

Nanomedicine Characterization Techniques

Gaurav Thakur¹, Subhendu Nair², Santosh K Patil³

Guide¹, Research Scholars^{2,3}

Department of Pharmaceutics

Smt. B.N.B. Swaminarayan Pharmacy College

Corresponding Author Email: *Gauravthakur700@gmail.com¹*

DOI: *<https://doi.org/10.5281/zenodo.19690138>*

ABSTRACT

Nanomedicine has emerged as a revolutionary approach in the diagnosis, prevention, and treatment of various diseases. Its unique physicochemical properties, such as high surface area-to-volume ratio, tunable surface chemistry, and size-dependent behavior, enable targeted drug delivery and enhanced therapeutic efficacy. Accurate characterization of nanomedicines is critical for understanding their biological interactions, stability, safety, and efficacy. This review comprehensively summarizes current nanomedicine characterization techniques, including physicochemical, morphological, structural, and biological methods. Techniques such as dynamic light scattering (DLS), transmission electron microscopy (TEM), atomic force microscopy (AFM), zeta potential analysis, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and in vitro/in vivo biological assays are discussed in detail. The review also highlights the importance of integrating multiple techniques to obtain reliable and reproducible data, which is essential for regulatory compliance and clinical translation of nanomedicines. Challenges and future perspectives in nanomedicine characterization are also addressed.

KEYWORDS: *Nanomedicine, Characterization techniques, Dynamic light scattering, Electron microscopy, Surface charge, Drug delivery, Nanoparticles*

INTRODUCTION

Nanomedicine involves the application of nanotechnology in the medical and pharmaceutical fields. Nanoparticles (NPs), nanoliposomes, dendrimers, nanomicelles, and other nanoscale formulations are widely employed for targeted drug delivery, diagnostic imaging, and therapeutic applications. The physicochemical properties of nanoparticles, such as particle size, shape, surface charge, and surface chemistry, significantly influence their pharmacokinetics, biodistribution, cellular uptake, and toxicity profile.

Accurate and comprehensive characterization of nanomedicines is therefore essential during the development process. Characterization enables the prediction of biological behavior, quality control, and optimization of formulations. It also facilitates compliance with regulatory requirements, ensuring safety and efficacy in clinical applications.

This review aims to provide a detailed overview of the key nanomedicine characterization techniques, their principles, advantages, limitations, and relevance in biomedical research.

CLASSIFICATION OF NANOMEDICINES

Nanomedicines are broadly classified based on their composition, structure, and functional properties. Understanding these classes is critical because the physicochemical characteristics of each type directly influence their biological behavior, targeting ability, stability, and therapeutic potential.

1. Lipid-Based Nanoparticles

Lipid-based nanoparticles are widely used in drug delivery due to their biocompatibility, biodegradability, and ability to encapsulate both hydrophilic and hydrophobic drugs.

- **Liposomes:** Spherical vesicles composed of one or more phospholipid bilayers encapsulating an aqueous core. Liposomes protect drugs from degradation, improve pharmacokinetics, and enable targeted delivery through surface modification (e.g., PEGylation).

Applications: Cancer therapy (doxorubicin-loaded liposomes), antifungal therapy, vaccine delivery.

- **Solid Lipid Nanoparticles (SLNs):** Composed of solid lipids stabilized by surfactants. SLNs offer controlled drug release, enhanced stability, and lower cytotoxicity compared to other carriers.

Applications: Oral, topical, and parenteral drug delivery

- **Nanostructured Lipid Carriers (NLCs):** Advanced lipid carriers combining solid and liquid lipids. NLCs reduce drug expulsion during storage and provide higher loading capacity.

2. Polymeric Nanoparticles

Polymeric nanoparticles are colloidal systems made from natural or synthetic polymers. They can be designed as nanospheres (matrix-type) or nanocapsules (core-shell).

- **Polymers:** PLGA (poly(lactic-co-glycolic acid)), chitosan, PEG, and polycaprolactone are commonly used.
- **Advantages:** Controlled and sustained drug release, tunable surface functionality for targeted therapy, and protection of labile drugs from degradation.
- **Applications:** Chemotherapy, gene delivery, vaccines, and imaging agents.

3. Inorganic Nanoparticles

Inorganic nanoparticles possess unique optical, magnetic, or electrical properties that make them valuable for diagnostics, imaging, and therapeutic applications.

- **Metal Nanoparticles:** Gold and silver nanoparticles are used for photothermal therapy, biosensing, and antimicrobial activity.
- **Metal Oxide Nanoparticles:** Iron oxide NPs are superparamagnetic, enabling magnetic resonance imaging (MRI) contrast and hyperthermia treatment.
- **Quantum Dots:** Semiconductor nanocrystals exhibiting size-tunable fluorescence, used in bioimaging and diagnostics.

Advantages: High stability, ease of surface functionalization, and ability to provide multimodal functionality (therapy + imaging).

Limitations: Potential long-term toxicity and accumulation in organs; surface modification is often required to enhance biocompatibility.

4. Dendrimers

Dendrimers are highly branched, three-dimensional macromolecules with numerous terminal functional groups, allowing precise drug conjugation.

- **Structure:** Central core, repetitive branching units (generations), and terminal functional groups.
- **Advantages:** Monodispersity, high drug loading capacity, and tunable surface chemistry.
- **Applications:** Gene delivery, targeted drug delivery, and diagnostic imaging.

5. Nanomicelles

Nanomicelles are self-assembled colloidal structures formed by amphiphilic molecules in aqueous solutions. They typically have a hydrophobic core and hydrophilic shell, which allows the encapsulation of poorly soluble drugs.

- **Advantages:** Enhanced solubility of hydrophobic drugs, prolonged circulation time, and passive tumor targeting via the enhanced permeability and retention (EPR) effect.
- **Applications:** Delivery of anticancer drugs, anti-inflammatory agents, and imaging probes.

6. Hybrid Nanoparticles

Hybrid nanoparticles combine the advantages of multiple classes of nanomedicines, such as lipid–polymer hybrids or inorganic–organic nanocomposites.

- **Advantages:** Improved stability, multifunctionality, and customizable drug release profiles.
- **Applications:** Theranostics (simultaneous therapy and diagnostics), combined drug delivery, and controlled release systems.

Table 1: Summary Table of Nanomedicine Classes

Class	Composition	Unique Feature	Typical Applications
Liposomes	Phospholipid bilayers	Biocompatible, encapsulate hydrophilic & hydrophobic drugs	Cancer therapy, vaccines
Solid Lipid Nanoparticles (SLNs)	Solid lipids + surfactants	Controlled release, high stability	Oral/topical delivery

Class	Composition	Unique Feature	Typical Applications
Polymeric NPs	Natural/synthetic polymers	Controlled release, surface functionalization	Chemotherapy, gene delivery
Metal NPs	Gold, silver	Optical/photothermal properties	Imaging, antimicrobial therapy
Metal Oxide NPs	Iron oxide, titanium dioxide	Magnetic properties	MRI, hyperthermia
Quantum Dots	Semiconductor nanocrystals	Fluorescent labeling	Bioimaging, diagnostics
Dendrimers	Branched polymers	High drug loading, tunable surface	Gene/drug delivery, imaging
Nanomicelles	Amphiphilic molecules	Solubilize hydrophobic drugs	Cancer therapy, drug delivery
Hybrid NPs	Lipid-polymer or inorganic-organic	Multifunctional, stable	Theranostics, combined therapy

PHYSICOCHEMICAL CHARACTERIZATION

Physicochemical characterization is a fundamental aspect of nanomedicine research, as it provides insights into the size, morphology, surface properties, chemical composition, and stability of nanoparticles. These properties directly influence **biological interactions, cellular uptake, circulation time, biodistribution, toxicity, and therapeutic efficacy**. Comprehensive characterization is essential for designing safe, effective, and reproducible nanomedicine formulations.

Key physicochemical parameters include:

- Particle size and size distribution
- Morphology and shape
- Surface charge (zeta potential)
- Chemical composition and functional groups
- Crystallinity and phase behavior

Accurate measurement of these parameters requires the integration of multiple analytical techniques.

1. Particle Size and Distribution

Particle size is one of the most critical attributes of nanomedicines. It affects **cellular uptake, blood circulation, tissue penetration, renal clearance,** and the **enhanced permeability and retention (EPR) effect** in tumors. Typically, nanoparticles in the **10–200 nm range** exhibit optimal tumor accumulation while avoiding rapid renal clearance or uptake by the reticuloendothelial system (RES).

Size distribution or polydispersity index (PDI) provides information on uniformity. A low PDI (<0.2) indicates a monodisperse system, whereas higher PDI values suggest aggregation or heterogeneity, which can impact biological performance.

Several techniques are commonly used to measure particle size:

a) Dynamic Light Scattering (DLS)

- **Principle:** DLS measures fluctuations in light scattering caused by the Brownian motion of nanoparticles in suspension. These fluctuations are analyzed to calculate the **hydrodynamic diameter**, which includes the particle core plus any surface coatings and solvation layers.
- **Advantages:**
 - Rapid and non-destructive measurement.
 - Provides average particle size and polydispersity index (PDI).
 - Suitable for colloidal suspensions in aqueous and organic solvents.
- **Limitations:**
 - Cannot resolve particle shape or internal structure.
 - Sensitive to aggregates; a small number of large aggregates can skew results.
 - Provides an intensity-weighted size, which may overestimate larger particles in polydisperse samples.

Example Application: Liposomes loaded with doxorubicin are typically characterized by DLS to confirm their hydrodynamic size (~100 nm) and ensure reproducibility in manufacturing batches.

b) Nanoparticle Tracking Analysis (NTA)

- **Principle:** NTA tracks the movement of individual nanoparticles in suspension using a laser beam and video microscopy. The **diffusion coefficient** of each particle is calculated, and the **particle diameter** is derived using the Stokes–Einstein equation.
- **Advantages:**
 - Provides particle-by-particle size distribution, offering higher resolution than DLS.
 - Measures particle concentration and can detect minor populations in heterogeneous samples.
- **Limitations:**
 - Requires moderate to high particle concentrations.
 - More time-consuming than DLS.
 - Sensitive to sample viscosity and operator handling.

Example Application: Polymeric nanoparticles for gene delivery are often evaluated by NTA to confirm a narrow size distribution (~80–150 nm) before in vivo administration.

c) Electron Microscopy (EM)

Electron microscopy provides **direct visualization** of nanoparticle morphology and core size. Unlike DLS or NTA, EM allows observation of individual nanoparticles, their aggregation state, and structural features.

- **Transmission Electron Microscopy (TEM):**
 - Uses an electron beam transmitted through an ultrathin sample.
 - Provides high-resolution images (1–2 nm) of **particle shape, core size, and internal structure**.
 - Useful for liposomes, dendrimers, and inorganic nanoparticles.
 - Limitation: Sample preparation may alter native morphology due to drying or staining.

- **Scanning Electron Microscopy (SEM):**

- Uses electrons scattered from the sample surface to generate images.
- Provides detailed **surface morphology and topography** of nanoparticles.
- Limitation: Typically provides surface detail rather than internal structure; non-conductive samples may require metal coating.

Example Application:

- Gold nanoparticles for photothermal therapy are visualized by TEM to confirm spherical shape and uniform size (~50 nm).
- Polymeric micelles are analyzed by SEM to verify smooth surface morphology and absence of aggregation.

Table: 2

Technique	Parameter Measured	Strengths	Limitations
DLS	Hydrodynamic diameter, PDI	Fast, reproducible	Sensitive to aggregation
NTA	Particle size distribution	Single particle resolution	Requires high concentration
TEM	Morphology, core size	High-resolution images	Sample preparation may alter shape
SEM	Surface morphology	Topographical detail	Cannot measure hydrodynamic size

2. Surface Charge (Zeta Potential)

Surface charge plays a crucial role in nanoparticle behavior, influencing **colloidal stability, cellular uptake, circulation time, and interactions with biomolecules**. Nanoparticles with high surface charge repel each other electrostatically, reducing aggregation and sedimentation, which enhances **formulation stability** during storage and in biological fluids.

- **Measurement Technique:**

Electrophoretic Light Scattering (ELS) is the most widely used technique. In ELS, an electric field is applied to the nanoparticle suspension, causing charged particles to move (electrophoresis). The velocity of particle movement is measured by light scattering, and the **zeta potential (ζ)** is calculated using the Smoluchowski equation.

- **Significance of Zeta Potential:**
 - High positive or negative values ($> \pm 30$ mV) indicate **strong electrostatic stabilization**, reducing the likelihood of aggregation.
 - Low zeta potential ($< \pm 20$ mV) often results in **particle flocculation**, which can compromise drug delivery performance.

- **Applications in Nanomedicine:**
 - Liposomes: PEGylation reduces surface charge and increases circulation time while maintaining colloidal stability.
 - Polymer nanoparticles: Cationic nanoparticles interact efficiently with negatively charged cell membranes, enhancing cellular uptake for gene delivery.
 - Inorganic nanoparticles: Surface modification with ligands or polymers tunes zeta potential to optimize biodistribution and reduce opsonization.

Example: Chitosan-coated PLGA nanoparticles exhibit a zeta potential of +35 mV, enhancing mucoadhesion and promoting drug delivery to mucosal surfaces.

3. Surface Chemistry and Functional Groups

The chemical composition and functional groups on nanoparticle surfaces influence **drug loading, targeting, stability, and protein interactions**. Surface characterization ensures successful **surface modifications, ligand attachment, or coating strategies**.

a) Fourier-Transform Infrared Spectroscopy (FTIR)

- **Principle:** FTIR measures the absorption of infrared radiation by molecular bonds at characteristic wavelengths. Each functional group (e.g., $-\text{OH}$, $-\text{NH}_2$, $-\text{COOH}$) produces a unique absorption peak, allowing qualitative identification.

- **Applications in Nanomedicine:**
 - **Confirmation of surface modification:** PEGylation or ligand conjugation can be verified by the appearance of characteristic peaks.
 - **Drug encapsulation:** Changes in peak positions or intensities indicate interaction between the drug and the carrier.

- **Functional group analysis:** Detects chemical changes after nanoparticle synthesis or post-modification.
- **Example:**
 - Gold nanoparticles functionalized with folic acid show new peaks around 1650 cm^{-1} (amide bond), confirming ligand conjugation.
- b) X-ray Photoelectron Spectroscopy (XPS)**
 - **Principle:** XPS measures the **binding energy of electrons** ejected from the nanoparticle surface when irradiated with X-rays. The elemental composition, chemical state, and surface coverage can be determined.
 - **Applications:**
 - Detection and quantification of surface ligands or coating materials.
 - Verification of oxidation states in inorganic nanoparticles (e.g., $\text{Fe}^{2+}/\text{Fe}^{3+}$ in iron oxide NPs).
 - Quality control for reproducible surface modification.
 - **Example:** PEGylated liposomes analyzed by XPS confirm the presence of carbon (C1s) and oxygen (O1s) peaks corresponding to PEG chains on the surface.

4. Crystallinity and Phase

The **physical state** of nanoparticles (crystalline or amorphous) significantly affects **drug solubility, stability, and release kinetics**.

a) X-ray Diffraction (XRD):

- Determines the crystalline structure of inorganic nanoparticles by measuring the diffraction pattern of X-rays passing through the sample.
- **Significance:**
 - Crystallinity affects drug loading and dissolution rates.
 - Identification of polymorphic forms is critical for regulatory compliance.

- **Example:**
 - Silver nanoparticles exhibit characteristic peaks at 38°, 44°, and 64° 2θ, confirming face-centered cubic (fcc) crystalline structure.

- b) **Differential Scanning Calorimetry (DSC):**
 - Measures thermal transitions such as melting points, glass transition, or crystallization.

- **Significance:**
 - Detects physical interactions between drug and carrier.
 - Confirms amorphous or crystalline state of encapsulated drugs.

- **Example:**
 - Paclitaxel-loaded PLGA nanoparticles show a shift in melting peak in DSC, indicating successful encapsulation and molecular interaction.

5. Morphology

Nanoparticle morphology—including **shape, surface roughness, and porosity**—influences **biodistribution, cellular internalization, and clearance**. Spherical particles are generally preferred for uniform biodistribution, while rod-shaped or filamentous particles may have prolonged circulation times.

a) Atomic Force Microscopy (AFM)

- **Principle:** AFM uses a sharp probe that scans across the sample surface. The deflection of the cantilever is measured to construct a **3D topographic map** at nanometer resolution.

- **Advantages:**
 - Provides three-dimensional imaging of nanoparticles.
 - Can be performed in air or liquid, preserving native state.
 - Measures surface roughness and particle height.

- **Limitations:**
 - Small scanning area; may not represent entire sample.
 - Time-consuming compared to DLS or NTA.

- **Applications:**
 - Visualization of liposomes and polymeric nanoparticles.
 - Surface roughness analysis for enhanced cellular adhesion.
 - Complementary to TEM/SEM for 3D morphological insight.
- **Example:** Polymeric micelles show uniform spherical shape with 50–100 nm diameter, confirmed by AFM 3D mapping.

BIOLOGICAL CHARACTERIZATION

Biological characterization evaluates the interaction of nanomedicines with biological systems, including cytotoxicity, cellular uptake, and in vivo behavior.

a) In Vitro Assays

- **Cytotoxicity assays:** MTT, LDH release, and live/dead staining measure cell viability.
- **Cellular uptake:** Confocal microscopy and flow cytometry track nanoparticle internalization.
- **Hemocompatibility:** Evaluates interaction with blood components, hemolysis, and platelet aggregation.

b) In Vivo Pharmacokinetics and Biodistribution

- **Imaging techniques:** MRI, PET, and fluorescence imaging monitor distribution and clearance.
- **Pharmacokinetic studies:** Assess half-life, circulation, and accumulation in target tissues.

ADVANCED ANALYTICAL TECHNIQUES

Modern nanomedicine research often employs hybrid and high-resolution analytical techniques:

- **Nuclear Magnetic Resonance (NMR):** Provides structural information and drug–carrier interactions.
- **Mass Spectrometry (MS):** Detects drug loading, release kinetics, and degradation products.
- **Small-angle X-ray scattering (SAXS):** Determines particle size distribution and shape in solution.

- **Cryo-electron microscopy (Cryo-EM):** Preserves native morphology and avoids drying artifacts.

CHALLENGES IN NANOMEDICINE CHARACTERIZATION

Despite advances, several challenges remain:

1. **Polydispersity:** Nanoparticles often have broad size distributions, complicating analysis.
2. **Aggregation:** Nanoparticles tend to agglomerate, affecting reproducibility.
3. **Surface modification:** Detecting and quantifying surface ligands is challenging.
4. **Biological complexity:** Protein corona formation in biological fluids alters nanoparticle behavior.
5. **Standardization:** Lack of universal protocols for characterization leads to inconsistent results.

INTEGRATION OF CHARACTERIZATION TECHNIQUES

No single technique can provide complete information. Combining multiple complementary techniques ensures accurate assessment:

Table: 3

Property	Techniques Used
Particle size	DLS, NTA, TEM, AFM
Surface charge	Zeta potential, XPS
Surface chemistry	FTIR, XPS, NMR
Crystallinity	XRD, DSC
Morphology	TEM, SEM, AFM, Cryo-EM
Biological activity	In vitro assays, in vivo imaging

REGULATORY PERSPECTIVE

Regulatory agencies, including the FDA and EMA, emphasize comprehensive physicochemical and biological characterization for nanomedicines. Characterization data are critical for Investigational New Drug (IND) applications and quality control during manufacturing. Standardized methods and validated analytical techniques are encouraged to ensure reproducibility, safety, and efficacy.

FUTURE PERSPECTIVES

- **High-throughput characterization:** Automation and AI-assisted imaging can accelerate analysis.
- **In situ characterization:** Techniques such as real-time NTA or microfluidic platforms enable monitoring nanoparticle behavior under physiological conditions.
- **Protein corona studies:** Advanced proteomics approaches will better predict biological interactions.
- **Standardization:** Global initiatives are underway to establish universally accepted characterization protocols.

CONCLUSION

Nanomedicine characterization is a multidisciplinary effort that integrates physicochemical, morphological, and biological analysis. Accurate characterization ensures safety, efficacy, and reproducibility, which are critical for clinical translation. While traditional techniques like DLS, TEM, and FTIR remain widely used, emerging high-resolution and in situ methods are enhancing our understanding of nanoparticle behavior in complex biological systems. The combination of multiple techniques, standardized protocols, and advanced analytical approaches will drive the next generation of safe and effective nanomedicines.

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Cite as:

Gaurav Thakur, Subhendu Nair, Santosh K Patil (2026). Nanomedicine Characterization Techniques. *Journal of Pharmaceutical Analysis and Drug Research*, 8(1), 25-39.

<https://doi.org/10.5281/zenodo.19690138>