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Protein Nanoparticles in Drug Delivery

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ABSTRACT

Protein-based nanoparticles (PNPs) have emerged as a promising platform for targeted and controlled drug delivery due to their biocompatibility, biodegradability, and functional versatility. Unlike synthetic polymers, proteins such as albumin, gelatin, and ferritin offer natural binding sites, intrinsic bioactivity, and modifiable surface chemistry, making them highly suitable for therapeutic applications. This review provides a comprehensive overview of protein nanoparticles in drug delivery, covering their preparation techniques, characterization methods, functionalization strategies, therapeutic applications, and clinical translation challenges. The review also highlights recent advances in surface modification, targeting strategies, and stimuli-responsive PNPs for site-specific drug release. Additionally, analytical challenges and future perspectives in protein nanoparticle-based drug delivery are discussed. This synthesis aims to provide researchers and clinicians with insights into the potential of protein nanoparticles to improve therapeutic efficacy and reduce systemic toxicity.

KEYWORDS: *Protein nanoparticles, drug delivery, albumin nanoparticles, gelatin nanoparticles, targeted delivery, stimuli-responsive nanoparticles, biocompatibility.*

INTRODUCTION

Nanotechnology has transformed modern medicine by enabling precise delivery of therapeutics to target sites while minimizing systemic side effects. Among nanocarriers, protein-based

nanoparticles have gained attention for their unique advantages, including biocompatibility, biodegradability, and minimal immunogenicity. Proteins can self-assemble into nanoparticles and can be modified chemically or genetically to enhance their drug-loading capacity, stability, and targeting potential.

Figure 1 illustrates the general concept of protein nanoparticles in drug delivery, where therapeutic cargo is encapsulated or conjugated to protein nanoparticles and delivered to the target site with controlled release.

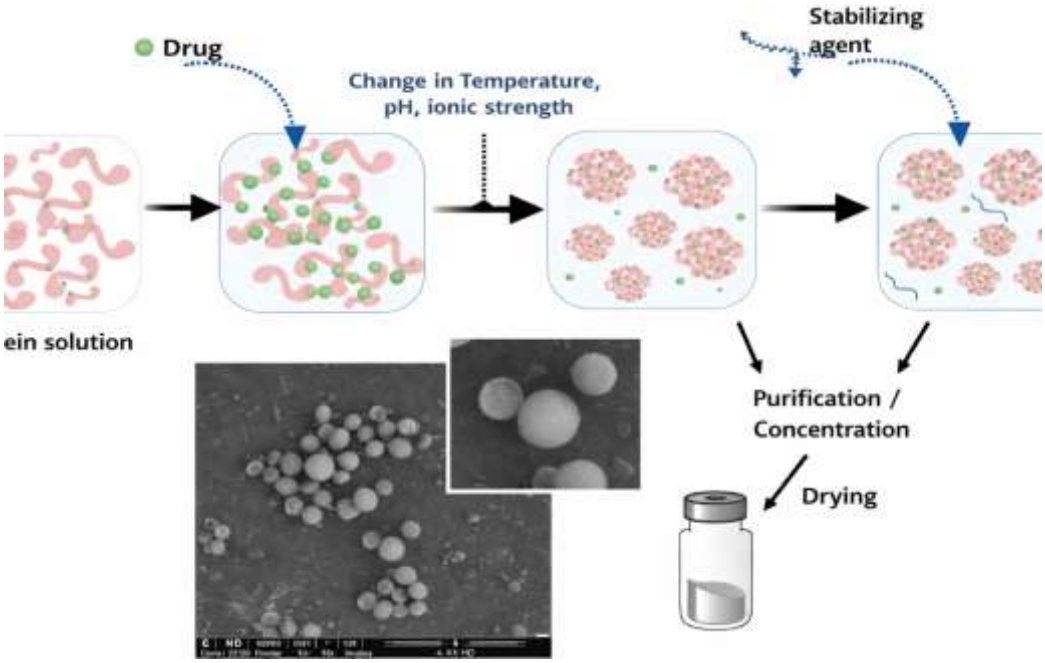

Figure 1: Schematic representation of protein nanoparticles for drug delivery.

TYPES OF PROTEIN NANOPARTICLES

Protein nanoparticles are versatile carriers for drug delivery due to their natural origin, biocompatibility, biodegradability, and ability to interact with drugs through physical and chemical means. Different proteins offer unique physicochemical properties, which influence nanoparticle size, drug encapsulation efficiency, release profiles, and targeting potential. Several proteins have been extensively explored for nanoparticle formulation:

1. Albumin Nanoparticles

Albumin, primarily human serum albumin (HSA) or bovine serum albumin (BSA), is the most widely studied protein for nanoparticle drug delivery. It is a 66.5 kDa globular protein abundant

in plasma, characterized by multiple functional groups such as amines, carboxyls, and thiols that allow conjugation with therapeutic molecules, imaging agents, or targeting ligands.

Advantages:

- **Non-immunogenic and biocompatible:** Albumin is naturally present in the human body, reducing the risk of immune responses.
- **High drug-binding capacity:** Hydrophobic pockets and charged residues facilitate loading of both hydrophilic and hydrophobic drugs.
- **Receptor-mediated targeting:** Albumin binds to gp60 and SPARC (secreted protein acidic and rich in cysteine) receptors, which are overexpressed in tumors, enhancing passive and active targeting.

Applications:

- **Paclitaxel delivery (Abraxane):** Albumin nanoparticles improve solubility, reduce hypersensitivity reactions, and allow higher drug accumulation in tumors.
- **Doxorubicin and methotrexate:** Albumin nanoparticles have been explored to reduce cardiotoxicity and improve efficacy.

Preparation considerations: Desolvation and nanoparticle albumin-bound (NAB) techniques are commonly used. Crosslinking can stabilize the nanoparticles, but careful optimization is needed to avoid protein denaturation.

2. Gelatin Nanoparticles

Gelatin is a natural polymer derived from hydrolyzed collagen, possessing abundant amino and carboxyl groups suitable for chemical modification. Gelatin nanoparticles are biodegradable, hydrophilic, and capable of forming both physical and chemically crosslinked structures.

Advantages:

- **Biodegradable and non-toxic:** Gelatin is enzymatically degradable by proteases, making it safe for in vivo applications.
- **Flexible surface chemistry:** Allows conjugation of targeting ligands or fluorescent markers.

- **Versatile drug encapsulation:** Can carry both hydrophilic drugs (e.g., peptides, proteins) and hydrophobic drugs (e.g., curcumin).

Applications:

- **Oral delivery of insulin:** Protects peptide drugs from gastrointestinal degradation.
- **Cancer therapy:** Encapsulation of doxorubicin and 5-fluorouracil enhances tumor targeting and reduces systemic toxicity.
- **Transdermal and nasal delivery:** Gelatin nanoparticles improve permeation of drugs across epithelial barriers.

Preparation considerations: Desolvation, coacervation, and emulsion-crosslinking methods are commonly used. The degree of crosslinking can control drug release rates.

3. Ferritin Nanoparticles

Ferritin is an iron-storage protein that self-assembles into a hollow, spherical cage (~12 nm internal cavity, ~12–14 nm overall diameter). Its cage-like architecture allows encapsulation of therapeutic molecules, imaging agents, or metal ions.

Advantages:

- **High drug-loading capacity:** The hollow core can accommodate a large amount of cargo.
- **Biocompatible and stable:** Ferritin is naturally occurring and stable under physiological conditions.
- **Targeting potential:** Surface modification with antibodies or peptides allows receptor-mediated uptake.

Applications:

- **Cancer therapy:** Ferritin nanoparticles have been used to deliver doxorubicin, cisplatin, and photosensitizers to tumors.
- **Imaging:** Encapsulation of contrast agents enables MRI or CT imaging.
- **Combination therapy:** Co-delivery of chemotherapeutics and imaging agents for theranostic applications.

Preparation considerations: Drugs can be loaded via pH-induced disassembly/reassembly or surface conjugation. Maintaining structural integrity during drug loading is critical.

4. Silk Fibroin Nanoparticles

Silk fibroin is a fibrous protein derived from **Bombyx mori** silk, composed of heavy and light chains rich in glycine, alanine, and serine. It forms β-sheet structures, which confer mechanical strength and controlled biodegradability.

Advantages:

- **Biocompatible and minimally immunogenic:** Suitable for long-term drug release.
- **Tunable degradation:** β-sheet content can be adjusted to control drug release kinetics.
- **Versatile drug loading:** Can encapsulate small molecules, proteins, and nucleic acids.

Applications:

- **Sustained anticancer drug release:** Silk fibroin nanoparticles release paclitaxel or doxorubicin over extended periods.
- **Gene therapy:** Delivery of siRNA or plasmid DNA using cationized silk fibroin nanoparticles.
- **Tissue engineering:** Silk nanoparticles can deliver growth factors for regenerative medicine applications.

Preparation considerations: Nanoparticles are prepared using desolvation, self-assembly, or microfluidic techniques. Controlling β-sheet content is critical for achieving desired release profiles.

5. Casein Nanoparticles

Casein, a phosphoprotein found in milk, naturally forms micellar structures (~100–300 nm), which can encapsulate hydrophobic drugs within their hydrophobic cores. Casein nanoparticles are biocompatible, biodegradable, and resistant to gastric degradation, making them suitable for oral drug delivery.

Advantages:

- **Self-assembly:** Casein micelles can form nanoparticles without toxic crosslinkers.

- **Protection of sensitive drugs:** Encapsulation shields bioactive molecules from enzymatic degradation.
- **Bioavailability enhancement:** Hydrophobic drug solubility improves significantly.

Applications:

- **Oral delivery of curcumin and resveratrol:** Enhances solubility and intestinal absorption.
- **Vitamin and nutraceutical delivery:** Protects labile compounds and facilitates controlled release.
- **Hydrophobic chemotherapeutics:** Casein nanoparticles improve systemic circulation and tumor accumulation.

Preparation considerations: Casein nanoparticles are typically formed via pH adjustment or coacervation, followed by stabilization through mild crosslinking if necessary.

PREPARATION TECHNIQUES OF PROTEIN NANOPARTICLES

The method used to prepare protein nanoparticles critically affects particle size, polydispersity, drug loading, release kinetics, and in vivo behavior. Various methods exploit the unique physicochemical properties of proteins, such as solubility, charge, and self-assembly tendency. Selection of a suitable technique depends on the type of protein, the drug to be encapsulated, and the intended route of administration.

1. Desolvation Method

The **desolvation method** is one of the most commonly employed techniques for protein nanoparticle fabrication. It relies on reducing protein solubility by adding a desolvating agent (e.g., ethanol, acetone, or isopropanol) to an aqueous protein solution. The decrease in solubility induces controlled aggregation of protein molecules into nanoparticles.

Mechanism:

1. Protein molecules are solubilized in aqueous buffer.
2. Desolvating agent is added dropwise under constant stirring.
3. Reduced solubility leads to aggregation, forming nanosized clusters.

4. Stabilization is achieved using crosslinking agents like glutaraldehyde, genipin, or carbodiimide.

Key Parameters Affecting Particle Characteristics:

- **Protein concentration:** Higher concentration favors larger particle formation.
- **Desolvating agent type and addition rate:** Slower addition yields uniform nanoparticles.
- **pH and ionic strength:** Proteins aggregate optimally near their isoelectric point.
- **Crosslinking duration:** Determines particle stability and drug release rate.

Advantages:

- Simple, reproducible, and easily scalable.
- Suitable for both hydrophilic and hydrophobic drugs.

Disadvantages:

- Use of chemical crosslinkers like glutaraldehyde can induce toxicity if not removed thoroughly.
- Requires careful control of aggregation to avoid polydisperse particles.

Applications: Albumin nanoparticles for paclitaxel, gelatin nanoparticles for insulin, and casein nanoparticles for curcumin.

2. Emulsification Method

The **emulsification method** produces nanoparticles by dispersing protein solutions in an immiscible phase (oil or water) to form droplets, followed by stabilization and crosslinking.

Mechanism:

1. Proteins are dissolved in the aqueous phase.
2. The aqueous phase is emulsified in a continuous phase (e.g., oil) using high-shear homogenization or ultrasonication.
3. Surfactants (e.g., Tween 80, Span 80) stabilize droplets.
4. Nanoparticles are formed upon solvent removal or crosslinking.

Key Parameters:

- **Emulsification speed:** Higher shear results in smaller nanoparticles.
- **Surfactant type and concentration:** Determines stability and prevents coalescence.
- **Protein concentration and oil-to-water ratio:** Influence particle size and encapsulation efficiency.

Advantages:

- Effective for encapsulating hydrophobic drugs and poorly soluble compounds.
- Can produce nanoparticles with narrow size distribution.

Disadvantages:

- Surfactant residues may induce toxicity or immune responses.
- Multiple washing steps are required to remove oil and surfactant.
- Process may denature sensitive proteins due to mechanical stress.

Applications: Gelatin nanoparticles for anticancer drugs, silk fibroin nanoparticles for hydrophobic drugs.

3. Coacervation (Phase Separation) Method

Coacervation involves inducing phase separation of proteins by altering pH, ionic strength, or solvent composition. The resulting coacervate droplets are crosslinked to form stable nanoparticles.

Mechanism:

1. Protein solution is prepared at a specific pH near its isoelectric point.
2. Addition of salt or organic solvents induces phase separation (coacervate droplets).
3. Crosslinking with mild agents stabilizes the droplets into nanoparticles.

Key Parameters:

- **pH control:** Critical for protein aggregation without denaturation.
- **Salt type and concentration:** Modulates coacervate size and density.
- **Temperature:** Can influence protein solubility and coacervate formation.

Advantages:

- High drug encapsulation efficiency, especially for sensitive macromolecules.
- Mild processing conditions preserve protein and drug integrity.

Disadvantages:

- Highly sensitive to environmental conditions (pH, temperature, ionic strength).
- Scale-up may be challenging due to precise control requirements.

Applications: Encapsulation of enzymes, peptides, and hydrophilic drugs in gelatin or albumin nanoparticles.

4. Self-Assembly Method

Certain proteins, such as ferritin, casein, and silk fibroin, possess the intrinsic ability to self-assemble into nanoparticles under specific conditions without external crosslinkers.

Mechanism:

1. Proteins are dissolved in aqueous solution at physiological pH or ionic strength.
2. Environmental triggers such as pH change, temperature variation, or metal ion binding induce spontaneous assembly into nanosized structures.
3. Drugs can be loaded during self-assembly or by post-assembly conjugation.

Key Parameters:

- Protein concentration and purity
- Temperature and pH conditions
- Presence of metal ions or small molecules that facilitate assembly

Advantages:

- Avoids toxic chemical crosslinkers.
- Preserves the native structure of proteins and drug molecules.
- Simple, reproducible process suitable for sensitive biomolecules like nucleic acids and peptides.

Disadvantages:

- Limited to proteins with natural self-assembly properties.
- Fine control of particle size and uniformity may be challenging.

Applications: Ferritin nanoparticles for doxorubicin, casein nanoparticles for hydrophobic nutraceuticals.

5. Spray Drying Method

Spray drying converts protein solutions into nanoparticles by atomizing the solution into fine droplets, which are rapidly dried with hot air or inert gas.

Mechanism:

1. Protein-drug solution or suspension is pumped through a nozzle to form microdroplets.
2. The droplets encounter heated air, causing rapid solvent evaporation.
3. Dry nanoparticles are collected in a cyclone separator or filter.

Key Parameters:

- Inlet and outlet temperature
- Atomization pressure and nozzle type
- Feed solution viscosity and concentration

Advantages:

- Scalable, fast, and reproducible method.
- Suitable for heat-stable proteins and drugs.
- Produces dry powders, facilitating storage and transport.

Disadvantages:

- High temperatures can denature sensitive proteins or degrade labile drugs.
- Particle aggregation may occur if not optimized.

Applications: Silk fibroin nanoparticles for sustained drug release, albumin nanoparticles for pulmonary or oral administration.

Table 1: Comparison of protein nanoparticle preparation techniques.

Method	Particle Size Control	Drug Loading	Advantages	Disadvantages
Desolvation	Good	Moderate	Simple, scalable	Crosslinker toxicity
Emulsification	Moderate	High	Hydrophobic drugs	Surfactant residues
Coacervation	Good	High	High encapsulation	Sensitive to pH/ions
Self-Assembly	Limited	Moderate	No toxic chemicals	Protein-specific
Spray Drying	Moderate	Moderate	Scalable, reproducible	Heat-sensitive drugs

CHARACTERIZATION OF PROTEIN NANOPARTICLES

Comprehensive characterization is crucial to ensure stability, efficacy, and safety. Common characterization parameters include:

1. Particle Size and Morphology

Dynamic light scattering (DLS) and scanning electron microscopy (SEM) are widely used to determine size, distribution, and morphology. Particle size affects cellular uptake, circulation time, and biodistribution.

2. Surface Charge (Zeta Potential)

Zeta potential measurement predicts nanoparticle stability. Proteins with high positive or negative zeta potential (> ±30 mV) resist aggregation due to electrostatic repulsion.

3. Drug Loading and Encapsulation Efficiency

Drug loading (% w/w) and encapsulation efficiency (% of initial drug encapsulated) are determined using UV-Vis spectroscopy, HPLC, or fluorescence techniques.

4. Stability Studies

Physical and chemical stability under different pH, temperature, and ionic strength conditions is assessed. Protein denaturation or aggregation can compromise efficacy.

5. Structural Characterization

Fourier-transform infrared spectroscopy (FTIR), circular dichroism (CD), and X-ray diffraction (XRD) provide information on secondary structure, chemical integrity, and crystallinity.

6. In Vitro Release Studies

Drug release kinetics are evaluated in physiological media to predict in vivo performance. Controlled release is crucial for maintaining therapeutic levels.

FUNCTIONALIZATION AND TARGETING STRATEGIES

Protein nanoparticles can be modified to enhance targeting, circulation time, and controlled release.

1. Surface PEGylation

Polyethylene glycol (PEG) chains on the surface reduce opsonization, increase circulation time, and minimize immune clearance.

2. Ligand Conjugation

Targeting ligands such as antibodies, peptides, or aptamers are conjugated to bind specific receptors on diseased cells, enabling active targeting.

3. Stimuli-Responsive Nanoparticles

Protein nanoparticles can be engineered to release drugs in response to pH, temperature, redox conditions, or enzymatic activity. This allows site-specific delivery to tumors or inflamed tissues.

4. Multifunctional Nanoparticles

Combining targeting ligands, imaging agents, and therapeutic drugs into one protein nanoparticle enables theranostics—simultaneous therapy and diagnostics.

THERAPEUTIC APPLICATIONS

Protein nanoparticles have demonstrated promising applications across multiple therapeutic areas:

1. Cancer Therapy

Albumin-bound paclitaxel (Abraxane) improves solubility, reduces hypersensitivity reactions, and targets tumor tissue via the enhanced permeability and retention (EPR) effect.

2. Vaccine Delivery

Protein nanoparticles enhance immunogenicity by providing adjuvant effects and controlled antigen release. Ferritin nanoparticles are explored for influenza and COVID-19 vaccines.

3. Gene Delivery

Cationic protein nanoparticles can complex with nucleic acids for efficient cellular uptake. Gelatin or albumin-based nanoparticles have been used for siRNA and plasmid DNA delivery.

4. Anti-Inflammatory Drugs

Protein nanoparticles improve oral bioavailability and target inflamed tissues in arthritis and inflammatory bowel disease.

5. Antimicrobial Delivery

Protein nanoparticles enhance the stability and efficacy of antimicrobial peptides and reduce off-target toxicity.

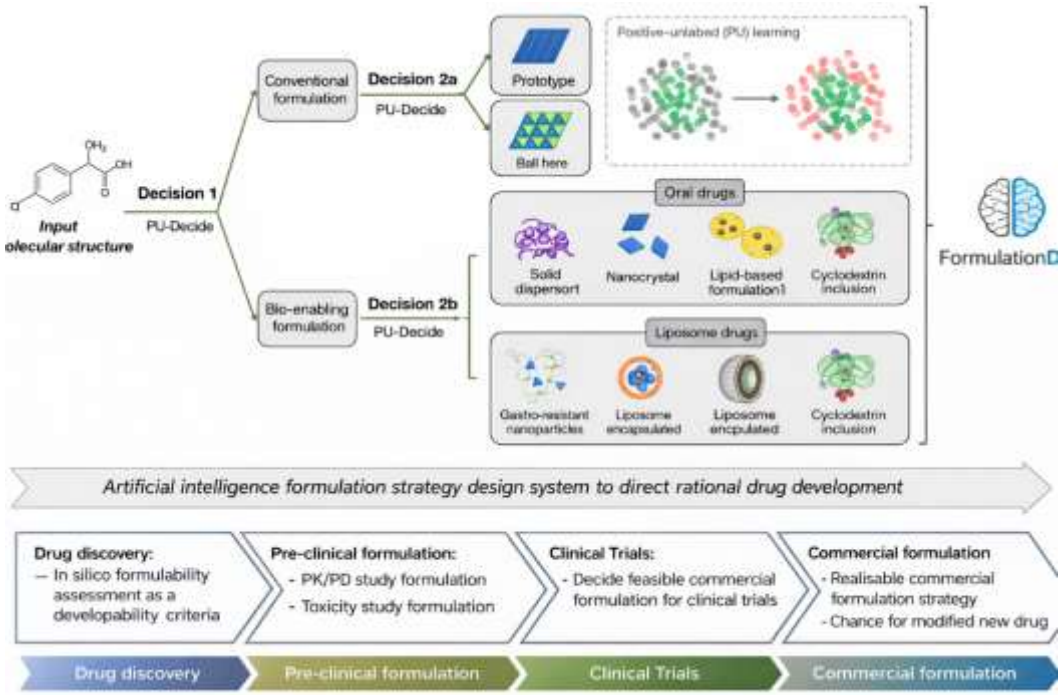

Figure 2: Applications of protein nanoparticles in drug delivery.

CLINICAL TRANSLATION AND CHALLENGES

Despite promising preclinical data, clinical translation of protein nanoparticles faces several challenges:

1. Immunogenicity and Toxicity

Although proteins are generally biocompatible, surface modifications or impurities can trigger immune responses.

2. Scale-Up and Reproducibility

Large-scale production requires consistent particle size, drug loading, and stability, which can be difficult to achieve.

3. Regulatory Hurdles

Complex formulations and functionalized nanoparticles face stringent regulatory scrutiny for

safety, efficacy, and quality control.

4. Analytical Challenges

Accurate characterization of protein nanoparticle-drug interactions, structural integrity, and release kinetics remains challenging due to protein complexity.

FUTURE PERSPECTIVES

Future research in protein nanoparticles should focus on:

- **Personalized Nanomedicine:** Designing nanoparticles tailored to patient-specific biomarkers.
- **Hybrid Nanoparticles:** Combining proteins with polymers or inorganic materials for multifunctional delivery.
- **Artificial Intelligence Integration:** AI-assisted formulation optimization to predict stability, release kinetics, and targeting efficiency.
- **Green Manufacturing:** Solvent-free or low-toxicity preparation methods for safer and scalable production.

CONCLUSION

Protein nanoparticles offer a versatile and biocompatible platform for drug delivery, capable of encapsulating diverse therapeutic agents and targeting specific tissues. Advances in preparation, functionalization, and stimuli-responsive designs have significantly improved drug bioavailability and therapeutic efficacy. Despite challenges in large-scale production, immunogenicity, and regulatory compliance, ongoing innovations in protein engineering and nanotechnology continue to expand the potential of protein nanoparticles in personalized medicine and targeted therapy. With continued research and clinical development, protein nanoparticles are poised to become a cornerstone of modern nanomedicine.

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