Latest Frontiers in Nanomaterial Characterization

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**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 behavior 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 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

1. **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 in 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. As a result of their academic interest and the potential technical applications in numerous domains, they can be the subject of considerable research. There are numerous techniques that can be used to create these nanostructures, including mechanical, chemical, and other approaches. To better understand how nanomaterials behave, it is crucial to thoroughly explore a number of their properties, including size, surface composition, surface energy, surface charge, and shape [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 medical, computers, energy materials, sensing, detection, and catalysis, nanoparticles themselves are of tremendous interest [2].

The use of these materials in commercial applications and the ability of the industry to adhere to laws will both be significantly impacted by reliable and trustworthy measurement methods for NPs. More dependable measurement methods will be needed as nanoparticle production scales up. Therefore, it is essential to fully characterize the nanomaterials created in various methods. In addition, we do not present only techniques that one might classify as ‘common’, but we also show examples of modern *in situ operando* techniques that are used to monitor the kinetics of nanoparticle formation and study through some recent advances in the topic the controlled defects that affect nanoparticle properties in a crucial manner.

A greater variety and quantities of nanomaterials are being created now than just ten years ago, necessitating the creation of more accurate and reliable techniques for their characterisation. Such characterization can, however, occasionally be lacking. This is due to the inherent challenges that come with adequately analyzing nanoscale materials as opposed to bulk materials (e.g., too small size and low number in some situations after laboratory-scale manufacture). In addition, not every research team can easily access a wide variety of characterization facilities due to the multidisciplinary nature of nanoscience and nanotechnology. In fact, it is frequently necessary to characterize NPs more broadly, necessitating a comprehensive strategy that combines complimentary approaches. [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 with appropriate spatial resolutions. 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.

With the aid of sophisticated characterization techniques and accurate results, it is possible to identify the advantages and disadvantages of specific nanomaterials. With various characterization techniques, it is simple to undertake detailed structural analysis, molecular interactions, surface purity analysis, and elemental identifications. As a result, additional special characterization methods are required in addition to the standard procedures available in materials research. The majority of characterization methods are non-destructive, direct measurement instruments, reproducible measurements and have the highest possible atomic-scale spatial resolution; as a result, they are particularly well suited for characterizing nanostructures for use in the biomedical field.

However, due to the interdisciplinary nature of the field, the lack of appropriate reference materials for the calibration of analytical tools, the challenges associated with sample preparation for analysis, and the interpretation of the data, there are significant challenges in the analysis of nanomaterials. The measurement of NP concentration in situ and online, particularly in a scaled-up production, as well as their analysis in complicated matrices are additional unsolved problems in NP characterization [5,6,7].

**III. MICROSCOPY TECHNIQUES**

Different methods for evaluating nanomaterials using microscopy have been introduced throughout the course of 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. On the basis of the image-producing source, microscopes can be categorized into two groups: optical or light microscopes (OM) and electron microscopes (EM).

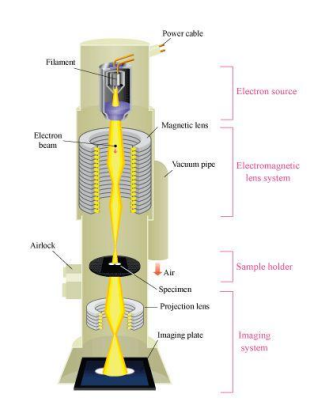
Due to its open design and simple operation, optical microscopy is employed in the characterisation and study of nanomaterials. Conventional optical microscopes have not been seriously examined so far for determining the size of nanoparticles, maybe because of perceived diffraction constraints, despite 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 clearly 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 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. In EM with short wavelengths, the employment of an accelerated electron beam causes diffraction effects to occur at considerably lower physical dimensions, aiding in the resolution of atomic features with sizes ranging from nanometers to micrometers in particle size. 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 modalities used to examine the specific physicochemical properties of nanomaterials are described below.

1. **Transmission Electron Microscope (TEM)**

TEM is the technique that is most frequently used to characterize nanomaterials. At a spatial resolution down to the level of atomic dimensions ( 1 nm), TEM gives direct images and chemical information about nanomaterials. The size, shape, localisation, dispersity, and aggregation of NPs in 2-dimensional pictures are all shown by TEM. A high-voltage electron beam produced by an electron gun is used in TEM to produce a picture. In the electron cannon, the tungsten filament cathode serves as an electron source. The anode then accelerates the electron beam, which is then focussed using electrostatic and electromagnetic lenses. The electrons from this beam then flow through the thin object and either scatter or strike a fluorescent screen at the microscope's base. The electron beam also leaves the specimen.[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. The functioning parts of a TEM are as follows: (i) an electron gun; (ii) an image-producing system; and (iii) an image-recording system [12,13].



**Figure 1: Layout and functioning of Transmission Electron Microscope**

**Sample Preparation-**

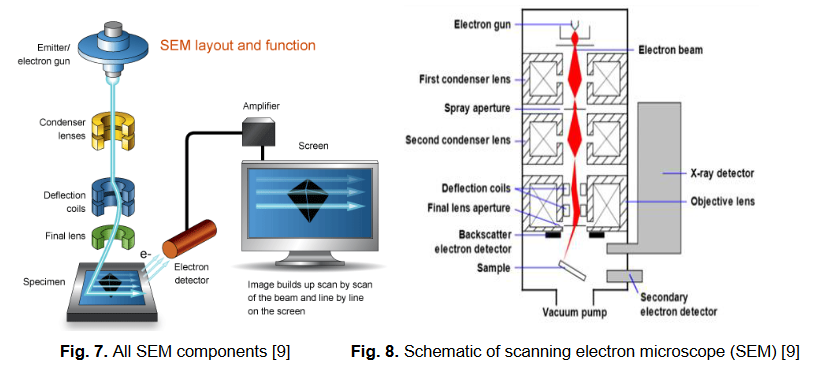
* Transmission electron microscopy or TEM works on the principle that electrons possess a wave-like character. The electrons are made to pass through the specimen and the image is formed on the fluorescent screen, either by using the transmitted beam or by using the diffracted beam.
* Electrons are emitted with increased acceleration potential from a heated filament into the vacuum. These will have a small wavelength. They have energy high enough to penetrate several microns distance in solids.
* When these electrons get focused, images with higher resolution are formed.

1. **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 tungsten filament lamp as the source similar to the TEM. The emitted electrons are controlled by a set of lenses until it hit the sample. The interaction of beam of electrons with the specimen generates signals which give 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 quantity of secondary electrons that enter the detector is greatly defined by the nature of the specimen. SEM provides different information about the NPs such as size, shape, aggregation, and dispersion. Even air-dried samples can be examined directly, the specimen does not need special treatment for visualization under the SEM.

SEM is an electronic and optical system which consists of the following components: (i) Electron gun (ii) Vacuum (iii) Column: condenser lens, scanning coil, objective lens, stigmator, sample holder and detector.



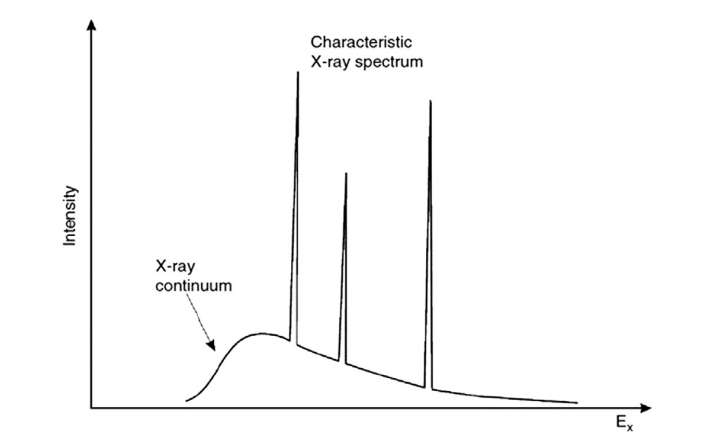
**Figure 2: Layout and Functioning of Scanning Electron Microscope**

Overall, the size, shape, and levels of aggregation and dispersion of nanomaterials can all be revealed by TEM and SEM both. 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]. The requirement for a high vacuum and narrow sample slice for electron-beam penetration in TEM measurement is a substantial trade-off.

1. **Energy Dispersive X-Ray Spectroscopy (EDX)**

EDX is used together with a scanning electron microscope (SEM), an EDX detector can generate more information about a sample than an SEM can alone. EDX spectroscopy can be employed for the detection of the elemental composition of a substance. When present in concentrations of at least 0.1%, elements with atomic numbers higher than boron can be discovered using EDX. The use of EDX comprises evaluating and identifying materials, identifying contamination, analyzing spot detection zones up to 10 cm in diameter, screening for quality control, and other tasks.

In a standard SEM, when samples collide with the electron beam, they interact with the beam and emit distinctive X-rays. Since no two elements have the same X-ray emission spectra, it is possible to distinguish between them and determine each element's concentration in the sample. The main electron beam's contact with the sample atom's nucleus produces the X-ray. When an atom's electron is excited by a primary electron beam, it is ejected from the nucleus and leaves an electron-hole [17]. The missing expelled electron will be replaced by an electron from the atom's outer shell (which has a higher energy), releasing the extra X-ray in the process. The emitted X-ray consists of an X-ray continuum (generated by the deceleration of electrons) and a characteristic X-ray (generated as a result of higher shell electrons filling the electron hole in the nucleus shell) 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.

1. **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. In order to create an image of interaction strength as a function of position, the strength of the interaction between the tip and the surface is measured along with the relative position of the tip. 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].

The distance between the tip and the surface during the surface sweep must be kept within an appropriate range in order to obtain the highest accuracy of the measured results. On the one hand, the large distance leads to a decrease in pulse aberration and decrease in signal-to-noise ratio; on the other hand, the very close distances lead to the insertion of large amounts of forces to the surface, which not only results in damages to the surface and head structure but also causes. For instance, the distance is adjusted as many angstroms to provide the optimum results in the contact mode of the AFM (repellant force between head and surface). The distance between the tip and the surface remains constant during constant surface scanning by an electronic feedback circuit [19].

1. **X-Ray Diffraction (XRD)**

A potent nondestructive method for characterizing crystalline materials is X-ray diffraction (XRD). It offers details on crystal textures, optimum orientations for crystals, and other structural factors like average grain size, crystallinity, strain, and crystal defects. It also offers details on structures and phases. By constructively interfering with a monochromatic beam of X-rays dispersed at particular angles from each set of lattice planes in a sample, one can create X-ray diffraction peaks. The distribution of atoms within the lattice controls the peak intensities. As a result, the X-ray diffraction pattern represents a material's unique signature of periodic atomic groupings. when a crystal is subjected to a monochromatic x-ray event [20]. The Crystal's atomic electrons are made to vibrate. Acceleration occurs with the same frequency as the incident ray's frequency. Then, these accelerated electrons radiate in all directions at the same frequency as the incident x-rays. if the incident radiation has a wavelength that is significantly larger than the crystal's size. The radiated X-rays are then in phase with one another. But since atomic dimensions are almost identical to X-ray wavelength. The electrons are out of phase with one another as they produce radiation. These radiations can interact with one another either positively or negatively, producing a diffraction pattern (maxima and minima) in particular directions.

### Scanning Tunneling Microscopy (STM)

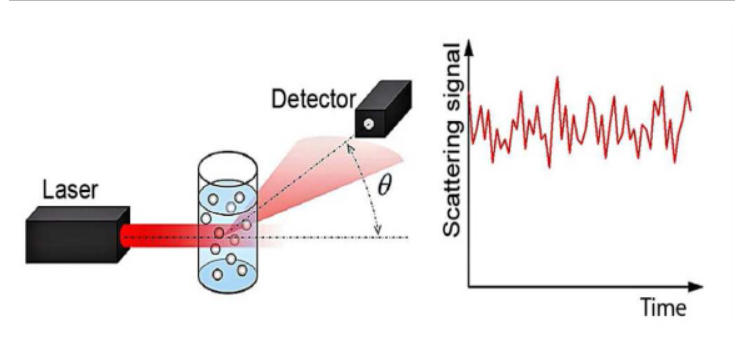
### So it is an imaging technique used to obtain ultra-high resolution images at the atomic scale, without using light or electron beams. STM was invented in 1981 by two IBM scientists named Gerd Binnig and Heinrich Rohrer. The principle of operation of the scanning tunneling microscope (STM), a type of microscope, is based on the quantum mechanical phenomenon known as tunneling, in which the wavelike characteristics of electrons 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. As the distance from the surface grows, the likelihood of detecting such tunneling electrons drops exponentially. This extraordinary sensitivity to distance is used by the STM. A few angstroms from the sample surface, a tungsten needle's sharp tip is placed. Electrons tunnel through the gap when a little voltage is supplied between the probe tip and the surface. The fluctuations in the tunneling 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 tunneling phenomenon is responsible for the atomic-resolution of STM. STM gauges the tunneling 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 tunneling 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 feedback loop that controls the z-direction is also used to maintain a constant tunneling current. The precise surface shape can be determined by tracking the tunneling current as the tip scans a surface.Measurements can be carried out at room temperature in solution as well as at low temperature under ultra-high vacuum (UHV) conditions. We can obtain various electrical properties of an object by analyzing the tunneling current, such as the local density of states (LDOS) from d*I*/d*V* and local barrier height from d*I*/d*z*.

### Dynamic light scattering (DLS)

### Dynamic light scattering (DLS) methods have been chosen as the major approach for measuring the size distribution of nanoparticles in liquid solutions on the bench. DLS can be used to rather quickly determine the size of biomolecules in solution; measurements only take a few minutes. A homogeneous monodisperse sample and an aggregation sample can be distinguished by DLS. Numerous researchers studying nano-colloids rely only on it for size characterisation 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.

### For the purpose of characterizing particle size, 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 really 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 macromolecules or small particles. Based on the variations that are collected from various sources, the signals obtained after scattering are examined. These oscillations, which are brought on by the dispersion particles' thermal motion and occur for incredibly brief periods, are handled by DLS. In order to investigate phase transitions in aggregates and quantify the elastic properties of gels, DLS may, for example, monitor the 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, size distribution of nanomaterials with high resolution compared to SEM  Electronic structure and chemical composition of nanomaterials can be investigated by coupling with other analytical methods. | Ultrathin samples in 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 natural state | Sample must be conducting, otherwise conductive coating is required  Requirement of dry samples  Non-physiological conditions in sample analysis (except ESEM)  In heterogeneous samples biased results of size distribution  Expensive equipment | Bals et al. 2023, Bryan et al 2011, Lin et al. 2014,  (24, 25, 14) |
| 3 | EDX | Elemental composition | High speed of data collection  Elemental coverage for almost  all elements  Surface sensitive | Accuracy decreases moving from the heavier elements to lighter elements  Poor energy resolution in many cases | Kepekçi et al 2021, Burdet et al 2015  (26, 27) |
| 3 | 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  Sample analysis in dry, aqueous or ambient environment | Overestimation of lateral dimensions  Poor sampling and time consuming  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) |
| 4 | XRD | Size, shape and structure for crystalline materials | Atomic-scale spatial resolution that is high | Only single conformation/binding state of sample accessible  Low intensity compared to electron diffraction | Zhang et al 2016 , Mourdikoudis et al 2018  (31, 28) |
| 5 | STM | Structure, Shape, Size and size distribution Dispersion Aggregation | High spatial resolution at atomic scale | Conductive surface required | Khan et al 2016, Kokab et al 2019  (32, 33) |
| 6 | 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 accurately determined for monodisperse samples | Insensitive correlation of size fractions with a specific composition,  Influence of small numbers of large particles  Limit in polydisperse sample measures Limited size resolution | Giambruno et al 2022, Jia et al 2023, Caputo 2019  (34, 35, 36) |

The unique physicochemical characteristics of nanomaterials, such as size, surface qualities, shape, composition, molecular weight, identity, purity, stability, and solubility, are crucially related to specific physiological interactions compared to those of their bulk material counterparts. Table 2 provides a summary of the many methods used for the characterization of nanomaterials based on their various characteristics.

**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) |

1. **Other techniques**

The list above does not include all of the widely used 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 are distinct, it is utilised to explore the properties of the nanomaterials, such as size, concentration, aggregation state, and even bioconjugation. [1].

Fluorescence spectroscopy (FS), in general, is a effective technique for pursuing the ligand binding or conformational changes of macromolecules due to its sensitivity to the environment of the chromophore [54]. FS can be used to determine the characteristics of biomolecule on the NP surface, including concentration, particle size, and spacer composition

Fluorescence spectroscopy (FS) is a useful tool for exploring the ligand binding or conformational changes of macromolecules due to its sensitivity to the chromophore's surroundings, [54]. FS can be used to analyse the concentration, particle size, and spacer composition of biomolecules on the NP surface. [55].

There are several thermal approaches which can be used to assess the thermal stability and the quantity of the nanomaterial conjugates [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 access material transitions such as melting, crystallisation, glass transition, and decomposition of nanomaterial-bioconjugates; consequently, analysis of the DSC measurements afterward can reveal the structure and stability of the subject material [58].

In addition to determining the size/size distribution, shape, and molecular weight of nanomaterials, centrifugation techniques, such as analytical ultracentrifugation (AUC), can be used to explore the conformation, structure, stoichiometry, and self-aggregation state of these materials [59]. The chromatography methods, such as high-performance liquid chromatography (HPLC) and hydrodynamic chromatography (HDC), can be utilised for the purification of nanomaterial bioconjugates when linked 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 roles 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 the tools they need to understand the complex properties of nanomaterials.

Several analytical techniques are required for the nanomaterial characterization. Identifying the type of Nanomaterial is required before determining the physical and chemical characteristics, including size, shape, aggregation state, surface coating, elemental composition, and oxidation state. 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 environment impact of nanomaterials is necessary.

There is a persistent need to develop new characterization techniques as well as a need to improve existing techniques to obtain reliable and reproducible information about NPs in increasingly complex matrices.

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