities and the force-sensing tip's physical support. [18]

   To get the best accuracy of the measured data, the distance between the tip and the surface during the surface sweep must be kept within an appropriate range. The large distance results in less pulse aberration and a lower signal-to-noise ratio; whereas, the very close distances result in the insertion of large amounts of forces to the surface, which not only damages the surface and head structure but also causes. [19]

5. **X-ray diffraction (XRD):** X-ray diffraction (XRD) is a non-destructive method for the characterization of crystalline materials. It gives information on crystal texturing, optimal crystal orientations, and other structural variables such as crystallinity, average grain size, crystal defects and strain. X-ray diffraction peaks can be created by constructively interfering with a monochromatic beam of X-rays distributed at specific angles from each set of lattice planes in a sample. The X-ray diffraction pattern represents the unique hallmark of periodic atomic groupings in a material. When a monochromatic X-ray event is applied to a crystal. [20]

   When X-rays incident on a crystal the atomic electrons in the Crystal are made to oscillate at the frequency same as the incident beam. These electrons radiate at the same frequency as the incident radiations. If the incident radiation has a wavelength that is much greater than the size of the crystal, the X-rays that are emitted are then in phase with one another. However, atomic dimensions are nearly equivalent to the X-ray wavelength of the emitted radiation, the electrons are out of phase with one another. These radiations can interact positively or negatively with one another, resulting in a diffraction pattern (maxima and minima) in specific directions.

6. **Scanning Tunneling Microscopy (STM):** Scanning Tunneling Microscopy was invented by two IBM scientists Heinrich Rohrer and Gerd Binnig in 1981. It does not utilize light or electron beams to produce ultra-high-resolution images at the atomic scale. The quantum mechanical

phenomenon known as tunnelling is the basis for the working principle of the scanning tunnelling microscope (STM), in which electrons' wave-like properties allow them to "tunnel" past the surface of a solid and into regions of space that are inaccessible to them according to the laws of classical physics. This kind of tunnelling electron is extremely unlikely to be seen closer to the surface. The STM makes use of this remarkable sensitivity to distance. A tungsten needle's sharp tip is placed a few angstroms from the sample surface. Electrons tunnel through the gap when a little voltage is supplied between the probe tip and the surface. The fluctuations in the tunnelling current that are detected by the probe as it scans the surface can be used to create a topographical representation of the surface.

The imaging process built on the quantum tunnelling phenomenon is responsible for the atomic resolution of STM. STM gauges the tunnelling current I produced by the bias voltage V placed between the material surface and the atomically sharp STM tip. For every 1 reduction in distance, the tunnelling current increases by an order of the current [21]. A piezoelectric scanner that delivers angstrom-order changes in distance is used to control the distance in the x, y, and z directions. A constant tunnelling current is also ensured by a feedback loop that regulates the z-direction. Tracking the tunnelling current as the tip scans a surface yields the exact surface contour. Both room temperature and low-temperature measurements under ultra-high vacuum (UHV) circumstances are possible. By examining the tunnelling current, we may determine the local density of states (LDOS) from dI/dV and the local barrier height from dI/dz, among other electrical features of an item.

7. **Dynamic light scattering (DLS):** Dynamic light scattering (DLS) techniques have been widely used for the determination of the size distribution of nanoparticles in solutions. It just takes a few minutes to measure the size of biomolecules in solution using DLS, making it a rather rapid method. DLS allows for the separation of an aggregation sample from a homogeneous monodisperse sample. Numerous researchers studying nano-colloids rely only on it for size characterization because of its market dominance. The method is used for particle characterization in a variety of industries where particle size is important, including paint, dyes, and, more critically, biological diagnosis and medical intervention systems. The method does, however, have some application restrictions, such as those related to working capacity and data content.

DLS is frequently employed to measure the Brownian motion of discrete particles in liquids. Contrary to other forms of microscopy, the calibrated size also takes into account the hydrodynamic diameter, the effects of fluid stabilizing and wetting agents, and the thickness of the electrical double layer. DLS measures a wider size distribution than TEM and SEM, which require the transfer of nano-colloids to a medium and dehydration [22]. DLS is based on the hypothesis of the time-resolved observation of coherent light scattered by objects like large molecules or small particles. Based on the variations that are collected from various sources, the signals obtained after scattering are examined. Oscillations, that are brought on by the dispersion particles' thermal motion and occur for incredibly brief periods, are handled by DLS. To investigate phase transitions in aggregates and quantify the elastic properties of gels, DLS can monitor vibrations in nanoparticle connections. The measuring of submicron-sized particles is the most frequent application of DLS.

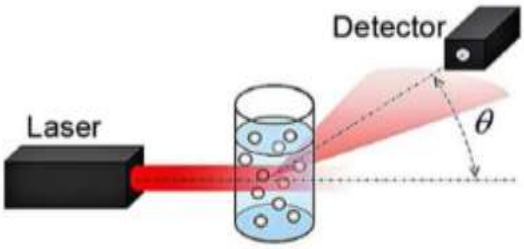

Figure 4: Schematic diagram for the working of DLS

A brief comparison of these techniques, their main strengths and limitations are summarized in the table.

Table 1: Comparison of Different Techniques used for Surface Characterization

S. No	Technique	Physicochemical characteristics analyzed	Strengths	Limitations	References
1	Transmission Electron Microscope (TEM)	Size and Shape Size distribution Heterogeneity Aggregation Dispersion	Measurement of the shape, size, and size distribution of nanomaterials with high resolution compared to SEM The electronic structure and chemical composition of nanomaterials can be investigated by coupling with other analytical methods.	Ultrathin samples are required Samples in non-physiological condition Sample damage/alternation Poor sampling Expensive equipment	Rice et al. 2013, Lin et al. 2014 (23, 14)
2	Scanning Electron Microscope (SEM)	Shape, Size and size distribution, Aggregation Dispersion	Measurement of the size, shape and size distribution of nanomaterials High-resolution Images of biomolecules in the natural state	Sample must be conducting, conductive coating is required Requirement of dry samples	Bals et al. 2023, Bryan et al 2011, Lin et al. 2014, (24, 25, 14)

				Non-physiological conditions in sample analysis (except ESEM) In heterogeneous samples biased results of size distribution Expensive equipment	
3	EDX	Elemental composition	High speed of data collection Elemental coverage for almost all elements Surface sensitive	Accuracy decreases when moving from the heavier elements to lighter elements Poor energy resolution in many cases	Kepekçi et al 2021, Burdet et al 2015 (26, 27)
4	Atomic Force Microscope (AFM)	Size and size distribution Shape Structure Sorption Dispersion Aggregation Surface properties (modified AFM)	3D sample surface mapping Sub-nanoscaled topographic resolution Samples in aqueous, dry or ambient environment	Time-consuming Poor sampling Broad analysis restricted to a nanomaterial's surface	Mourdikoudis et al. 2018, Akhtar et al. 2019, Baalousha et al 2013, Lin et al. 2014 (28, 29, 30, 14)
5	XRD	Size, shape and structure for crystalline materials	Atomic-scale spatial resolution that is high	Only the single conformation/ binding state of the sample is accessible	Zhang et al 2016 , Mourdikoudis et al 2018 (31, 28)

				Low intensity compared to electron diffraction	
6	STM	Structure, Shape, Size and Size Distribution Dispersion Aggregation	High spatial resolution at the atomic scale	Conductive surface required	Khan et al 2016, Kokab et al 2019 (32, 33)
7	Dynamic light scattering (DLS)	Hydrodynamic size distribution	Non-destructive/invasive technique Rapid and more reproducible measurement Measurements can be in any liquid media, Hydrodynamic sizes are accurately determined for monodisperse samples	Insensitive relationship between size fractions and a particular composition Impact of small numbers of massive particles Measurement ceiling for polydisperse samples limited resolution in size	Giambruno et al 2022, Jia et al 2023, Caputo 2019 (34, 35, 36)

In contrast to their equivalents in bulk materials, the distinct physicochemical properties of nanomaterials—such as size, surface qualities, shape, composition, molecular weight, identity, purity, stability, and solubility—have a critical role in determining particular physiological interactions. A review of the several techniques for characterizing nanomaterials according to their diverse properties is given in Table 2.

Table 2 : Physicochemical Characteristics of Nanomaterials and Suitable Evaluation Modalities

Parameter Studied	Technique used	References
Size Distribution	SEM, TEM, XRD, UV-Vis, DLS	Awwad et al.(2020), Demissie et al.(2020), Aziz et al.(2020), Verma et al.(2020), Mustapha et al.(2020), Phuruangrat et al. (2021), (37, 39, 40, 41, 42)

Shape and Size	XRD, SEM, TEM	Baaloudj et al.(2021), Patel et al.(2021), Pillai et al.(2020), Kuriakose et al.(2020), Verma et al (2020), Santhi et al. (2020), Kennan et al.(2020), Vazques et al.(2021) (43, 44, 45, 46, 47, 48, 49, 50)
Composition	EDX, ICP-OES	Awwad et al.(2016), Demissie et al.(2020), Aziz et al.(2020), Verma et al.(2020), Mustapha et al. (2021)
Surface Properties	FTIR, MS, XPES	Awwad et al.(2020), Vazquez et al.(2021)
Structure	XPS, Raman, XRD	Verma et al.(2020), Mustapha et al.(2020), Phuruangrat et al. 2021
Stability	TGA	Mohanan et al.(2020),
Dispersion	SEM, TEM, STM	Lin et al.(2019), Bezza,et al.(2020), Poperenko et al.(2020) (51, 52, 53)

8. **Other techniques:** The list above does not include all of the widely employed spectroscopic methods for examining the physicochemical properties of nanomaterials. Utilising UV-visible absorbance spectroscopy is one such method. When the absorption profiles of the nanomaterials differ, it is utilised to explore the properties of the nanomaterials such as size, concentration, aggregation and even bioconjugation. Fluorescence spectroscopy (FS) is a useful tool for exploring the binding of ligands or modifications to macromolecule conformational changes due to its sensitivity to the chromophore's surroundings. FS can be used to analyse the concentration, particle size, and spacer composition of biomolecules on the NP surface. [54, 55]

   There are several thermal approaches which can be employed to evaluate the amount of conjugated nanomaterials and their thermal stability. [56] Thermal gravimetric analysis (TGA) is a technique that can be used to track the weight change that is temperature-dependent in bulk materials, such as different nanomaterial bioconjugates .[57] Differential scanning calorimetry (DSC) can be used for the determination of melting, crystallisation, glass transition, and decomposition of nanomaterial-bioconjugates; consequently, analysis of the DSC measurements afterwards can reveal the structure and stability of the subject material. [58]

   Centrifugation methods, such as analytical ultracentrifugation, can be utilized to evaluate the structure, conformation, stoichiometry and self-aggregation state of these materials in addition to evaluating size/size distribution, shape, and molecular weight of nanomaterials. [59] Chromatography methods such as hydrodynamic chromatography (HDC) and high-performance liquid chromatography (HPLC) can be used to purify nanomaterial bioconjugates when paired with reverse-phase, ion-exchange-phase, or size-exclusion-phase columns. [60, 61].

IV. CONCLUSION

In this comprehensive review, we have explored the fascinating field of nanomaterial characterization in depth. Numerous fields, including electronics, medicine, energy, and

environmental science, have been transformed by nanomaterials. Surfaces and interfaces of nanomaterials play a predominant role in determining their properties. Understanding their properties at the nanoscale is paramount for harnessing their full potential. Characterization techniques have emerged as a crucial link between synthesis and application, providing researchers with the tools they need to understand the complex properties of nanomaterials.

The characterization of nanomaterials necessitates the utilization of several analytical approaches. Before evaluating the physical and chemical properties, such as size, shape, aggregation state, surface coating, elemental composition, and oxidation state, it is necessary to ascertain the type of nanomaterial. Crystallinity and size distribution have been shown by structural characterization techniques like X-ray diffraction (XRD) and Transmission Electron Microscopy (TEM). Surface roughness, porosity and functionalization can be investigated by Surface characterization techniques Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Scanning Tunneling Microscopy (STM) is essential for examining atomic-scale surface features whereas Energy Dispersive X-ray Spectroscopy (EDX) finds widespread application in nanomaterial characterization, enabling precise elemental analysis for materials across diverse fields. Sample preparation, instrumentation constraints, and the interpretation of complex data can pose hurdles. Furthermore, a careful evaluation of the safety and environmental impact of nanomaterials is necessary.
To get precise and reproducible data regarding NPs in ever-more complicated matrices, it is constantly required to develop new characterization procedures and enhance those that already exist.

REFERENCES

[1] S. Mourdikoudis, R. M. Pallares and N.T.K Thanh, " Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties" Nanoscale, 10, 12871-12934, 2018.
[2] A. Lagashetty, A. Bhavikatti, Nanotechnology-an overview. Mater. Sci. Indian J. Rev.,5:274–276, 2019
[3] P. Prieto, V. Nistor, K. Nouneh, M. Oyama, M. Abd-Lefdil and R. Diaz, Appl. Surf. Sci., 258, 8807, 2012
[4] W. Wang, X. Chen, Q. Cai, G. Mo, L. S. Jiang, K. Zhang, Z. J. Chen, Z. H. Wu and W. Pan, Eur. Phys. J. B, 65, 57, 2008
[5] S. Sharma, S. Jaiswal, B. Duffy, A. K. Jaiswal, "Nanostructured Materials for Food Applications: Spectroscopy, Microscopy and Physical Properties" Bioengineering, 6, 26, 2019
[6] V. Parkash, A. Gaur, R. Agnihotri, "Nanoparticles from Fungal Resources: Importance and Applications" Springer International Publishing: Cham, Switzerland, pp. 1–18, 2020
[7] W. Jiang, K. Yang, R.W. Vachet, B. Xing, "Interaction between Oxide Nanoparticles and Biomolecules of the Bacterial Cell Envelope as Examined by Infrared Spectroscopy", Langmuir, 26, 18071–18077, 2010
[8] T. L. Hayes, R. F. W. Pease, " The scanning electron microscope: Principles and applications in biology and medicine. Advances in biological and medical physics, 12, 85– 137, 1968.
[9] S. L. Pal, U. Jana, P. K. Manna, G. P. Mohanta, and R. Manavalan, "Nanoparticle: an overview of preparation and characterization," Journal of Applied Pharmaceutical Science, vol. 1, no. 6, pp. 228–234, 2011.
[10] R. Joerger, T. Klaus, and C. G. Granqvist, "Biologically produced silver carbon composite materials for optically functional thin-film coatings," Advanced Materials, vol. 12, no. 6, pp. 407–409, 2000.
[11] M. Kannan, "Transmission Electron Microscope -Principle, Components and Applications Illumination system (Electron gun and condenser lenses) Electron gun" 2018
[12] K. Kanakamedala, "Characterization of Tin-Oxide (SnO 2 -Ni) Based Sensors" International Journal of Emerging Technology and Advanced Engineering, 9, 91-100, 2019
[13] H.H. Radamson, "Electron Microscopy. In: Analytical Methods and Instruments for Micro- and Nanomaterials" Lecture Notes in Nanoscale Science and Technology, vol 23. Springer, Cham., 2023
[14] C. Lin, S. Lin, P.C. Wang & R. Sridhar, "Techniques for physicochemical characterization of nanomaterials. Biotechnology Advances" 32(4), 711, 2014

[15] J. B. Hall M.A. Dobrovolskaia, A.K. Patri, S.E. McNeil, "Characterization of nanoparticles for therapeutics" Nanomedicine (London), 2:789–803,2007.
[16] D. B. Williams, C.B. Carter, "The transmission electron microscope", Transmission electron microscopy. Springer; 20093–22.
[17] M. Abd. Mutalib, M.A. Rahman, M.H.D. Othman, A.F. Ismail, J. Jaafar, Chapter 9 - Scanning Electron Microscopy (SEM) and Energy-Dispersive X-Ray (EDX) Spectroscopy, Elsevier, 161-179, 2017
[18] Y.F. Dufrene. Atomic force microscopy, A powerful tool in microbiology. J Bacteriol, Oct;184 (19):5205-13,2002.
[19] N. A. Amro, L. P. Kotra, K. Wadu-Mesthrige, A. Bulychev, S. Mobashery, and G.-Y. Liu. **"**High-resolution atomic force microscopy studies of the Escherichia coli outer membrane: structural basis for permeability", Langmuir **16:**2789-2796, 2010
[20] Y. Higashi, T. J. Smith, J. M. Jez, T. M. Kutchan, "Crystallization and Preliminary X-ray Diffraction Analysis of Salutaridine Reductase from the Opium Poppy Papaver somniferum." Acta Crystallogr.Sect. F Struct. Biol. Cryst. Commun, 66(Pt 2), 163–166, 2010.
[21] Chu, Ping, and D. L. Mills. "Plasmonic Response of STM Tips." Physical Review B - Condensed Matter and Materials Physics 2011
[22] G. Oskam, "Metal Oxide Nanoparticles: Synthesis, Characterization and Application", J. Sol. Gel Sci. Technol. 37, 161–164, 2006
[23] J. Bals, M. Epple, "Deep learning for automated size and shape analysis of nanoparticles in scanning electron microscopy" RSC Adv., 19;13(5):2795-2802, 2023
[24] M. Bryan Tyson, K. Abu Al-Rub Rashid, Ardavan Yazdanbakhsh, Zachary Grasley, "A quantitative method for analyzing the dispersion and agglomeration of nano-particles in composite materials", Composites Part B: Engineering, Volume 42, Issue 6, 1395-1403, 2011
[25] H. Safari, J. Bruce J. Balcom, A. Afrough, "Characterization of pore and grain size distributions in porous geological samples – An image processing workflow," Computers & Geosciences, 156, 104895, ISSN 0098-3004,2021
[26] S.B. Rice, C. Chan, S.C. Brown, P. Eschbach, L.Han, D.S. Ensor, A.B. Stefaniak, J. Bonevich, A.E. Vladár, A.R. Hight Walker, "Particle size distributions by transmission electron microscopy: an interlaboratory comparison case study", Metrologia.50(6):663-678,2013
[27] M. Shadpour, R. Shima, 4 - Microscopic characterization techniques for layered double hydroxide polymer nanocomposites, In Woodhead Publishing Series in Composites Science and Engineering, 157-203,2020
[28] S. Mourdikoudis, R. M. Pallares, and N. T. K. Thanh, "Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties" Nanoscale,**10**, 12871-12934, 2018
[29] I. Akhtar, M. A. Rehman, W. Choi, "Three-dimensional atomic force microscopy for ultra-high-aspect-ratio imaging," Applied Surface Science, 469, 582-592, 2019
[30] M. Baalousha, J.R. Lead, "Characterization of natural and manufactured nanoparticles by atomic force microscopy: Effect of analysis mode, environment and sample preparation, Colloids and Surfaces A: Physicochemical and Engineering Aspects", 419, 238-247,2013
[31] Xi. F. Zhang, Z. G. Liu, W. Shen and S. Gurunathan. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. Int J Mol Sci.,17(9): 1534,2016
[32] M.K. Khan, Q.Y. Wang, M.E. Fitzpatrick, 1 - Atomic force microscopy (AFM) for materials characterization, Editor(s): Gerhard Hübschen, Iris Altpeter, Ralf Tschuncky, Hans-Georg Herrmann, Materials Characterization Using Nondestructive Evaluation (NDE) Methods, Woodhead Publishing, 1-16,2016
[33] T. Kokab, A. Munir, A. Shah, S. Kurbanoglu, M. A. Zia, S. A. Ozkan, "The Effect of Nanomaterials on the Drug Analysis Performance of Nanosensors" Editor(s): Sibel A. Ozkan, Afzal Shah, New Developments in Nanosensors for Pharmaceutical Analysis, Academic Press, 79-118,2019
[34] R. Giambruno, J. Rupert, E. Zacco, "Experimental methods to study protein-nucleic acid interactions" Editor(s): Timir Tripathi, Vikash Kumar Dubey, Advances in Protein Molecular and Structural Biology Methods, Academic Press, 137-161,2022
[35] Z. Jia, J. Li, L. Gao, D. Yang, A. Kanaev, Dynamic Light Scattering: A Powerful Tool for in Situ Nanoparticle Sizing. Colloids Interfaces 7, 15,2023
[36] F. Caputo, A. Arnould, M. Bacia, W.L. Ling, E. Rustique, I. Texier, A. P. Mello, and A. Couffin, "Measuring Particle Size Distribution by Asymmetric Flow Field Flow Fractionation: A Powerful Method for the Preclinical Characterization of Lipid-Based Nanoparticles" Mol Pharm. 16(2): 756–767, 2019

[37] M. Awwad, M. Nidá Salem, M. Marwa, M. Aqarbeh and M. Abdulaziz. Green synthesis, characterization of silver sulfide nanoparticles and antibacterial activity evaluation. Chemistry International, Volume 6, Issue 1, 2020.
[38] M. G. Demissie, K.S. Fedlu, Gemechu Deressa Edossa, synthesis of zinc oxide nanoparticles using leaf extract of *Lippia adoensis* (Koseret) and evaluation of Its antibacterial activity, 2020
[39] C. Aziz, A. Bruska, synthesis of dysprosium doped cobalt ferrites nanoparticles by sol-gel auto-combustion method and influence of grinding techniques on structural, morphological, and magnetic properties, Journal of Magnetism and Magnetic Materials, 542, 2022
[40] M. Verma, M. Mitan, H. Kim, D. Vaya, efficient photocatalytic degradation of Malachite green dye using facilely synthesized cobalt oxide nanomaterials using citric acid and oleic acid, Journal of Physics and Chemistry of Solids, 155, 110125, 2021
[41] S. Mustapha, J.O. Tijani, M.M. Ndamitso, facile synthesis and characterization of TiO2 nanoparticles: X-ray peak profile analysis using Williamson–Hall and Debye–Scherrer methods. Int Nano Lett 11, 241–261, 2021
[42] A. Phuruangrat, S. Thongtem & T. Thongtem, synthesis, characterization, and UV light-driven photocatalytic properties of $CeVO_4$ nanoparticles synthesized by sol-gel method, Journal of the Australian Ceramic Society 57:597–604,2021
[43] O. Baaloudj & N. Nasrallah, A comparative study of ceramic nanoparticles synthesized for antibiotic removal: catalysis characterization and photocatalytic performance modelling Environmental Science and Pollution Research, 28,13900–13912, 2021
[44] H. G. Patel, S.H. Chaki, M. Rohitkumar, M. Kannaujiya, R. Zubin & M.P. Deshpande, Sol-gel synthesis and thermal characterization of SnO_2 nanoparticles, Physica B: Condensed Matter, 613, 412987, 2021
[45] A.M. Pillai, V.S. Sivasankarapillai, A. Rahdar, J. Joseph, Green synthesis and characterization of zinc oxide nanoparticles with antibacterial and antifungal activity, Journal of Molecular Structure, 1211, 128107,2020
[46] S. Kuriakose, H. Hitha, A. Jose, M. John, T. Varghese, Structural and optical characterization of lanthanum tungstate nanoparticles synthesized by chemical precipitation route and their photocatalytic activity, Optical Materials, 99, 109571,2020
[47] J. Verma, A. S. Khanna, R. Sahney and A. Bhattacharya. Super protective anti-bacterial coating development withsilica–titania nano core–shells. *Nanoscale Adv.*, **2**, 4093-4105,2020
[48] K. Santhi, M. Navaneethan, S. Harish, S. Ponnusamy, C. Muthamizhchelvan, Synthesis and characterization of TiO_2 nanorods by hydrothermal method with different pH conditions and their photocatalytic activity, Applied Surface Science, Volume 500,144058, 2020
[49] K. Kannan, D. Radhika, A.S Nesaraj, K. K. Sadasivuni, K.R. Reddy, D. Kasai, V. Raghu, Photocatalytic, antibacterial and electrochemical properties of novel rare earth metal oxides-based nanohybrids, Materials Science for Energy Technologies, Volume 3,853-861, 2020
[50] M. Gomez-Vazquez, B. A. Correa-Piña, L. F. Zubieta-Otero, A. M. Castillo-Paz, S.M. Londoño-Restrepo, M. E. Rodriguez-García, synthesis and characterization of bioinspired nano-hydroxyapatite by wet chemical precipitation, Ceramics International, 47, 23, 32775-32785,2021
[51] T.C. Lin, C. Cao, M. Sokoluk, Aluminum with dispersed nanoparticles by laser additive manufacturing. Nat Commun **10**, 4124, 2019.
[52] F. A. Bezza, S.M. Tichapondwa, & E.M.N. Chirwa, Fabrication of monodispersed copper oxide nanoparticles with potential application as antimicrobial agents. Sci Rep **10**, 16680 2020
[53] L. Poperenko, S. Rozouvan, V. Staschuk, "Third-order Nonlinear Dispersion Properties of Surface Plasmon Resonance in Gold Nanoparticles. Journal of Nano and Electronic Physics", 12(3), 03031, 2020
[54] F. H. Rodrigues, G. G. Delgado, T. S. Costa, L. Tasic, "Applications of fluorescence spectroscopy in protein conformational changes and intermolecular contacts" BBA Adv. 28;3:100091, 2023.
[55] S. J. Hurst, A. K. R. Lytton-Jean, C. A. Mirkin. Maximizing DNA loading on a range of gold nanoparticle sizes. Anal Chem.,78:8313–8318,2006
[56] J.M. Rami, C.D. Patel, C.M. Patel, M.V. Patel, "Thermogravimetric analysis (TGA) of some synthesized metal oxide nanoparticles," Materials Today: Proceedings, Volume 43, Part 1,655-659,2021
[57] H. S. N. Jayawardena, S. H. Liyanage, K. Rathnayake, U. Patel, M. Yan, "Analytical Methods for Characterization of Nanomaterial Surfaces. Anal Chem, 2;93(4):1889-1911,2021
[58] P. Gill, T. T. Moghadam, B. Ranjbar, "Differential scanning calorimetry techniques: applications in biology and nanoscience," J Biomol Tech.,21(4):167-93,2010
[59] G. B. Edwards, U. M. Muthurajan, S. Bowerman, K. Luger, "Analytical Ultracentrifugation (AUC): An Overview of the Application of Fluorescence and Absorbance AUC to the Study of Biological Macromolecules," Curr Protoc Mol Biol.,133(1):e131,2020

[60] B. W. J. Pirok, N. Abdulhussain, T. Aalbers, B. Wouters, R. A. H. Peters, and P. J. Schoenmakers, "Nanoparticle Analysis by Online Comprehensive Two-Dimensional Liquid Chromatography combining Hydrodynamic Chromatography and Size-Exclusion Chromatography with Intermediate Sample Transformation," Anal. Chem., 89, 17, 9167–9174,2017

[61] C. Fornaguera and C. Solans, "Analytical Methods to Characterize and Purify Polymeric Nanoparticles" International Journal of Polymer Science, 2018.

