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NANOPARTICLE MEDIATED DRUG DELIVERY SYSTEMS FOR CANCER TREATMENT

Chapter 4: Synthesis and Characterization Techniques of Nanoparticles

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Abstract

Nanotechnology has emerged as an important multidisciplinary field involving the design, preparation, characterization, and application of materials with dimensions in the nanoscale range. Nanoparticles exhibit distinctive physicochemical characteristics compared with bulk materials because reduction in particle size produces a high specific surface area and changes in surface energy, optical behavior, electrical properties, magnetic characteristics, and chemical reactivity. These properties have created extensive opportunities for the development of nanoparticles in pharmaceutical sciences, particularly as carriers for drugs, proteins, peptides, nucleic acids, vaccines, and imaging agents.

Pharmaceutical nanoparticles can improve the apparent solubility and dissolution rate of poorly water-soluble drugs, protect labile therapeutic molecules from degradation, modify pharmacokinetic behavior, facilitate controlled drug release, and provide opportunities for passive or active targeting. Polymeric nanoparticles, lipid nanoparticles, metallic nanoparticles, magnetic nanoparticles, silica nanoparticles, and other nanostructured systems have therefore been investigated for oral, parenteral, transdermal, ocular, pulmonary, nasal, buccal, and targeted drug-delivery applications.





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Nanoparticles can be prepared using top-down and bottom-up approaches. Physical techniques include milling, laser ablation, evaporation-condensation, and high-pressure homogenization, whereas chemical and physicochemical approaches include nanoprecipitation, emulsion-solvent evaporation, ionic gelation, coacervation, microemulsion, sol-gel synthesis, and chemical reduction. Biological or green synthesis has received increasing attention because plant extracts, microorganisms, enzymes, proteins, and other biological materials can act as reducing, stabilizing, or capping agents.

Characterization is an essential component of nanoparticle development because particle size alone cannot adequately define the quality or performance of a nanomedicine. Dynamic light scattering, polydispersity index, zeta potential, scanning electron microscopy, transmission electron microscopy, atomic force microscopy, Fourier-transform infrared spectroscopy, X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, X-ray photoelectron spectroscopy, drug-loading studies, encapsulation efficiency, in-vitro release testing, and stability assessment provide complementary information regarding nanoparticle quality. The integration of these techniques provides a comprehensive understanding of nanoparticle structure, composition, surface properties, physical state, drug distribution, release behavior, and stability.

This chapter provides a comprehensive overview of the major synthesis approaches used for nanoparticles, with particular emphasis on pharmaceutical preparation methods, critical formulation and process variables, characterization techniques, stability assessment, applications, safety considerations, quality-by-design principles, scale-up, and future perspectives.

Keywords: Nanoparticles; Nanotechnology; Nanoprecipitation; Polymeric nanoparticles; Ionic gelation; Green synthesis; Characterization; Drug delivery.

1. Introduction

Nanotechnology involves the manipulation of matter at dimensions where size-dependent properties become important and can be exploited for specific technological or biomedical applications [1-3]. The nanoscale environment is associated with a substantial increase in surface-area-to-volume ratio, which can influence dissolution, adsorption, chemical reactivity, cellular interactions, and transport behavior [1,2].

In pharmaceutical sciences, nanotechnology has been increasingly explored to overcome limitations associated with conventional drug-delivery systems. Many therapeutic compounds exhibit poor aqueous solubility, low bioavailability, rapid degradation, unfavorable





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pharmacokinetics, or nonspecific distribution, which may limit their therapeutic effectiveness. Nanoparticle-based delivery systems can be designed to modify some of these properties by incorporating the drug into a nanoscale carrier [4-7].

The reduction of particle dimensions can increase the specific surface area of poorly soluble drug substances and consequently increase the surface available for interaction with dissolution media. According to the Noyes-Whitney relationship, dissolution rate is influenced by surface area, concentration gradient, diffusion coefficient, and diffusion-layer characteristics. Therefore, reducing the particle size of a poorly soluble drug can potentially enhance dissolution behavior when other factors are appropriately controlled [21].

Nanoparticles can also act as protective carriers for chemically or biologically unstable molecules. Encapsulation within a polymeric, lipid, or inorganic nanocarrier may reduce direct exposure of the drug to environmental factors or biological enzymes.[4,7] The carrier may additionally provide controlled release, thereby maintaining drug concentration over a prolonged period [5,7].

The term nanoparticle encompasses several structurally different systems [1]. Polymeric nanoparticles may occur as nanospheres or nanocapsules, lipid nanoparticles may contain solid or partially liquid lipid matrices, and metallic nanoparticles may consist of elemental metals or metal oxides [1,22]. Consequently, the synthesis technique must be selected according to the composition, intended function, drug properties, and desired critical quality attributes of the final system [15, 22].

An important principle in pharmaceutical nanotechnology is that nanoparticle synthesis and characterization are interdependent processes [15]. A change in polymer concentration, solvent composition, mixing intensity, surfactant concentration, pH, or temperature during synthesis can modify particle size, surface charge, morphology, encapsulation efficiency, and release behavior [15,23]. Consequently, nanoparticle formulation development requires systematic optimization of both material attributes and manufacturing conditions [23].

1.2. Classification of Nanoparticles

Nanoparticles can be classified according to composition, structure, morphology, preparation method, or intended application [1-3].





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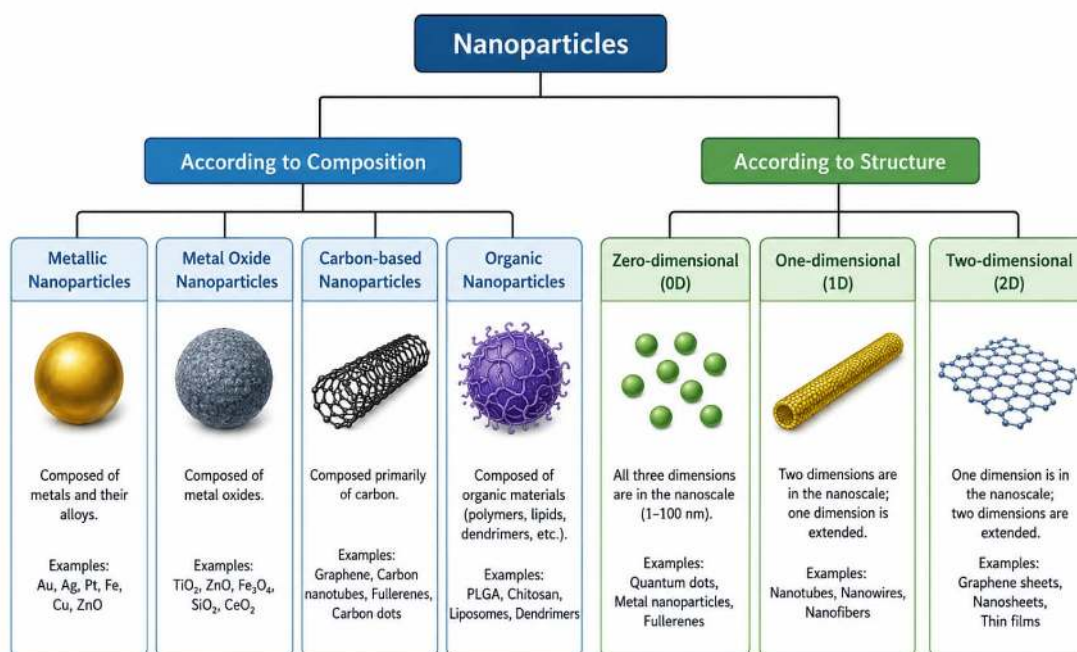


Fig.1 Classification of nanoparticles according to composition and structure

1.2.1 Polymeric Nanoparticles

Polymeric nanoparticles are nanosized carriers prepared from natural or synthetic polymers. Frequently investigated polymers include poly (lactic acid), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone, chitosan, gelatin, alginate, and other biodegradable or biocompatible materials [22–24]. Polymeric nanoparticles may be classified into Nano spheres and Nanocapsules. Nano spheres possess a matrix structure in which the drug is dissolved, dispersed, entrapped, or adsorbed within the polymeric matrix. Nanocapsules generally consist of an inner core surrounded by a polymeric membrane or shell [22].

The degradation characteristics of the polymer can be exploited to achieve controlled drug release. PLGA is particularly important because its degradation products, lactic acid and glycolic acid, can enter normal metabolic pathways. Polymer molecular weight, lactide-to-glycolide ratio, end-group chemistry, crystallinity, and concentration can influence degradation and drug-release behavior [24,25].

1.2.2 Lipid-Based Nanoparticles

Lipid-based nanocarriers include solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid Nanocapsules, liposomes, and related systems. SLNs contain a lipid matrix that remains solid at physiological temperature, whereas NLCs generally contain mixtures of solid





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and liquid lipids that create a less ordered matrix. Lipid nanoparticles can improve the delivery of poorly water-soluble compounds and may provide controlled or sustained drug release [27]. Their performance depends on lipid type, lipid ratio, surfactant concentration, drug solubility in the lipid phase, particle size, surface properties, and lipid crystallinity [26].

1.2.3 Metallic Nanoparticles

Metallic nanoparticles include gold, silver, platinum, copper, selenium, and related metal-based systems. Their distinctive optical, catalytic, electrical, and biological properties have resulted in applications in diagnostics, imaging, antimicrobial therapy, bio sensing, and drug delivery.

Gold nanoparticles are particularly important in biomedical nanotechnology because their optical properties can be manipulated through control of size, shape, and surface chemistry. Silver nanoparticles have been extensively investigated for antimicrobial applications [12,28].

1.2.4 Magnetic Nanoparticles

Magnetic nanoparticles commonly contain iron oxide materials such as magnetite or maghemite [3]. Their response to external magnetic fields can be exploited for magnetic targeting, separation, imaging, magnetic hyperthermia, and theranostic applications.

Surface coating of magnetic nanoparticles can improve colloidal stability and biocompatibility and can provide functional groups for drug loading or ligand attachment [29].

1.2.5 Silica Nanoparticles

Silica nanoparticles can be prepared with controlled surface chemistry and, in some cases, porous structures [3]. Mesoporous silica nanoparticles have attracted interest as drug-delivery systems because their pore size, surface area, and surface chemistry can be modified to accommodate different therapeutic molecules [30].

1.2.6 Carbon-Based Nanoparticles

Carbon-based nanomaterials include fullerenes, carbon nanotubes, graphene-derived materials, and carbon dots. Their optical, electrical, mechanical, and surface properties have resulted in applications in sensing, imaging, drug delivery, and biomedical engineering [3].

1.3. Approaches to Nanoparticle Synthesis

Nanoparticle synthesis is broadly classified into top-down and bottom-up approaches [2, 3].

1.3.1 Top-Down Approach

The top-down approach begins with a bulk material and reduces its dimensions to the nanoscale. Mechanical milling, high-pressure homogenization, laser ablation, lithography, and related techniques are representative top-down methods.





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The main advantage of top-down methods is that they can produce nanoparticles directly from bulk materials without requiring complex molecular synthesis. However, high energy requirements, structural defects, contamination, and broad size distributions may occur depending on the technique and processing conditions [2,3].

1.3.2 Bottom-Up Approach

Bottom-up synthesis involves the formation of nanoparticles from atoms, ions, molecules, polymers, or molecular assemblies. Nucleation, precipitation, condensation, self-assembly, reduction, and controlled aggregation contribute to nanoparticle formation.

Nano precipitation, chemical reduction, sol-gel synthesis, ionic gelation, coacervation, and biological synthesis are common bottom-up methods [12].

Bottom-up methods can provide considerable control over particle formation because nucleation and growth can be influenced through changes in concentration, temperature, pH, solvent composition, reaction time, and stabilizer concentration [2,3].

2. Methods of Nanoparticle Synthesis

2.1. Physical Methods of Nanoparticle Synthesis

2.1.1 Ball Milling

Ball milling is a mechanical top-down technique in which bulk material is subjected to repeated impact, collision, and friction against milling media. The mechanical forces progressively reduce the size of the starting material.

Particle size obtained through ball milling depends on milling speed, milling duration, ball-to-material ratio, milling-media characteristics, material hardness, and processing atmosphere. Prolonged or high-energy milling may introduce crystal defects or contamination from the milling equipment.

Ball milling is useful for certain inorganic and pharmaceutical materials but is less attractive when extremely narrow particle-size distributions or contamination-free products are required [2].

2.1.2. High-Pressure Homogenization

High-pressure homogenization involves forcing dispersion through a narrow homogenization gap at high pressure. High shear, turbulence, cavitation, and collision forces reduce the dimensions of dispersed particles [3].





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The technique is particularly useful for lipid-based nanoparticles and Nano suspensions. Pressure, number of homogenization cycles, temperature, and formulation viscosity can affect the resulting particle size [26].

2.1.3. Laser Ablation

Laser ablation involves irradiation of a solid target with a high-energy laser beam. Energy absorbed by the target produces vaporization or removal of material followed by rapid cooling, nucleation, and nanoparticle formation.

The technique can produce relatively pure nanoparticles because chemical reducing agents are not necessarily required. However, specialized equipment and careful control of laser energy, wavelength, pulse duration, and solvent conditions are required [2].

2.1.4. Evaporation-Condensation

Evaporation-condensation involves vaporization of a starting material followed by controlled condensation to form Nano sized particles. Temperature, pressure, carrier gas, evaporation rate, and condensation conditions influence the resulting particle characteristics [2].

2.2. Chemical Methods of Nanoparticle Synthesis

2.2.1. Sol-Gel Method

The sol-gel process is commonly used for the preparation of inorganic and metal-oxide nanoparticles.[2] It involves hydrolysis and condensation of molecular precursors followed by formation of a colloidal sol and subsequently a three-dimensional gel network. The resulting gel can undergo drying, calcination, or other thermal treatment to obtain the desired nanoscale material. Precursor concentration, water-to-precursor ratio, catalyst concentration, pH, temperature, aging time, and drying conditions influence the final structure.

2.2.2. Chemical Reduction:

Chemical reduction is extensively used for metallic nanoparticle synthesis. [12, 28] A metal precursor is reduced to its elemental state, followed by nucleation and growth of metallic particles. A stabilizing or capping agent may adsorb on the newly formed particle surface and reduce uncontrolled aggregation. The final particle size and morphology depend on the relative rates of nucleation and growth. [28]

The process can be represented as:

Metal precursor → Reduction → Atomic metal → Nucleation → Growth → Stabilization → Nanoparticles





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Reducing-agent concentration, metal precursor concentration, pH, temperature, reaction time, and stabilizer concentration are important variables affecting particle characteristics [12, 28].

2.3. Nano precipitation

Nano precipitation is one of the most important bottom-up techniques used for preparing polymeric nanoparticles [31-33]. The technique is based on rapid solvent displacement and precipitation of polymer when a polymer-containing organic phase is introduced into an aqueous non-solvent phase [31].

2.3.1 Principle

In a conventional Nano precipitation process, the drug and polymer are dissolved in a suitable water-miscible organic solvent. The organic phase is then introduced into an aqueous phase containing a stabilizer or surfactant when required [31].

Rapid diffusion of the organic solvent into the aqueous phase decreases the solubility of the polymer. Polymer super saturation is followed by nucleation, growth by condensation, and subsequent growth by coagulation or aggregation, resulting in nanoparticle formation [31]

The simplified mechanism can be represented in Figure 2.

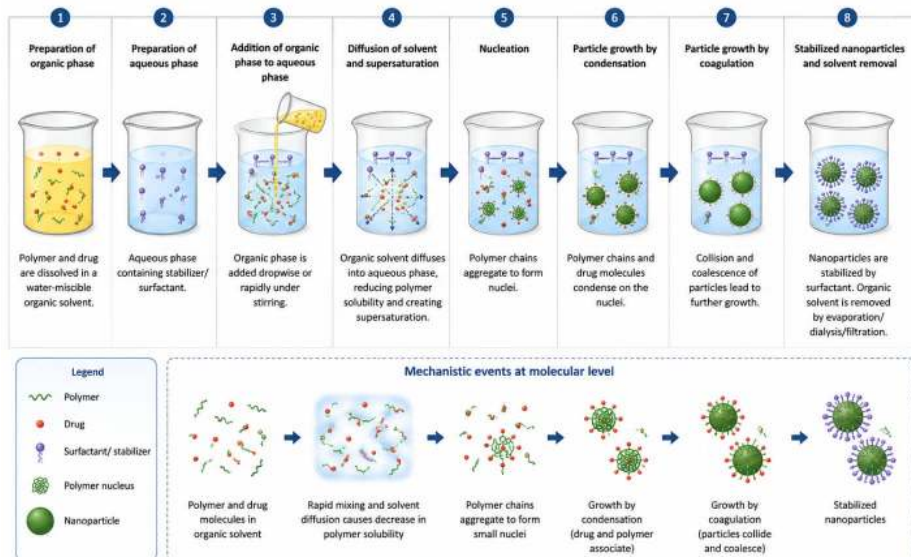


Fig. 2 General Mechanism of Nanoparticles

The final particle characteristics depend strongly on the balance between nucleation and growth [31, 32].





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2.3.2 General Procedure

The polymer and drug are first dissolved in a suitable organic solvent. The aqueous phase is prepared separately, with surfactant or stabilizer incorporated when necessary. The organic phase is then added to the aqueous phase under controlled stirring or mixing conditions. Nanoparticle formation occurs rapidly because of solvent diffusion and polymer precipitation. The organic solvent is subsequently removed using evaporation, dialysis, ultrafiltration, or another appropriate technique. The nanoparticles may then be purified by centrifugation, filtration, dialysis, or ultrafiltration depending on particle composition and formulation requirements [31].

2.3.3 Factors Affecting Nano precipitation

Polymer Concentration

Increasing polymer concentration may increase particle size because higher polymer content increases solution viscosity and the amount of material available for particle growth.

Drug Concentration

Drug concentration can influence nucleation, drug-polymer interactions, encapsulation efficiency, and particle size.

Solvent Selection

The organic solvent must dissolve the selected polymer and, where applicable, the drug while allowing appropriate diffusion into the aqueous phase. Solvent miscibility with water influences solvent-displacement kinetics and nanoparticle formation.

Aqueous-to-Organic Phase Ratio

The ratio between the aqueous and organic phases influences super saturation and solvent diffusion and can therefore affect particle size and size distribution [31, 32].

Addition Rate

Rapid addition may produce different nucleation conditions compared with slow addition. Controlled addition is therefore important for reproducibility.

Mixing Conditions

Stirring intensity and mixing geometry determine the rate at which the organic and aqueous phases interact. Poor mixing may produce heterogeneous particle populations, whereas excessively intense mixing may affect particle stability depending on formulation composition [31].





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Surfactant Concentration

Surfactants can reduce interfacial tension and provide steric or electrostatic stabilization. Excessive surfactant concentration, however, may complicate purification and may influence biological compatibility.

2.3.4 Advantages

Nanoprecipitation is relatively simple and requires comparatively mild processing conditions [31-33]. The method can produce nanoparticles with relatively narrow size distributions when formulation and process conditions are properly controlled. It is particularly suitable for many biodegradable polymers and hydrophobic drug molecules [31, 32].

2.3.5 Limitations

The method requires careful selection of a suitable solvent/non-solvent system [31]. Residual organic solvent must be appropriately removed when the formulation is intended for pharmaceutical or biomedical use. Highly water-soluble drugs may also require modification of the conventional process because they may partition preferentially into the aqueous phase.

Flash nanoprecipitation and microfluidic nanoprecipitation have been investigated to improve mixing, reproducibility, and scale-up [33, 34].

2.4. Emulsion-Solvent Evaporation:

Emulsion-solvent evaporation is a widely used technique for the preparation of polymeric nanoparticles [22, 35].

In the single-emulsion process, the drug and polymer are dissolved or dispersed in an organic phase. This organic phase is emulsified into an aqueous phase containing a stabilizer. High-speed homogenization or sonication is used to reduce the organic droplets.

The organic solvent is then evaporated, causing polymer precipitation and formation of nanoparticles [35].





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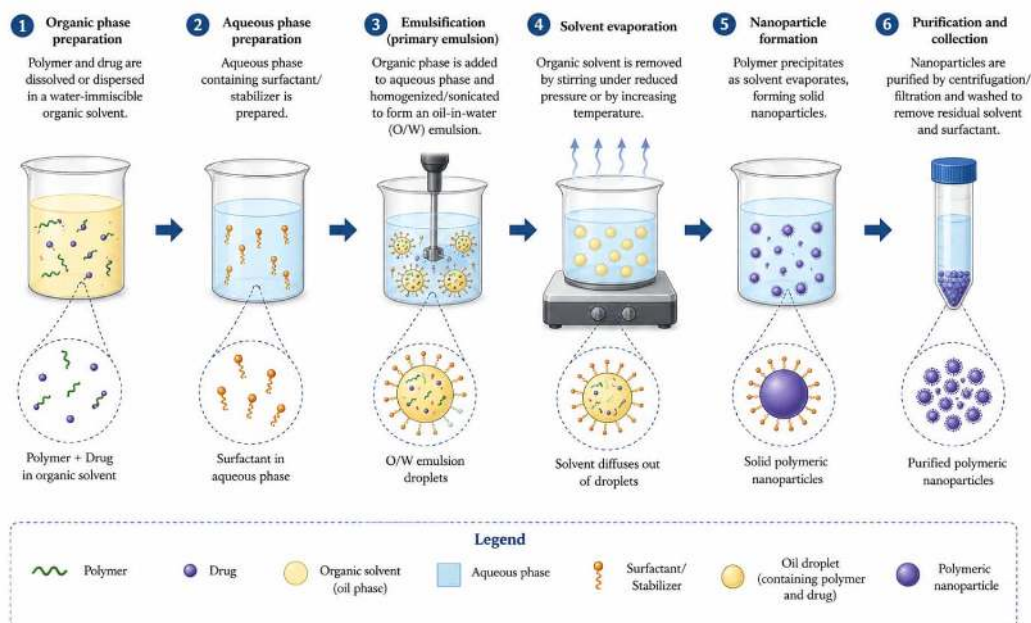


Fig.3 Preparation of polymeric nanoparticles by single-emulsion solvent evaporation

For hydrophilic drugs, proteins, peptides, and nucleic acids, a double-emulsion water-in-oil-in-water technique can be used. The hydrophilic drug is first dissolved in an internal aqueous phase, which is emulsified into an organic polymer solution and subsequently dispersed into an external aqueous phase.

Important variables include polymer concentration, drug-to-polymer ratio, surfactant concentration, organic solvent, homogenization speed, sonication time, phase-volume ratio, and solvent-evaporation rate [35, 36].

2.5. Ionic Gelation

Ionic gelation is a mild technique commonly used for preparing chitosan nanoparticles. Chitosan is a cationic natural polymer derived from chitin and possesses biodegradability, biocompatibility, mucoadhesive characteristics, and functional groups that permit chemical modification [37, 38]. The method is based primarily on electrostatic interactions between oppositely charged polymer chains and cross-linking ions.[37] Chitosan can interact with anionic cross-linkers such as sodium tripolyphosphate, producing ionically cross-linked nanoparticles .





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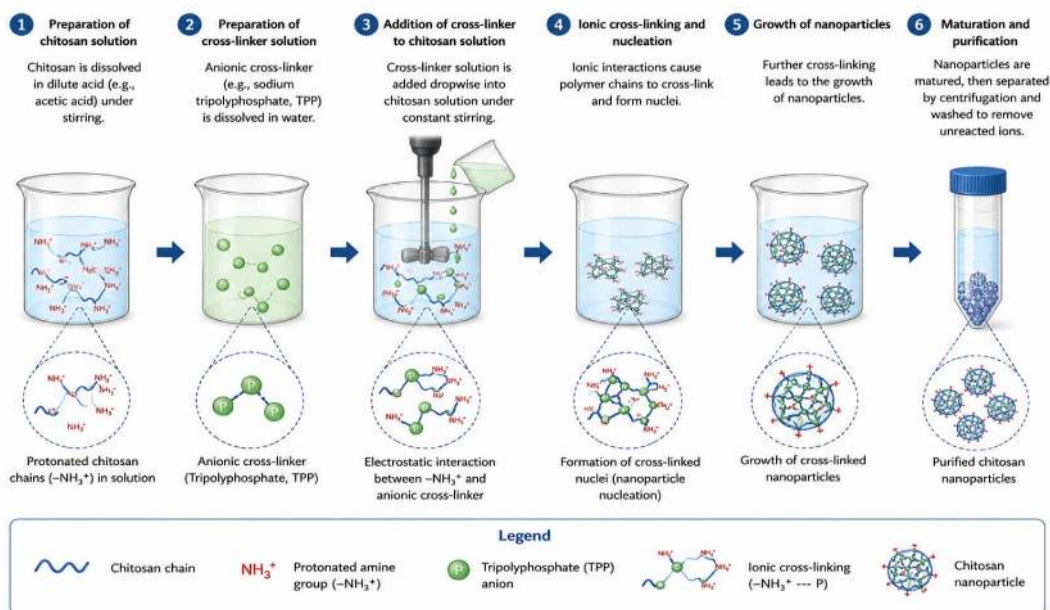


Fig.4 Preparation of chitosan nanoparticles by ionic gelation

The major formulation variables include chitosan concentration, degree of deacetylation, molecular weight, pH, cross-linker concentration, chitosan-to-cross-linker ratio, drug concentration, and mixing conditions [40]. A major advantage is the possibility of preparing nanoparticles under relatively mild aqueous conditions, making the method suitable for certain proteins, peptides, nucleic acids, and other sensitive molecules. However, chitosan nanoparticles can aggregate under unfavorable pH, ionic-strength, temperature, or storage conditions. Therefore, stabilization and storage conditions must be carefully evaluated.

2.6. Micro emulsion Method

Microemulsion methods use surfactant-stabilized nanoscale domains as reaction or particle-formation environments. Depending on the composition, oil-in-water, water-in-oil, or bicontinuous structures can be formed. The confined nanoscale domains can restrict particle growth and may facilitate production of relatively small nanoparticles. The major formulation variables include surfactant concentration, co-surfactant concentration, oil-to-water ratio, temperature, precursor concentration, and reaction conditions.

A limitation is the potential difficulty associated with removing large amounts of surfactant and other formulation components from the final pharmaceutical product [2].





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2.7. Green and Biological Synthesis:

Green synthesis represents an alternative approach in which biological materials participate in nanoparticle formation. Plants, plant extracts, microorganisms, enzymes, proteins, polysaccharides, and other biomolecules have been investigated as reducing, stabilizing, or capping agents. Plant extracts are particularly attractive because they contain diverse phytochemicals, including polyphenols, flavonoids, terpenoids, proteins, sugars, and organic acids. These compounds can participate in reduction of metal ions and subsequent stabilization of nanoparticles [12].

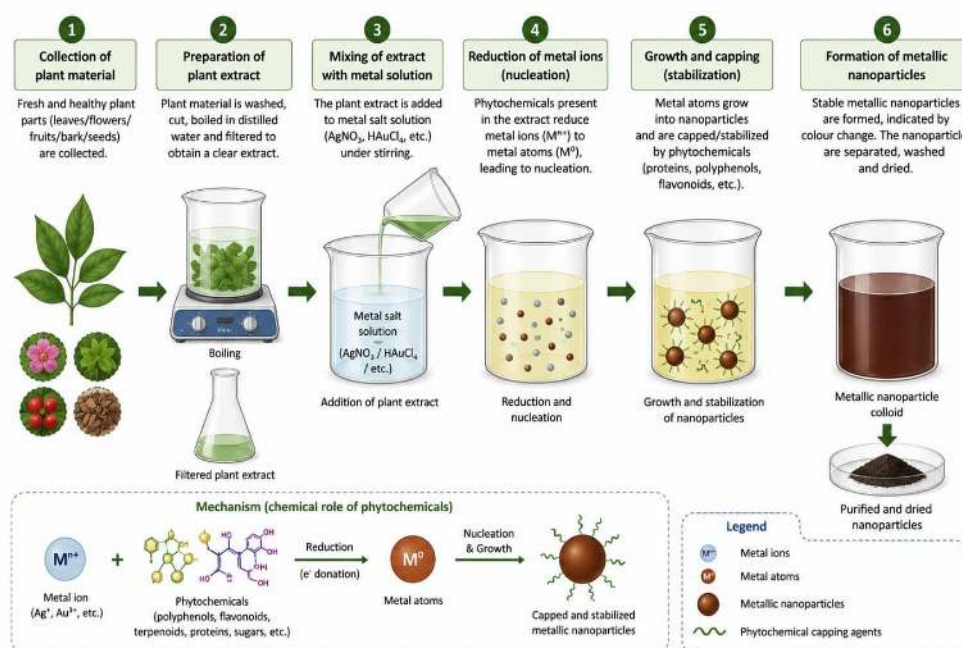


Fig. 5 Green Synthesis of metallic nanoparticles using plant extracts

The formation of metallic nanoparticles is often accompanied by changes in solution color and optical properties, although color change alone is insufficient to establish nanoparticle formation. UV-visible spectroscopy, FTIR, XRD, SEM, TEM, DLS, and zeta-potential analysis are commonly combined to confirm nanoparticle formation and characterize the resulting systems. The principal advantages of green synthesis include potential reduction in toxic reducing agents, use of renewable biological resources, and relatively simple processing however, variability in biological source, extraction conditions, phytochemical composition, and reaction parameters can compromise reproducibility [13, 14]. Critical Formulation and Process Variables Nanoparticle quality is strongly influenced by both material attributes and process conditions [15, 23].





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Table No: 01 Different Variable and its Effect on nanoparticles

Variable	Effect on nanoparticles
Polymer concentration	Influences size, viscosity, drug loading and release
Molecular weight of polymer	Influences viscosity, degradation and release
Drug/polymer ratio	Influences loading and encapsulation
Surfactant concentration	Influences stabilization and size
Solvent type	Influences precipitation and diffusion
Phase ratio	Influences nucleation and particle growth
pH	Influences ionization and surface charge
Temperature	Influences viscosity, diffusion and reaction rate
Mixing speed	Influences particle-size distribution
Sonication	Influences droplet/particle size
Reaction time	Influences growth and maturation
Drying conditions	Influence aggregation and redispersibility

The systematic evaluation of these variables is important for developing a robust nanoparticle manufacturing process [23].

3. Characterization of Nanoparticles

Nanoparticle characterization should be considered a multidimensional process because no single analytical method can adequately describe particle size, morphology, surface chemistry, composition, physical state, drug loading, and stability simultaneously [15-20].

3.1 Particle Size

Particle size is a major critical quality attribute because it can influence surface area, dissolution, cellular uptake, biodistribution, aggregation, and drug release. Dynamic light scattering is widely used for determining hydrodynamic particle diameter in liquid dispersion. [16, 41] DLS analyzes fluctuations in scattered light generated by Brownian motion and converts these measurements into hydrodynamic size [41]. An important consideration is that DLS measures the hydrodynamic diameter of particles in dispersion, whereas TEM measures the physical dimensions of particles prepared for electron microscopy. Consequently, the two techniques may produce different size values without either result necessarily being incorrect [16].





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3.2 Polydispersity Index

PDI provides an estimate of the breadth of the particle-size distribution obtained from DLS. Lower PDI generally indicates greater uniformity, whereas higher PDI indicates greater heterogeneity. PDI should be interpreted together with the intensity, volume, or number distributions and with knowledge of the formulation and measurement conditions [41].

3.3 Zeta Potential

Zeta potential describes the electrokinetic potential associated with the particle-liquid interface. It provides useful information about surface charge and contributes to interpretation of colloidal stability. Strong electrostatic repulsion can reduce particle-particle interaction and aggregation. However, zeta potential should not be treated as the sole predictor of stability because steric stabilization, polymer adsorption, ionic strength, pH, and medium composition can also strongly influence aggregation [41].

3.4 Scanning Electron Microscopy

SEM provides high-resolution information about the external morphology and surface characteristics of nanoparticles. SEM can be used to examine particle shape, surface roughness, aggregation, and approximate particle dimensions. Sample drying and preparation can influence morphology, so sample-preparation conditions should be standardized [18].

3.5 Transmission Electron Microscopy

TEM provides direct high-resolution visualization of nanoparticles. It can provide information about particle morphology, internal structure, shell thickness, and particle dimensions. TEM is particularly valuable for differentiating structural architectures such as Nano spheres and Nanocapsules [15].

3.6 Atomic Force Microscopy:

AFM generates nanoscale surface-topographical information by scanning a probe over the sample surface. The technique can provide three-dimensional information regarding particle height, surface roughness, and morphology. AFM can be useful for soft or polymeric nanoparticles because imaging does not necessarily require conductive coatings used in some electron microscopy preparations [18].

3.7. Fourier-Transform Infrared Spectroscopy:

FTIR spectroscopy is widely used to identify functional groups and investigate possible chemical or molecular interactions among drugs, polymers, surfactants, and other formulation components. The spectrum of the pure drug can be compared with the polymer, physical mixture, and final nanoparticle formulation. Retention of characteristic absorption bands may





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support preservation of the major functional groups, whereas shifts, broadening, intensity changes, or disappearance of peaks may indicate molecular interactions or changes in the physical environment. FTIR interpretation should be performed cautiously because peak changes can result from hydrogen bonding, physical-state changes, encapsulation, overlapping bands, or differences in sample preparation rather than necessarily indicating chemical degradation [15].

3.8. X-Ray Diffraction:

XRD is used to investigate crystalline structure and physical state [18]. Crystalline materials generally produce characteristic diffraction peaks, whereas amorphous materials produce broad diffuse halos. Comparison of the XRD pattern of the pure drug with that of the nanoparticle formulation can reveal changes in drug crystallinity. Reduction or disappearance of characteristic drug peaks may suggest reduced crystallinity or amorphization, although the result should preferably be confirmed by DSC and other complementary techniques. Crystallinity can influence drug solubility, dissolution, stability, and release behavior [15].

3.9. Differential Scanning Calorimetry

DSC measures changes in heat flow associated with thermal transitions. Important thermal events include melting, crystallization, glass transition, and other phase transitions. DSC can be used to compare the thermal behavior of pure drug, polymer, physical mixture, and nanoparticle formulation. Changes in the position, intensity, or shape of a drug melting endotherm can provide evidence of changes in crystallinity or molecular dispersion. DSC should be interpreted together with XRD and FTIR because each technique provides different information regarding the physical and chemical state of the formulation [15].

3.10. Thermo gravimetric Analysis

TGA measures sample mass changes as a function of temperature. The technique can provide information about moisture, residual solvent, thermal decomposition, and overall thermal stability. TGA is particularly useful when nanoparticle formulations contain multiple components with different thermal-decomposition profiles [18].

3.11. UV-Visible Spectroscopy

UV-visible spectroscopy can be used for quantitative drug estimation when the drug possesses an appropriate chromophore. In metallic nanoparticle synthesis, UV-visible spectroscopy can also provide information about optical properties such as surface Plasmon resonance [12]. For pharmaceutical formulations, the analytical method should be appropriately validated for





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parameters such as specificity, linearity, accuracy, precision, range, and robustness according to the intended application.

3.12. X-Ray Photoelectron Spectroscopy

XPS provides information regarding elemental composition and chemical states at or near the nanoparticle surface. The technique is particularly valuable when surface functionalization, coating, ligand attachment, oxidation state, or surface chemistry is important [18]. Surface chemistry can strongly influence protein adsorption, cellular interactions, colloidal stability, and targeting behavior [42].

3.13. Energy-Dispersive X-Ray Spectroscopy

EDX or EDS is frequently coupled with SEM or TEM to provide elemental information. The technique can help confirm the presence of specific elements in metallic, inorganic, or composite nanoparticles [18]. For example, EDX can complement microscopic evidence of silver or gold nanoparticles by confirming the corresponding elemental composition [12].

3.14. Surface Area and Porosity

Brunauer-Emmett-Teller (BET) analysis can be used to determine specific surface area and, in appropriate systems, pore characteristics [18]. Surface area is particularly relevant to porous silica nanoparticles, adsorbent nanomaterials, and some drug-delivery systems because it can influence drug adsorption and loading [30].

3.15. Drug Content and Encapsulation Efficiency

Drug content determines the quantity of active pharmaceutical ingredient present in a defined quantity of nanoparticle formulation. Quantitative determination may be performed using UV-visible spectroscopy, HPLC, LC-MS, or another validated analytical method depending on the drug. Encapsulation efficiency describes the percentage of the initial drug that becomes incorporated into or associated with nanoparticles. High encapsulation efficiency is generally desirable because it indicates efficient utilization of the drug during formulation. Encapsulation efficiency is influenced by drug solubility, polymer-drug affinity, polymer concentration, drug-to-polymer ratio, solvent characteristics, surfactant concentration, and preparation technique [31].

3.16. Drug Loading

Drug loading represents the amount of drug incorporated relative to the total nanoparticle mass. Drug loading is an important parameter because high drug loading can reduce the quantity of





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carrier required to deliver a particular dose. A formulation can show high encapsulation efficiency but relatively low drug loading when a large amount of polymer is required to incorporate a small quantity of drug.

3.17. In-Vitro Drug Release

In-vitro drug-release studies are conducted to determine the rate and extent of drug release under controlled experimental conditions. Release from polymeric nanoparticles can occur through diffusion, desorption of surface-associated drug, polymer swelling, polymer degradation, polymer erosion, or combinations of these mechanisms. Nanoparticles prepared by Nano precipitation may show an initial burst-release phase followed by a slower sustained-release phase. The burst phase may be associated with drug located near or on the particle surface, whereas prolonged release may result from diffusion through the polymeric matrix and/or polymer degradation [31]. Release studies may be conducted using dialysis bags, diffusion cells, sample-and-separate techniques, or other validated approaches depending on the nanoparticle formulation and drug properties.

3.18. Mathematical Modelling of Drug Release

Mathematical models can be used to investigate the kinetics and possible mechanism of drug release.

Zero-Order Model

$$Q = Q_0 + K_0t$$

Zero-order release describes a system in which a relatively constant amount of drug is released per unit time.

First-Order Model

$$\log Q = \log Q_0 - K_1t/2.303$$

First-order behavior describes release in which the rate depends on the amount of drug remaining in the system.

Higuchi Model

$$Q = KH\sqrt{t}$$

The Higuchi model is commonly used to describe diffusion-controlled release from matrix systems.

Korsmeyer-Peppas Model

$$M_t/M_\infty = Kt^n$$





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The release exponent n can provide information regarding the mechanism of drug transport when the model is applied within its appropriate assumptions. Model selection should consider statistical fit, experimental design, and mechanistic plausibility rather than relying only on the highest correlation coefficient.

3.19. Stability of Nanoparticles

Stability studies are essential for determining whether nanoparticles maintain their intended quality attributes throughout storage [15, 22]. Important parameters include particle size, PDI, zeta potential, morphology, drug content, encapsulation efficiency, physical appearance, crystallinity, and drug-release behavior. Nanoparticle dispersions can undergo aggregation, sedimentation, Ostwald ripening, particle growth, drug leakage, oxidation, hydrolysis, or other chemical and physical changes during storage. Temperature, humidity, light, pH, ionic strength, oxygen exposure, and container properties can influence nanoparticle stability. Lyophilization can improve storage stability by converting aqueous nanoparticle dispersion into a dry product. However, freezing and drying can cause particle aggregation or changes in particle structure, making appropriate cry protectants or lyoprotectants necessary in some formulations [15].

3.20. Safety and Toxicity

Nanoparticle safety is influenced by particle size, morphology, surface chemistry, surface charge, composition, aggregation state, dose, route of administration, and biodistribution. Nanoparticles can interact with proteins, cell membranes, immune cells, and intracellular structures because of their high surface area and nanoscale dimensions. Potential biological effects include oxidative stress, inflammatory responses, membrane disruption, mitochondrial effects, genotoxicity, and changes in cellular signaling depending on the nanoparticle type and exposure conditions. Therefore, safety assessment should be integrated into nanoparticle development rather than considered only after formulation optimization. For pharmaceutical nanoparticles, relevant studies may include cytotoxicity, Hemocompatibility, immunotoxicity, genotoxicity, pharmacokinetics, biodistribution, and tissue accumulation depending on the route and intended application [17].

4. Applications of Nanoparticles in Pharmaceutical Sciences

4.1 Oral Drug Delivery

Nanoparticles can enhance the dissolution and apparent solubility of poorly water-soluble drugs and may influence interaction with gastrointestinal mucus and epithelial surfaces.





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4.2 Parenteral Drug Delivery

Intravenous nanoparticles can be engineered to modify circulation, biodistribution, drug release, and interactions with specific biological targets.

4.3 Transdermal Drug Delivery

Nanoparticles can potentially improve drug deposition within or permeation across the skin by modifying drug solubility, partitioning, and interaction with skin structures [43].

4.4 Ocular Drug Delivery

Nanoparticles can prolong drug residence time and potentially improve ocular delivery by reducing rapid clearance from the ocular surface [44].

4.5 Pulmonary Drug Delivery

Nanoparticles have been investigated for pulmonary delivery of small molecules, proteins, nucleic acids, and vaccines [4].

4.6 Nasal and Buccal Delivery

Chitosan-based nanoparticles are particularly interesting for mucosal delivery because of their mucoadhesive properties and ability to interact with epithelial surfaces.[37-40]

4.7 Cancer Drug Delivery

Nanoparticles are widely investigated for anticancer drug delivery because they can accommodate therapeutic agents, facilitate controlled release, and support surface modification for targeting [4, 28].

4.8 Gene Delivery

Nanoparticles can protect nucleic acids from degradation and facilitate cellular uptake.[4] Lipid and polymeric nanoparticles have become important platforms for delivery of RNA and other nucleic-acid therapeutics.

4.9 Vaccine Delivery

Nanoparticles can act as carriers or adjuvant platforms for antigens and nucleic acids and can influence immune-cell interactions.

4.10 Diagnostic and Theranostic Applications

Metallic, magnetic, silica, and other nanoparticles can combine diagnostic imaging with therapeutic functions, creating multifunctional theranostic systems [28,29].





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4.11. Nanoparticles in Targeted Drug Delivery

Targeting can be broadly divided into passive and active targeting.

Passive targeting exploits differences in physiological distribution and pathological microenvironments, whereas active targeting involves modification of nanoparticle surfaces with ligands capable of recognizing specific cellular or molecular targets. Potential targeting ligands include antibodies, peptides, aptamers, carbohydrates, folic acid, and other receptor-binding molecules [4, 42]. Surface modification can alter particle charge, protein adsorption, circulation behavior, cellular uptake, and biological distribution. However, successful attachment of a targeting ligand does not automatically guarantee improved therapeutic efficacy because biological barriers, protein-corona formation, receptor availability, and particle heterogeneity can influence targeting performance [42, 46].

5. Quality-by-Design Approach:

Quality-by-Design (QbD) provides a systematic approach for developing nanoparticle formulations by linking material attributes and process parameters with critical quality attributes [23].

Critical Material Attributes:

- Polymer molecular weight
- Polymer composition
- Drug physicochemical properties
- Drug-to-polymer ratio
- Surfactant type
- Surfactant concentration
- Solvent characteristics
- Lipid composition

Critical Process Parameters:

- Mixing speed
- Addition rate
- Homogenization pressure
- Sonication time
- Temperature
- Processing time





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- Solvent evaporation rate
- Drying conditions

Critical Quality Attributes:

- Particle size
- PDI
- Zeta potential
- Morphology
- Drug content
- Encapsulation efficiency
- Drug loading
- Residual solvent
- Release profile
- Stability

Design of experiments can be used to investigate the effect of formulation and process variables systematically and to identify an optimized design space.[23]

6. Scale-Up and Manufacturing Challenges:

Laboratory-scale nanoparticle preparation does not always translate directly to industrial manufacturing [31,33]. Changes in mixing geometry, flow conditions, heat transfer, mass transfer, solvent-removal efficiency, and equipment configuration can alter nanoparticle formation during scale-up. Flash Nano precipitation, microfluidic systems, continuous-flow reactors, and other intensified manufacturing approaches are being investigated to improve reproducibility and scalability. [33, 34] Industrial manufacturing also requires control of residual solvents, microbial contamination, batch-to-batch variation, sterility where appropriate, and long-term stability.[15,33]

7. Regulatory and Quality Considerations

Nano medicines require careful control of quality, safety, and efficacy because changes in nanoscale properties can influence biological behavior [15, 17]. Important quality attributes include particle size distribution, morphology, surface charge, chemical composition,





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crystallinity, drug loading, encapsulation efficiency, residual solvent, impurities, surface modification, and stability [15, 18]. Regulatory assessment should consider both conventional pharmaceutical quality attributes and nano-specific characteristics that may influence biological performance [15]. Analytical methods used for release testing and characterization should be appropriately validated and capable of detecting relevant changes in critical quality attributes.

8. Characterization Techniques

8.1 Advanced Characterization Techniques:

Modern nanoparticle characterization increasingly incorporates techniques capable of resolving particle heterogeneity rather than relying exclusively on ensemble-average measurements. Asymmetric flow field-flow fractionation can separate nanoparticles according to hydrodynamic size and can be coupled with detectors such as multi-angle light scattering or other analytical systems [47]. Single-particle analytical approaches can provide information regarding particle-to-particle variability in size, composition, drug loading, or surface modification [19]. Nano-flow cytometry and related technologies have been investigated for characterizing individual lipid nanoparticles, extracellular vesicles, and other nanoscale systems [20].

These advanced approaches are particularly relevant to clinical translation because two nanoparticle batches may have similar mean particle sizes while differing substantially in heterogeneity [19,20].

8.2. Common Characterization Techniques and Their Applications

Table No: 02. Common Characterization Techniques and Their Applications

Characterization technique	Principle/measurement	Major information obtained
DLS	Brownian motion/light scattering	Hydrodynamic size
PDI	Distribution analysis	Size heterogeneity
Zeta potential	Electrophoretic mobility	Surface charge
SEM	Electron-surface interaction	Surface morphology





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TEM	Electron transmission	Size and internal morphology
AFM	Probe-surface interaction	3D topography
FTIR	Infrared absorption	Functional groups/interactions
XRD	X-ray diffraction	Crystallinity/phase
DSC	Heat-flow changes	Thermal transitions
TGA	Mass change with temperature	Thermal stability/solvent
UV-Vis	Optical absorption	Drug quantification/optical properties
HPLC	Chromatographic separation	Drug content/impurities
XPS	Photoelectron emission	Surface composition
EDX	Characteristic X-rays	Elemental composition
BET	Gas adsorption	Surface area/porosity
AF4	Field-flow separation	Size distribution/heterogeneity

No single characterization method is sufficient to establish complete nanoparticle quality [15, 19,20].

8.3. Comparative Evaluation of Major Synthesis Techniques

Table No: 03. Comparative Evaluation of Major Synthesis Techniques

Method	Major principle	Advantages	Limitations	Pharmaceutical relevance
Ball milling	Mechanical size reduction	Simple, solvent-free	High energy, possible contamination	Nanosizing of solids
High-pressure homogenization	Cavitation/shear	Scalable	High energy and pressure	Nanosuspensions, lipid systems





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Laser ablation	Laser-induced material removal	High purity	Expensive equipment	Metallic nanoparticles
Sol-gel	Hydrolysis/condensation	Good structural control	Drying/thermal treatment	Silica/oxide nanoparticles
Chemical reduction	Reduction/nucleation	Simple and versatile	Chemical residues	Metallic nanoparticles
Nanoprecipitation	Solvent displacement	Simple, rapid, reproducible	Solvent removal	Polymeric nanoparticles
Emulsion-solvent evaporation	Emulsion + solvent removal	Suitable for many polymers	Multiple processing steps	Drug-loaded polymeric NPs
Ionic gelation	Electrostatic cross-linking	Mild aqueous process	Stability concerns	Chitosan nanoparticles
Microemulsion	Confined nanoscale domains	Size control	Surfactant removal	Drug/inorganic nanoparticles
Green synthesis	Biological reduction/capping	Environmentally attractive	Reproducibility	Metallic/biogenic nanoparticles

9. Troubleshooting in Nanoparticle Preparation

9.1. Excessively Large Particle Size

Large particle size may result from excessive polymer concentration, inadequate stabilization, inappropriate solvent selection, poor mixing, or excessive particle growth. Possible corrective approaches include reducing polymer concentration, optimizing stabilizer concentration, improving mixing, modifying the solvent/non-solvent ratio, or changing the addition rate [31].

9.2. High PDI

A high PDI may indicate uncontrolled nucleation, aggregation, inadequate mixing, or inappropriate formulation composition. Process optimization and improved mixing can help produce a more uniform particle population [31, 41].





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9.3. Low Encapsulation Efficiency

Low encapsulation may result from poor drug-polymer affinity, high drug solubility in the external phase, unsuitable solvent selection, or an inappropriate drug-to-polymer ratio [22,31].

9.4. Aggregation during Storage

Aggregation may result from insufficient surface stabilization, unfavorable ionic strength, temperature changes, freeze-thaw stress, or inadequate drying conditions [15,37].

10. Future Perspectives

Future nanoparticle research is expected to focus increasingly on multifunctional, stimuli-responsive, targeted, and biologically responsive systems [4,28].

Stimuli-responsive nanoparticles can be designed to respond to pH, temperature, enzymes, redox conditions, light, magnetic fields, or other physiological triggers [4].

Green synthesis is expected to continue developing as researchers seek more sustainable nanoparticle manufacturing processes [12-14].

Artificial intelligence and machine learning may facilitate prediction of nanoparticle characteristics from formulation and process variables and may reduce the experimental burden associated with formulation optimization [48].

Continuous manufacturing and microfluidic systems may improve batch reproducibility and facilitate scale-up of nanoparticle production [33, 34].

Advanced single-particle characterization is expected to become increasingly important because mean particle-size measurements alone may fail to identify small but clinically relevant subpopulations of nanoparticles [19, 20].

The future of pharmaceutical nanotechnology will therefore depend not only on the ability to produce nanoparticles but also on the ability to reproducibly manufacture, accurately characterize, safely administer, and consistently control complex nanoformulations [15, 17].

11. Conclusion

Nanoparticles represent an important platform for modern pharmaceutical technology because their size, composition, surface chemistry, morphology, and internal structure can be engineered to modify drug delivery and biological performance.

Top-down methods reduce bulk materials to nanoscale dimensions, whereas bottom-up methods construct nanoparticles through nucleation, precipitation, condensation, self-assembly, or related molecular processes.





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Nano precipitation, emulsion-solvent evaporation, ionic gelation, micro emulsion, and related techniques are particularly important in pharmaceutical nanoparticle development because they can produce drug-loaded systems under controlled conditions.

The selection of synthesis technique should be based on drug properties, carrier characteristics, desired particle size, drug-loading requirements, route of administration, stability, scalability, and intended therapeutic application.

Comprehensive characterization using DLS, PDI, zeta potential, SEM, TEM, AFM, FTIR, XRD, DSC, TGA, chromatographic methods, and drug-release studies is necessary to establish the quality and performance of nanoparticle systems.

Future progress will depend on integration of pharmaceutical formulation science with advanced materials engineering, green synthesis, continuous manufacturing, quality-by-design principles, computational optimization, and single-particle analytical technologies.

References

1. Zielińska A, Carreiró F, Oliveira AM, Neves A, Pires B, Venkatesh DN, et al. Polymeric nanoparticles: production, characterization, toxicology and ecotoxicology. *Molecules*. 2020;25(16):3731.
2. Khan I, Saeed K, Khan I. Nanoparticles: properties, applications and toxicities. *Arab J Chem*. 2019;12(7):908-931.
3. Khan Y, Sadia H, Ali Shah SZ, Khan MN, Shah AA, Ullah N, et al. Classification, synthetic, and characterization approaches to nanoparticles and their applications in various fields of nanotechnology: a review. *Catalysts*. 2022;12(11):1386.
4. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnology*. 2018;16:71.
5. Pawar SP, Ahire MS, Shewale VV, Jadhav S, Mahajan KS, Sonawane DD. Silver nanoparticles: A comprehensive review on synthesis, properties and applications. *Research Journal of Pharmaceutical Dosage Forms and Technology*. 2025;17(4):279-85.
6. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. *Adv Drug Deliv Rev*. 2013;65(1):36-48.
7. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids Surf B Biointerfaces*. 2010;75(1):1-18.
8. Pelaz B, Alexiou C, Alvarez-Puebla RA, Alves F, Andrews AM, Ashraf S, et al. Diverse applications of nanomedicine. *ACS Nano*. 2017;11(3):2313-2381.





WISELEAF SCIENTIFIC VENTURES

9. Pawar PS, Ahire SM, Shewale VV, Jadhav S, Mahajan SK. Nanotechnology in herbal medicines: a review. *J Hosp Pharmacy*. 2025;20(1 Suppl 3):1-9.
10. Sharma A, Sharma US. Liposomes in drug delivery: progress and limitations. *Int J Pharm*. 1997;154(2):123-140.
11. Ahire SM, Pawar PS, Jadhav SP, Shewale VV, Patil CD, Mahajan SK, Vishwas S. Nanotechnology-driven antioxidants and immunomodulators for polycystic ovary syndrome (PCOS) therapy. *Nanotechnology NextGen: From Treatment to Transformation in Polycystic Ovary Syndrome (PCOS)*. 2025 Jul 15:184.
12. Singh J, Dutta T, Kim KH, Rawat M, Samddar P, Kumar P. 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *J Nanobiotechnology*. 2018;16:84.
13. Iravani S. Green synthesis of metal nanoparticles using plants. *Green Chem*. 2011;13:2638-2650.
14. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *J Adv Res*. 2016;7(1):17-28.
15. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*. 2018;10(2):57.
16. Mourdikoudis S, Pallares RM, Thanh NTK. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale*. 2018;10:12871-12934.
17. Fadeel B. Hide and seek: nanomaterial interactions with the immune system. *Front Immunol*. 2019;10:133.
18. Bhattacharjee S. DLS and zeta potential—what they are and what they are not? *J Control Release*. 2016;235:337-351.
19. Current and near-future technologies to quantify nanoparticle therapeutic loading efficiency and surface coating efficiency with targeted moieties. *Nanomaterials*. 2025;15.
20. Characterization of lipid-based nanomedicines at the single-particle level. *Adv Drug Deliv Rev*. 2022.
21. Sinko PJ, Singh Y. *Martin's Physical Pharmacy and Pharmaceutical Sciences*. 7th ed. Philadelphia: Wolters Kluwer; 2020.
22. Mohanraj VJ, Chen Y. Nanoparticles—a review. *Trop J Pharm Res*. 2006;5(1):561-573.





WISELEAF SCIENTIFIC VENTURES

23. Jadhav SP, Ahire SM, Chitte NR, Pawar PS, Sonawane I, Shewale V. Crossing the Blood–Brain Barrier: Challenges, Strategies and Emerging Technologies. *Asian Journal of Research in Pharmaceutical Sciences*. 2026 Jul;16(3):234-42.
24. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers*. 2011;3(3):1377-1397.
25. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev*. 2012;64:72-82.
26. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv Drug Deliv Rev*. 2002;54 Suppl 1:S131-S155.
27. Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *Int J Pharm*. 2009;366(1-2):170-184.
28. Shahalaei M, Azad AK, Wan Mohd Azizi Wan Sulaiman, et al. A review of metallic nanoparticles: present issues and prospects focused on preparation methods, characterization techniques, and theranostic applications. *Front Chem*. 2024;12:1398979.
29. Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, et al. Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chem Rev*. 2008;108(6):2064-2110.
30. Vallet-Regí M, Colilla M, Izquierdo-Barba I. Silica-based mesoporous materials for drug delivery. *J Pharm Sci*. 2007;96(10):2410-2420.
31. Martínez Rivas CJ, Tarhini M, Badri W, Miladi K, Greige-Gerges H, Nazari QA, et al. Nanoprecipitation process: from encapsulation to drug delivery. *Int J Pharm*. 2017;532(1):66-81.
32. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int J Pharm*. 1989;55:R1-R4.
33. Martínez-Muñoz OI, Mora-Huertas CE. Nanoprecipitation technology to prepare carrier systems of interest in pharmaceuticals: an overview of patenting. *Int J Pharm*. 2022;614:121440.
34. Karnik R, Gu F, Basto P, Cannizzaro C, Dean L, Kyei-Manu W, et al. Microfluidic platform for controlled synthesis of polymeric nanoparticles. *Nano Lett*. 2008;8(9):2906-2912.





WISELEAF SCIENTIFIC VENTURES

35. Nava-Arzaluz MG, Piñón-Segundo E, Ganem-Rondero A, Lechuga-Ballesteros D. Single emulsion-solvent evaporation technique and modifications for the preparation of pharmaceutical polymeric nanoparticles. *Curr Pharm Des.* 2012;18(21):???.
36. Piñón-Segundo E, Nava-Arzaluz MG, Lechuga-Ballesteros D. Pharmaceutical polymeric nanoparticles prepared by the double emulsion-solvent evaporation technique. *Curr Pharm Des.* 2012;18(21):???
37. Naskar S, Koutsu K, Sharma S. Chitosan-based nanoparticles as drug delivery systems: a review on two decades of research. *J Drug Target.* 2019;27(4):379-393.
38. Desai KG. Chitosan nanoparticles prepared by ionotropic gelation: an overview of recent advances. *Crit Rev Ther Drug Carrier Syst.* 2016;33(2):107-158.
39. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J Control Release.* 2004;100(1):5-28.
40. Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan—A versatile semi-synthetic polymer in biomedical applications. *Prog Polym Sci.* 2011;36(8):981-1014.
41. Bhattacharjee S. DLS and zeta potential—what they are and what they are not? *J Control Release.* 2016;235:337-351.
42. Monopoli MP, Åberg C, Salvati A, Dawson KA. Biomolecular coronas provide the biological identity of nanosized materials. *Nat Nanotechnol.* 2012;7:779-786.
43. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, et al. Nanoparticles and microparticles for skin drug delivery. *Adv Drug Deliv Rev.* 2011;63(6):470-491.
44. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. *AAPS J.* 2010;12:348-360.
45. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. *Nat Rev Mater.* 2021;6:1078-1094.
46. Elkomy MH, Ali AA, Eid HM. Chitosan on the surface of nanoparticles for enhanced drug delivery: a comprehensive review. *J Control Release.* 2022;351:923-940.
47. Jadhav SP, Ahire SM, Pawar PS, Mahajan SK, Sonawane DD. Exploring Natural Oils: Role in Antimicrobial Activity and Preservation. *Asian Journal of Research in Pharmaceutical Sciences.* 2026 Jan 5;16(1):59-64.
48. Recent advances in artificial intelligence and machine learning for nanomedicine formulation and drug delivery. *Pharmaceutics.* 2024;16.
49. Nanoparticles and nanomaterials: a review from the standpoint of pharmacy and medicine. *Pharmaceutics.* 2025;17.
50. Basic and advanced spectrometric methods for complete nanoparticles characterization in bio/eco systems: current status and future prospects. *Molecules.* 2023;28.





WISELEAF SCIENTIFIC VENTURES

51. Biofabrication of nanoparticles: sources, synthesis, and biomedical applications. *Front Bioeng Biotechnol.* 2023;11.
52. Dynamic light scattering and transmission electron microscopy in drug delivery: a roadmap for correct characterization of nanoparticles and interpretation of results. *Mater Horiz.* 2023;10.

