



Applications of Different Analytical Tools for Characterization of Nano Particles-A Review

J. Pravallika^{*1}, K. Bhavya Sri², D. Anil³ and M. Sumakanth⁴

¹Research student, Department of pharmaceutical analysis, RBVRR women's college of pharmacy, Barkatpura, Hyderabad-500027, India

²Associate professor, Department of pharmaceutical analysis, RBVRR women's college of pharmacy, Barkatpura, Hyderabad-500027, India

³Research Student, 1937 Jerry Ave, Charles City, Iowa, University of Alaska Fairbanks

⁴Professor and Principal, Department of pharmaceutical chemistry, RBVRR women's college of pharmacy, Barkatpura, Hyderabad-500027, India

Received: 14 Oct 2023/Accepted: 9 Nov 2023/Published online: 01 Jan 2024

*Corresponding Author Email: bhavya.kagga@gmail.com

Abstract

Nanostructures, a rapidly evolving class of materials, have created significant interest due to their wide range of applications. The physical properties of nanoparticles include size, crystal structure, composition of elements, and many others have been described using a wide variety of methods. Certain physical properties can be analyzed using several approaches in various situations. Through the integration of these varied analytical instruments, researchers acquire a thorough comprehension of the structure, surface characteristics, and behavior of nanoparticles. This information is essential for creating customized nanoparticles with the right properties for uses in electronics, catalysis, medicine, and other fields. Since the importance of nanoparticles in fundamental research as well as applications is steadily rising. It is crucial that scientists from multiple sectors overcome challenges in characterizing nanomaterials in a reproducible and reliable manner following their synthesis and further phases of processing (e.g., Annealing). This review's main goal is to provide an overview of current understanding on the applications, advances, advantages, and disadvantages of the many experimental methods available for characterizing nanoparticles.

Keywords

Nano particles, Particle size, Surface area characterization, Surface charge.

INTRODUCTION

Nanotechnology is the design, synthesis, and use of materials with sizes ranging from atoms to molecules to macromolecules, to create novel materials at the nanoscale. Fundamental elements of nanotechnology are nanoparticles [1].

Pharmaceutical nanoparticles are characterized as solid, potentially biodegradable drug carriers that are submicron-sized (i.e., less than 100 nm in diameter). Nanospheres and Nano capsules have been referred to as nanoparticles. Nanoparticle may be one dimensional, like graphene, or zero

dimensional, like nano dots, with its length, width, and height all fixed at a single location. Two-dimensional materials, like carbon nanotubes, have length and width. Three-dimensional, like gold nanoparticles having all three dimensions such as length, width, and height [2].

There are several sizes of nanoparticles created throughout the synthesis process. Characterization techniques should offer direction for maintaining quality assurance and evaluating the toxicity and safety of nanomaterials. It is necessary to look at physicochemical properties (e.g., size, charge,

chemical composition, shape, coating) and molecule structure, their freezing point, elemental composition, soluble properties, boiling point, flash point, and pH levels for nanomaterials. Centrifugation and nanofiltration constitute the separation techniques used to separate and purify mixed materials for purpose of the characterization of nanomaterials. Analytical ultracentrifugation is useful in examining the shape, size distribution, molecular weight, and conformation, structure, self-aggregation state, and stoichiometry of nanomaterials [3]. Electron microscopy is used to select the required size nanoparticles. Transmission and scanning electron microscopy are typically used to measure size of nanoparticle.

Size determining becomes more challenging when there are nanoparticles occurring in the gaseous phase. scanning mobility particle size approach can be used to solve this problem as it measures particle size in gaseous phase and can be measured quickly and accurately with this technique. The surface area plays a crucial role in identifying of nanoparticles. The efficiency and properties of a nanoparticle are significantly influenced by its surface area to volume ratio [4].

Classification of Nanoparticles

1. Organic nanoparticles.
2. Inorganic nanoparticles: Metal, Ceramic, Semiconductor, Polymeric and Lipid-based Nano Particles.
3. Carbon-based nanoparticles [5].

Characterization of Nanoparticles

1. Size and surface morphology
2. Specific surface area
3. Surface charge and Electrophoretic mobility
4. Surface Hydrophobicity
5. Density
6. Molecular weight measurement of nano particles
7. Drug Entrapment efficiency.
8. Kinetic study
9. Stability of nano particles
10. Drug-Excipient compatibility studies.
11. *In-vitro* release studies
12. Lamellarity
13. Phase behavior
14. Biological and Chemical characterization (liposomes)

Importance of surface characterization of nanoparticles

Nanoparticles may be produced mechanically or chemically and have a wide range of possible technological uses; characterization has become

more and more relevant in academic study across several domains. Characteristics of nanoparticles change dramatically with size due to their enormous ratio of surface area to volume, characterization is a crucial step in understanding properties of nanoparticles at various molecular stages. The chemical, electrical, mechanical, and optical characteristics of the nanoparticles are affected by other features such texture, strain, shape anisotropy, crystal phase, crystal flaws, and crystal dimensions. As a result of the large surface area to volume ratio of these particles, surface analysis has a crucial part in behavior of the particles and helps to explain their characteristics.

For example, the incorporation of particles made of silicon, alumina, and titanium dioxide improve the tensile strength of the metal matrix made of aluminum. In this case, surface characterization was crucial in determining the aluminum matrix's surface characteristics, such as surface chemistry [6].

MATERIALS AND METHODS

ANALYTICAL TOOLS FOR CHARACTERIZATION OF NANO PARTICLES

Particle size: Transmission electron microscopy, atomic force microscopy, Scanning electron microscopy.

Surface charge: Electrophoresis.

Surface area: Brunauer-Emmett-Teller.

Crystal structure: X-Ray Diffraction.

Particle mass: Mass spectrometry.

Particle counters: Particle number.

Surface morphology

The various shapes and sizes of nanoparticles influence many aspects of their characteristics. Shapes include irregular shapes as well as tubular, cylindrical, flat, spherical, and conical forms. The surface morphology of nanoparticles can be classified into two categories: crystalline and amorphous, exhibiting both homogeneous and irregular surface structures.

SEM and TEM imaging approaches are also accustomed to determining surface morphological properties of nanoparticles. Imaging methods are used to analyze the nanoparticles in liquid medium that forms layer on surface. Additionally, gaseous phase particles record and examine surface morphology [7,8].

PARTICLE SIZE CHARACTERIZATION OF NANOPARTICLES

The key factors influencing stability, drug loading, and drug release rate are the size distribution and particle size. Surface distribution and particle size are determined using a variety of analytical techniques including microscopic techniques they are.

Microscopic techniques

1. Transmission electron microscopy
2. Scanning electron microscopy

These two methods primarily used to study the morphology of nanoparticle. These methods were employed by numerous researchers to demonstrate the synthesized nanoparticles roughly uniform dimensions and shape [9].

Transmission electron microscopy (TEM)

TEM is an imaging method that provides very high-resolution images of the size and shape of nanoparticles using electrons. It is especially beneficial to characterize specific nanoparticles.

Principle

An electron beam is focused on an object to create an image. Higher resolution is achieved because electrons have a wavelength that is significantly smaller than that of light. Compared to optical microscopy, transmission electron microscopy uses electrons with a wavelength of approximately 0.005 nm, which is substantially shorter. Because of this characteristic, TEM can achieve a resolution that is approximately 100,000 times greater compared to that of light microscopy [10,11].

Two modes of operation are available for the conventional transmission electron microscope:

Image mode: A thin sample can be imaged in high resolution by projecting electrons onto a camera after the electron beam has passed through it.

Diffraction mode: In this mode, a fluorescent screen with a diffraction pattern is illuminated by an electron beam generated by the sample.

Components

Electron source: A gun that utilizes either thermionic or field emission technique provides the electrons needed for illumination.

Condenser lens:

The primary condenser (Electromagnetic type) lens utilizes an aperture or slit that restricts the entry of wide-angle electrons to focus the beam. The second condenser lens focuses the beam onto a tiny portion of the sample.

Objective lens:

The electrons that travel within a thin sample are collected by the objective lens. The correct operating mode is selected at the rear aperture of the objective lens.

Projective lens: After an image is focused by the objective lens, it is enlarged by the projection and intermediate lenses.

Detector: An electron-sensitive camera receives the magnified image in order to capture a digital image of sample [12,13].

Working

Through its condenser lenses, the electron gun focuses electrons created by heating a tungsten filament onto the specimen. Magnetized lenses further focus the electron beam on the sample. The condenser lens's column tube helps create a vacuum, allowing electrons to generate an unobstructed image without colliding with air molecules that might deflect them. When electrons reach the analyte, they are transmitted and focused by magnetic lenses, creating a large, clear image. To produce a monochromatic image, a specific energy level is selected, and the electron beam passes through a monochromator. Both phosphor screens and CCD camera image processing system entrances can serve as the imaging surface [14,15].

Advantages:

Provides the most powerful magnification possible up to a million times and more. It develops high quality, detailed image of the samples. It generates images with highest resolution possible and yields information relating to the surface characteristics, size, shape, and structure.

Disadvantages:

Developed contrast mechanisms that complicate the interpretation of images and electron beam might destroy the sample.

Applications:

TEM imaging technique is helpful across food and agricultural sectors in detection of contaminants, as well as in various cancer treatments. It is utilized in nanotechnology to research zinc oxide and other nanoparticles. TEM images can provide information on topography, morphology, composition, and crystallography [16-19].

Scanning electron microscope [SEM]

It is employed for determining the morphologies, sizes, and shapes of formed nanoparticles. SEM operates using a similar principle as the optical microscope, except that instead of measuring photons, it measures electron scattering from the sample. This is because an electric potential can accelerate electrons, which results in a shorter wavelength than photons. As a result, images can be magnified to 200,000 times using this. It generates high-resolution nanoparticle images and operates well for surface imaging, providing precise details about the external shape of nanoparticles.

Components

1. Electron gun
2. Vacuum
3. Electromagnetic lenses to focus electrons.
4. Sample chambers.
5. Electron detectors are different types they are.

6. Secondary electrons include Everhart-Thornley detector,
7. Back scattered electrons include Solid state detector and x-rays includes spectrometer with energy dispersive capability.
8. Computers systems for recording and viewing images.

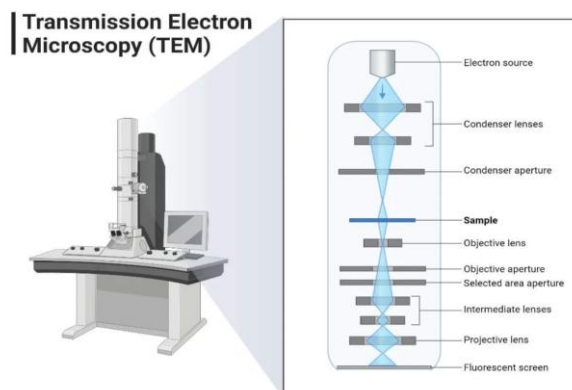


Figure1. Transmission electron microscopy.

Working principle

The Scanning electron microscope creates images by using electrons rather than light. Vacuum is required to operate, and metallic filament is heated and then at the microscope's top electron beam is produced. The electron beam travels through the microscope's column in a vertical direction. It passes through electromagnetic lenses before the beam is focused and directed downward towards analyte. Different electrons, either secondary or backscattered, are emitted from analyte once electrons hit analyte. The secondary or backscattered electrons are gathered by detectors, which then converting them nonelectrical signals are sent to the viewing screen in order to produce an image [8,10,20].

Advantages:

Comprehensive 3D (three-dimensional) topographical imaging and versatile data obtained through various sensors. Compared to transmission electron microscope, sample preparation is simple. Interpreting images is quick and easy.

Disadvantages:

The use of SEM is limiting to inorganic, solid samples that are adequate in size to fit inside of a vacuum chamber with a moderate vacuum pressure. Resolution is limited to very few nano meters. Ems need to be kept away from any potential electric, magnetic, or vibrational interference. They are large, expensive devices. Atomic level details are not observable.

Applications:

Used to analyse and detect surface fractures. Surface contaminations can be examined, and microstructures information can be obtained. SEM is essential tool in research fields including science, biology, forensic science, medical and metallurgy [21-23].

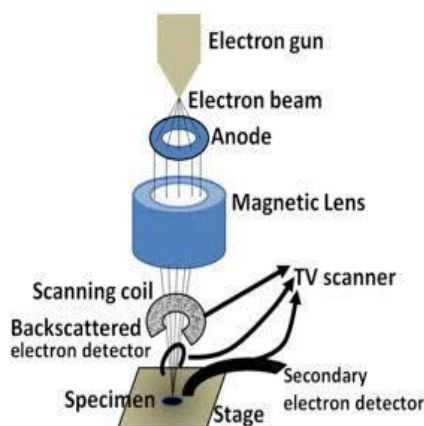


Figure 2. Scanning electron microscopy.

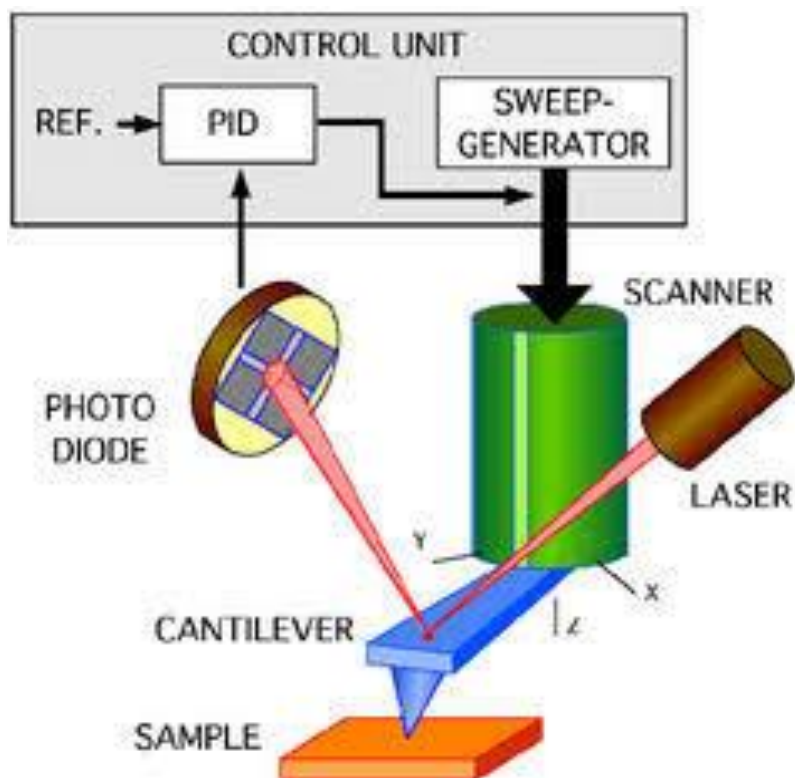


Figure 3. Atomic Force Microscope.

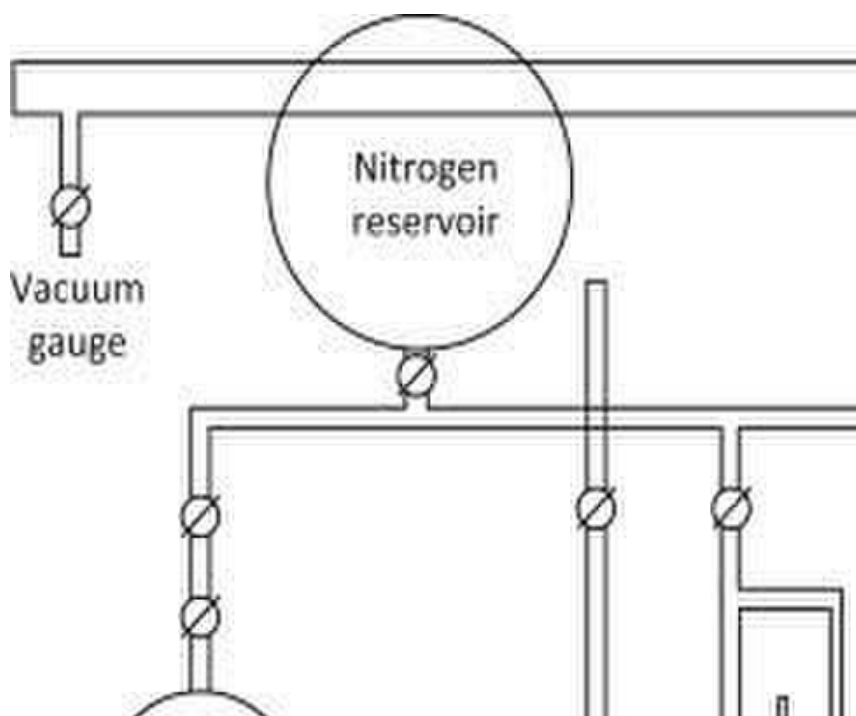


Figure 4. Brunauer Emmett teller surface area analysis.

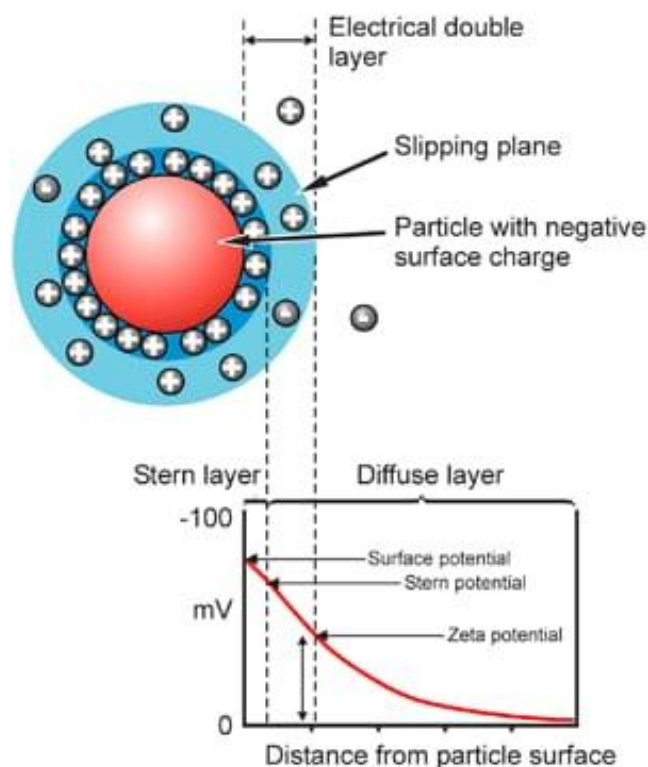


Figure 5. Zeta potential

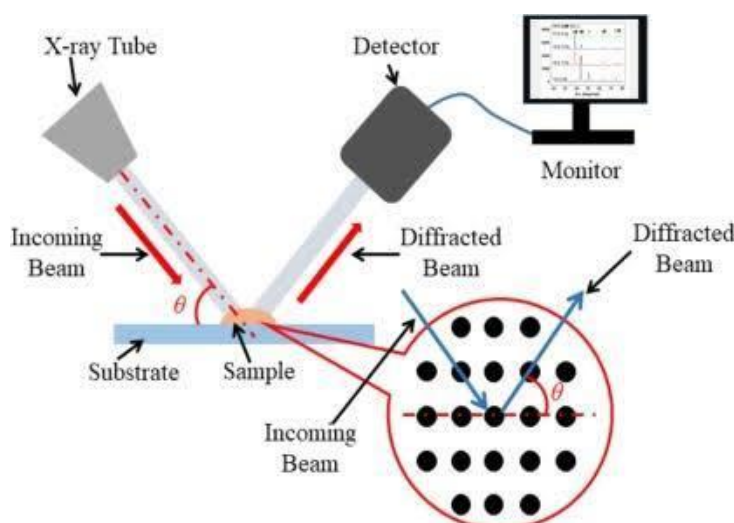


Figure 6. X-Ray Diffraction

Atomic force microscopy [AFM]

AFM is an approach that provides nanoscale resolution information about the size and morphology of nanoparticles by scanning their surface with a sharp tip.

Components

1. Sharp-tipped cantilever.
2. Scanner: X, Y, Z positions are controlled by a scanner.
3. Feedback control and consists of loop.

Working principle

Optical lever is used to measure the cantilever's lateral and vertical deflections in order to obtain the AFM image resolution. Laser beam is focused to reflect off the cantilever to operate the optical lever. A position-sensitive photodetector made of a four-segment photodetector strikes by reflected laser beam. The laser spot location on the detector is specified by the variations between the signal segments in the photodetector, which in turn indicates the cantilever's angular deflections. The AFM tip is precisely positioned by piezo-tube actuators. By voltage gradient, a class of materials

known as piezoelectric ceramics can expand or contract. As a result, devices can be precisely positioned using piezo ceramics in all three dimensions (X, Y, and Z). Therefore, it is possible to resolve both individual and group particles using the AFM [24,25].

To measure the forces between the little probe and surface that is being imaged, an AFM construction requires a force sensor. The force sensor uses Hook's law to determine the relationship between a cantilever's motion and applied force is:

$$F = -k * d$$

K represents a constant that is based on the cantilever's dimensions and material. D is the cantilever's motion. The "light lever" method, which involves reflecting light into a photodetector from the cantilever's back, can be used to measure the motion of the device.

For example, the light beam travels across the photo-detector's surface when the cantilever goes up and down. Subsequently, the cantilever's motion is directly proportional to the photo-detector's output. The light lever technique in the AFM is consistently able to measure motions as small as 1 nm. An object's motion is maintained proportionate to another object through feedback control. Feedback control is used in automated airplanes and temperature controls. While measuring a scan, feedback control keeps the probe in a "fixed" relationship with the surface [10,26,27].

Advantages:

Sample sizes are accurately measured. It is equipped with 3D imaging and used to quantify surface roughness. It can measure particles from one nanometer to eight micrometers in size in just a single scan.

Disadvantages:

It is limited to scan single nanosized images at a time of 150x150 nm. The sample may experience thermal drift due to their limited scanning duration. During detection, damage to the tip and sample may occur.

Applications:

Used to characterize natural colloidal substance as a function of pH and topography of particles dispersed in a solid matrix and to study the lipid nano capsules morphology including shape, size, stability, and dynamic process [28-30].

SURFACE CHARACTERIZATION OF NANO PARTICLES Brunauer-Emmett-Teller (BET) technique for surface area analysis

Its name originates from initial surname of its creators Brunauer, Emmett, and Teller. Principle is based on physical adsorption of gases on a solid

surface. Determines a nanoparticle specific surface area, porosity, and pore size distribution [31].

An inert gas, usually argon, krypton, or nitrogen, physisorbs on the surface of analyte to measure the area of its specific surface. It is important for determining the surface area under an isothermal temperature. 77K is appropriate temperature for liquid nitrogen, while other inert gases have different requirements.

The sample's characteristics determine which inert gas is suitable for the analysis. The solid sample's surface attracts the tiny gas molecules, which cause a porous structure to open and create an entire monolayer of adsorption gas and the sample is heated in a non-nitrogen atmosphere once a molecule of gaseous monolayer has developed. This permits surface of analyte to release the molecules of adsorbed nitrogen gas. It is then possible to quantify emitted gas molecules and calculate their surface area and porosity [32-35].

SURFACE CHARGE

Nanoparticles are comprised of different metallic and non-metallic substances with fixed charge on the surface. The properties of nanoparticles are significantly influenced by their surface charge. On the target site, the positively or negatively charged nanoparticles can interact with their opposing charges to form a complex. The nanoparticle surface charge depends upon both composition and dispersion medium [36].

Surface charge analysis by Zeta Potential

By adding a solution to a cell with two gold electrodes the zeta potential can be determined. When voltage is applied particles will travel in direction of the electrode containing the opposite charge. Velocity of particles as a function of voltage is measured using the Doppler technique [37].

When particles pass through a laser beam, the scattered light's intensity changes at a particular frequency that is directly related to the particle's speed. Particle speed is measured across a range of voltages to measure the zeta potential.

The zeta potential, sometimes called the electrokinetic potential, measures a "fully effective" electrical charge on the surface of colloidal nanoparticles and is a useful tool for determining their charge stability. Charges on a nanoparticle are "screened" by a greater quantity of ions containing opposite charges near the surface of the particle. Together, a layer of ions with opposite charges and surface charge are known as electrical double layer. The layer of oppositely charged ions travels with nanoparticle.

The Zeta Potential measures the potential difference among the bulk fluid in which the particles are dispersed and the fluid layer containing particles of ions which are charged oppositely and attached to the surface of the nanoparticle. Nanoparticles with a positive Zeta Potential will be attracted to negatively charged surfaces, and vice versa.

The magnitude of Zeta Potential gives information about particle stability. Higher magnitude potentials are indicative of greater electrostatic repulsion and, as a result, greater stability [38,39].

Particles tend to aggregate or agglomerate at 0–5 mV.

Particles have very little stability at 5–20 mV.

Particle stability is moderate at 20–40 mV.

Particles extremely stable at 40+ mV.

X-ray diffraction

This technique analyzes the diffraction patterns that are generated when X-rays interact with a sample for determining the crystalline structure and chemical composition of manufactured nanoparticles [40].

Components

1. X-ray tube: It is a source of X-ray.
2. Incident beam optics: which prepares X-ray before it hits analyte.
3. Sample holder.
4. Detector: It counts total amount of X-rays that the analyte has scattered.

Working principle

When a specimen is subjected to a monochromatic, parallel beam of X-rays, samples atomic lattice functions like a three-dimensional diffraction grating, diffracting the X-ray beam at angles a crystalline solid's diffraction pattern can be created using a diffractometer. X-ray beams are reflecting off parallel layers of atoms between molecules at various diffraction angles during XRD analysis. The X-ray beam can cause constructive or destructive interference because it only has one wavelength.

A diffractogram will show a peak when the reflected light rays are in phase (constructive interference) at specific angles. A particular molecule or combination of the molecules can be identified from the diffraction pattern. Different molecules can be identified by their unique set of diffraction peaks, like a fingerprint. A database containing known diffraction data is typically examined in order to identify the molecules [41-46].

Advantages: It is a versatile and effective nondestructive method for characterizing crystalline substances.

Disadvantages: When working with extremely small amounts of nanoparticles, XRD instruments can have limitations about sensitivity and resolution. Dynamic processes may be difficult to adequately capture,

the crystalline structure alterations or phase transitions^[61-66].

Applications: Used for analysis of food and pharmaceutical formulations. Biomolecules containing unparalleled higher resolution structures and the complexes from solid state are obtained from X-ray crystallography [47-49].

RESULTS AND DISCUSSION

Case study of transmission electron microscopy used as sample surface and particle size analysis.

Novel application of well-established methodology in the characterization of nanoparticles for drug delivery systems.

Using TEM, the prepared hybrid and inorganic silica particles morphology and shape were examined [50].

Materials required.

The following were used ethanol, poly(vinyl) formal, uranyl acetate, chloroform, and Milli Q water.

Procedure

1. Formvar film-coated grid preparation.
2. Carbon coating for grids.
3. Samples of native and radiolabeled nanoparticles.
4. Inorganic silica nanoparticles filled with traces of budesonide.
5. Block copolymer carrier required for samples of 7-ethyl-10-hydroxy camptothecin.

Images captured by transmission electron microscopy were obtained from samples derived from native nanoparticles and nanoparticles marked with Stannous chloride labelling method. Inorganic silica nanoparticles that are loaded with native micelles of the anticancer medication camptothecin as well as budesonide. Each sample was dried and then placed into a TEM that was connected to a digital camera and operated by software. TEM was used to examine a porous structure of hybrid particles of silica packed with budesonide [51-56].

RESULTS

The information obtained from TEM technique is very precise. During the labelling process, the nanoparticles' size, smooth surface, and spherical morphology remain unchanged. The sample was found to contain a unique layer of irregularly shaped Stannic oxide particles at the ending of the stannous chloride labelling method. Hybrid-silica-xerogel particles that formed to act as budesonide carriers for effective local therapy of inflammatory bowel disorders.

PHARMACEUTICAL APPLICATIONS

Nanoparticles can be used for drug delivery systems because their precise size, shape, and surface characteristics can be precisely controlled for targeted delivery, increasing therapeutic efficacy and reducing adverse effects [57-59].

Therapeutic applications of Polymeric nanoparticles:

They are used to develop innovative methods for delivering drugs to treat diseases linked to the brain and neurodegenerative conditions. By using the endocytosis and transcytosis pathways, polymeric NPs transport cargo-loaded molecules across blood-brain barrier [60-63].

Therapeutic applications of Lipid-based nanoparticles:

These are primarily used to treat cancers of the GI tract, lungs, breast, pancreas, and prostate cancers [64-67].

Drugs and gene delivery using carbon-based nanoparticles:

Studies conducted using invitro and in vivo demonstrates that graphene used for transporting anti-cancer medications to tumor cells in a way that prevents damage to healthy or normal cells.

Drug delivery into the brain using nanoparticles:

The blood-brain barrier has become significant challenge limiting the development of novel drugs for central nervous system [68].

CONCLUSION

These analytical instruments are significant for characterizing nanoparticles and helping researchers understanding their optical, chemical, and physical characteristics. Scientists can gain valuable insights into the properties and potential uses of nanoparticles by combining these techniques. To understand the evolution of nanoparticles and create reliable formulations, surface characterization of nanoparticles is crucial. When these analytical tools are combined, we can perform targeted surface analysis on nanoparticles. scanning electron microscopy is a crucial instrument for obtaining accurate data regarding the fundamental structural characteristics of various food types. Three-dimensional visualization, quantitative and qualitative data on various physical attributes, including size, roughness, surface texture, and morphology, are best suited for atomic force microscopy.

It emphasizes the significance of considering factors that include sampling, calibration of instruments and data interpretation to ensure reliable and accurate outcomes. These instruments are essential in research and development as well as quality control

in a number of fields, creating the way to products and solutions based on nanotechnology that have particular attributes for industrial and biomedical uses. As these analytical tools continue to advance, researchers will be better equipped to understand the complexities of nanoparticle surfaces, opening novel opportunities for their application in innovative technologies.

ACKNOWLEDGEMENT:

I need to recognize our cherished Principal prof. M. Sumakanth mam and staff of the Department of Pharmaceutical Analysis for giving the opportunity.

REFERENCES

- [1] Hasan S. *A Review on Nanoparticles: Their Synthesis and Types*. Res J Recent Sci, 4: 1-3, (2015).
- [2] Sovan Lal Pal, Utpal Jana, P. K. Manna, G. P. Mohanta, R. Manavalan. *Nanoparticle: An overview of preparation and characterization*. 01 (06): 228-234, (2011).
- [3] Lin, P.C., S. Lin, P. C. Wang, and R. Sridhar. *Techniques for physicochemical characterization of nanomaterials*. Biotechnology Advances, 32 (4): 711-726, (2014).
- [4] WA. Elkhateeb, M. Akramand GM. Daba. *Nanoparticles: Characterization, Biological Synthesis and Applications*. J Microbiol Biotechnol, 6(2): 000196, (2021).
- [5] KA. Altammar. *A review on nanoparticles: characteristics, synthesis, applications, and challenges*. Front Microbiol, 17(14):1155622, (2023).
- [6] DR Baer, MH Engelhard, GE Johnson, J Laskin, J Lai, K Mueller, Munusamy P, (Eds.). *Surface characterization of nanomaterials and nanoparticles: Important needs and challenging opportunities*. J Vac Sci Technol A.31(5):50820, (2013).
- [7] Ibrahim Khan, Khalid Saeed, Idrees Khan. *Nanoparticles: Properties, applications and toxicities*, Arabian Journal of Chemistry, Volume 12, Issue 7, (2019).
- [8] Wu, J.S., Kim, A.M., Bleher, R. et al. Imaging and elemental mapping of biological specimens with a dual-EDS dedicated scanning transmission electron microscope. Ultramicroscopy, 128: 24-31, (2013).
- [9] M. Mohan Varma, K. T. Sunil Kumar and I. Durga Srivalli. *A Review on nanoparticles: synthesis, characterization and applications*. wjpmr, 7(8):169 – 179, (2021).
- [10] R. Gopirajah, C. Anandha rama krishnan. *Characterization Methods for Nanoparticles Food Nanotechnology*. 375-396. (2019).
- [11] K. Bhavyasri, B. Anila reddy, M. Sumakanth. *"Analytical methods for quality control of nano formulations- A review"*. Asian Journal of Pharmaceutical and Clinical Research, 16(10): 1-6, (2023).

- [12] Stefanos Mourdi koudis ORCID logoab, Roger M. Pallares ORCID logoab and Nguyen T. K. Thanh ORCID logo*ab. *Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties (Review Article)* *Nanoscale*. 10: 12871-12934, (2018).
- [13] David J. Smith. *Surface and Interface Characterization by Electron Optical Methods*. 16:31-42, (1988).
- [14] Xi-Feng Zhang, Zhi-Guo Liu, Wei Shen, and Sangiliyandi Gurunathan. *Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches*. *International journal of molecular science*, 17(9): 1534, (2016).
- [15] Dr. M. Kannan. *A Textbook on Fundamentals and Applications of Nanotechnology*. 9: 94-101.
- [16] Smith, D. J. "Chapter 1: Characterization of nanomaterials using transmission electron microscopy." In *Nanocharacterisation*, Eds: Angus I. Kirkland, Sarah J. Haigh, Cambridge: RSC Nanoscience and Nanotechnology, 1-29, 2015.
- [17] Molpeceres J., Aberturas MR., Guzman M. *Biodegradable nanoparticles as a delivery system for cyclosporine: preparation and characterization*. *J Microencapsul*. 17: 599-614, (2000).
- [18] Bozzola, J.J., Russell, L.D. "Electron Microscopy: Principles and Techniques for Biologists." Jones and Bartlett Publishers, 1998.
- [19] Kwon, E.J., (Eds.). "Imaging Nanoparticles in Cancer: A Real- Time Method to Visualize Biodistribution, Tumor Specificity, and Gene Delivery." *Journal of Controlled Release*, 220: 222-233, 2015.
- [20] Michael E. Stowell, Edward J. Dennis. "Introduction to Scanning Electron Microscopy". *Journal: Methods in Cell Biology*, 96:1-20, (2010).
- [21] K D. Vernon-Parry. *Scanning Electron Microscopy: an introduction*. 13 (4):40-44, (2000).
- [22] Vega-Gálvez, A., K. Ah-Hen, M. Chacana, J. Vergara, J. Martínez-Monzó, P. García-Segovia, R. Lemus Mondaca, and K. Di Scala. "Effect of temperature and air velocity on drying kinetics, antioxidant capacity, total phenolic content, colour, texture and microstructure of apple (Var. Granny Smith) alices." *Food Chemistry*, 132 (1): 51-59, (2012).
- [23] Salame, Pareshe & Pawade, Vijay & Bhanvase, Bharat. *Characterization Tools and Techniques for Nanomaterials*, 3: 83-111, (2018).
- [24] Ramesh Raliya, Ramprakash Saran, K. Choudhary, J. C. Tarafdar. *Biosynthesis and Characterization of Nanoparticles*. of *Advancement in Medical and Life Sciences*, 1-4, (2014).
- [25] Palmer, A. F., P. Wingert, and J. Nickels. "Atomic force microscopy and light scattering of small unilamellar actin-containing liposomes." *Biophysical Journal*, 85 (2): 1233-1247, (2003).
- [26] Scaif, J., and P. West. "Part I: Introduction to nanoparticle characterization with AFM". In *Pacific Nanotechnology*. Inc. Santa Clara, CA 95054, 1-9, (2006).
- [27] Sum, C.-P., A. Shrestha, and A. Kishen. "Characterizing bacteria adhesion to substrate and early biofilm formation using atomic force microscopy: A review." *Journal of the Indian Institute of Science*, 93 (1): 47-56, (2013).
- [28] Bernardes-Filho, R. and O. B. G. de Assis. "Development of an algorithm for tip-related artifacts identification in AFM biological film imaging." *Brazilian Archives of Biology and Technology*, 48 (4): 667-674, (2005).
- [29] Binnig, G., C. F. Quate, and Ch. Gerber. "Atomic force microscope." *Physical Review Letters*, 56 (9): 930-933, (1986).
- [30] Lead, J. R., D. Muirhead, and C. T. Gibson. "Characterization of freshwater natural aquatic colloids by Atomic Force Microscopy (AFM)." *Environmental Science and Technology*, 39 (18): 6930-6936, (2005).
- [31] Brunauer, S., Emmett, P. H., & Teller, E. "Adsorption of Gases in Multimolecular Layers." *Journal of the American Chemical Society*, 60(2): 309-319, (1938).
- [32] Raja PMV, Barron AR. *BET Surface Area Analysis of Nanoparticles*. Rice University; 2022.
- [33] Thommes, M., Kaneko, K., Neimark, A. V., Olivier, J. P., Rodriguez-Reinoso, F., Rouquerol, J., & Sing, K. S. W. "Physisorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC Technical Report)." *Pure and Applied Chemistry*, 87(9-10):1051-1069, (2015).
- [34] Sing, K. S. W. "Reporting physisorption data for gas/solid systems with special reference to the determination of surface area and porosity (Recommendations 1984)." *Pure and Applied Chemistry*, 57(4): 603-619, (1985).
- [35] Bououdina, M.S., Rashdan, J.L., Bobet, Y., Ichiyanagi. *Nanomaterials for biomedical applications: synthesis, characterization, and applications*. *J. Nanomaterial*, 240 -501, (2013).
- [36] Kumal, R.R., Karam, T.E., and Haber, L.H. *Determination of the surface charge density of colloidal gold nanoparticles using second harmonic generation*. *J. Phys. Chem.* 119:16200-16207, (2015).
- [37] Marsalek, R. *Particle size and zeta potential of ZnO*. *APCBEE Proc*. 9: 13-17, (2014).
- [38] Hunter, R. J. "Zeta Potential in Colloid Science: Principles and Applications." *Pure and Applied Chemistry*, 53(8): 1689-1713, (1981).
- [39] Schleh, C., Semmler-Behnke, M., Lipka, J. et al. *Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration*. *Nanotoxicology*, 6: 36-46, (2012).

- [40] Arjun Sai Sreekar Aeila (Eds.). *Nanoparticles - the Future of Drug Delivery*. Indo American Journal of Pharmaceutical Research, 9(12):631-636, (2019).
- [41] Tampo, H., P. Fons, A. Yamada, K.-K. Kim, H. Shibata, K. Matsubara, S. Niki, H. Yoshikawa, and H. Kanie. "Determination of crystallographic polarity of ZnO layers." *Applied Physics Letters*, 87 (14): 141904, (2005).
- [42] Dorofeev, G.A., Streletskii, A.N., Povstugar, I.V. (Eds.). *Determination of nanoparticle sizes by the X-ray diffraction method*. *Colloid J*, 74: 678-688, (2012).
- [43] Sharma, R., Bisen, D.P., Shukla, U., and Sharma, B.G. *X-ray diffraction: a powerful method of characterizing nanomaterials*. *Recent Res. Sci. Technol*, 4: 77-79, (2012).
- [44] Bridget Ingham, B. *X-ray scattering characterization of nanoparticles*, *Crystallography Reviews*. Rev. 21(4): 229-303, (2015).
- [45] Chandrasekaran, R. "X-ray diffraction of food polysaccharides." *Advances in Food and Nutrition Research*, 42: 131-210, (1998).
- [46] Zobel, H. F., S. N. Young, and L. A. Rocca. "Starch Gelatinization: An X-Ray Diffraction Study." *Cereal Chemistry*, 65(6):443-446, (1998).
- [47] Lutterotti, L. "Total Pattern fitting for the Combined Size-Strain-Stress-Texture Determination in Thin Film Diffraction." *Journal of Applied Crystallography*, 43(2): 386-392, (2010).
- [48] Garman, E. F., & Weik, M. "Radiation damage to biological macromolecules: some answers and more questions." *Journal of Synchrotron Radiation*, 24(1): 1-6, (2017).
- [49] Holton, J. M., & Frankel, K. A. "The minimum crystal size needed for a complete diffraction data set." *Acta Crystallographica Section D: Biological Crystallography*, 66(4): 393-408, (2010).
- [50] M. Petrushevska, K. Pavlovska, J. Laskova, P. Zdravkovski, Dodov MG. *Transmission Electron Microscopy: Novel Application of Established Technique in Characterization of Nanoparticles as Drug Delivery Systems*. *Pril (Makedon Akad Nauk Umet Odd Med Nauki)*, 40(2):67-72, (2019).
- [51] Geskovski N, Kuzmanovska S, Simonoska, Crcarevska M, Calis S, Dimchevska S, Petrusevska M, Zdravkovski P, Goracinova K. *Comparative biodistribution studies of technetium-99m Radiolabeled amphiphilic nanoparticles using three different reducing agents during the labeling procedure*. *J Labelled Comp Radiopharm*, 56(14): 689-95, (2013).
- [52] Petrovska-Jovanovska V, Geskovski N, Crcarevska MS, Memed O, Petruševski G, Chachorovska M, Petrusevska M, (Eds.). *Formulation and characterization of ORMOSIL particles loaded with budesonide for local colonic delivery*. *Int J Pharm*, 484(1-2):75-84, (2015).
- [53] B. Djurdjic, S. Dimchevska, N. Geskovski, M. Petrusevska, V. Gancheva, G. Georgiev, P. Petrov, Goracinova. *Synthesis and self-assembly of amphiphilic poly(acrylic acid)-poly(ϵ -caprolactone)-poly(acrylic acid) block copolymer as novel carrier for 7-ethyl-10-hydroxy camptothecin*. *J Biomater Appl*, 29(6): 867-81, (2015).
- [54] Atherton OE, Robins RW, Rentfrow PJ, Bobo D, Robinson KJ, (Eds.). *Nanoparticle-based medicines: a review of FDA approved materials and clinical trials to date*. *Pharm Res* 33: 2373-2387, (2016).
- [55] M. Costanzo, F. Carton, M. Malatesta. *Microscopy techniques in nanomedical research*. *Microscopie*, 3: 66-71, (2017).
- [56] M. Malatesta. *Transmission electron microscopy for nanomedicine: novel applications for long-established Techniques*. *Eur J Histochem*, 60(4): 2751, (2016).
- [57] Villaverde, G. Baeza, A. Beilstein. *Targeting strategies for improving the efficacy of nanomedicine in oncology*. *J. Nanotechnol*, 10: 168-181, (2019).
- [58] Torchilin, P. Vladimir. "Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery." *Nature Reviews Drug Discovery*, 13(11): 813-827, (2014).
- [59] L Zhang, FX Gu, JM Chan, AZ Wang, RS Langer, OC Farokhzad. "Nanoparticles in medicine: therapeutic applications and developments." *Clinical Pharmacology & Therapeutics*, 83(5): 761-769, (2008).
- [60] Saraiva, C. Praca, C. Ferreira, R. Santos, T. Ferreira, L. Bernardino. "Nanoparticle-mediated brain drug delivery: Overcoming blood-brain barrier to treat neurodegenerative diseases." *Journal of Controlled Release*, 235: 34-47, (2016).
- [61] W. M. Pardridge, "Blood-brain barrier delivery." *Drug Discovery Today*, 12(1-2): 54-61, (2007).
- [62] Tosi, G. Costantino, L. Ruozi, B. Forni, F. Vandelli, M. A. "Polymeric nanoparticles for the drug delivery to the central nervous system." *Expert Opinion on Drug Delivery*, 4(1):45-57, (2007).
- [63] Allen, T. M., Cullis, P. R. *Liposomal drug delivery systems: from concept to clinical applications*. *Advanced Drug Delivery Reviews*, 65(1):36-48, (2013).
- [64] Narahari, N. Palei, Bibhash, C. Mohanta, Mohana L. Sabapathi, Malay, K. Das. *Lipid-based nanoparticles for diagnosis and therapy*. *Organic Materials as Smart Nanocarriers for Drug Delivery*, 415-470, (2018).
- [65] Torchilin, V. P. *Recent advances with liposomes as pharmaceutical carriers*. *Nature Reviews Drug Discovery*, 4(2): 145-16, (2005).
- [66] Liu, D. Yang, F. Xiong, F. & Gu, N. "The smart drug delivery system and its clinical potential." *Theranostics*, 6(9): 1306-1323, (2015).

- [67] Mehnert, W. and K. Mader. "*Solid lipid nanoparticles.*" *Advanced Drug Delivery Reviews, Lipid Assemblies for Drug Delivery*, 47 (2): 165–196, (2001).
- [68] Divya, D. Jadhav, Anita, A. Singh, Bhavana, D. Tambe. *Nanoparticles as drug delivery system.* *International Journal of Information Research and Review*, 8 (6): 7304-7308, (2021).