Characterization and Application of Nanomaterials 2025, 8(1), 8291.
https://doi.org/10.24294/can8291

Article

Polymeric nanoparticles for protein and peptide delivery

Nijhawan Monika, Neerude Sirisha, Sneha Nawale*

Department of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Hyderabad 500090, India
*** Corresponding author:** Sneha Nawale, sneha.nawale11@gmail.com

CITATION

Monika N, Sirisha N, Nawale S. Polymeric nanoparticles for protein and peptide delivery. Characterization and Application of Nanomaterials. 2025; 8(1): 8291. https://doi.org/10.24294/can8291

ARTICLE INFO

Received: 30 July 2024
Accepted: 7 November 2024
Available online: 2 December 2024

Abstract: Protein- and peptide-based medications are recognized for their effectiveness and lower toxicity compared to chemical-based drugs, making them promising therapeutic agents. However, their application has been limited by numerous delivery challenges. Polymeric nanostructures have emerged as effective tools for protein delivery due to their versatility and customizability. Polymers' inherent adaptability makes them ideal for meeting the specific demands of protein-delivery systems. Various strategies have been employed, such as enzyme inhibitors, absorption enhancers, mucoadhesive polymers, and chemical modifications of proteins or peptides. This study explores the hurdles associated with protein and peptide transport, the use of polymeric nanocarriers (both natural and synthetic) to overcome these challenges, and the techniques for fabricating and characterizing nanoparticles.

Keywords: drug delivery; nanoparticles (NPs); protein delivery; therapeutic effect

1. Introduction

Protein is derived from the Greek word "PROTOS", meaning the first or the supreme. Proteins are large organic molecules composed of amino acids linked together in a linear chain by peptide bonds, with proteins typically containing more than 50 amino acids. Peptides are short polymers formed by the linkage of amino acids in a specific order (a peptide contains < 50 amino acids). Proteins exhibit various structural forms: the primary structure, which is the sequence of amino acids; the secondary structure, consisting of regularly repeating local structures stabilized by hydrogen bonds; the tertiary structure, representing the three-dimensional configuration of the polypeptide; and the quaternary structure, formed by the assembly of multiple protein molecules (polypeptide chains) [1]. Proteins and peptides can be classified into different types, including polypeptides, oligopeptides, fibrous proteins, globular proteins, and oligomeric proteins, based on the number of amino acids present.

Proteins are essential to the body as they participate in various biological roles in the form of enzymes that catalyze virtually all chemical reactions (e.g., 6GDH), help in the transport of hemoglobin of erythrocytes, contract muscles (actin and myosin), maintain the structure of the body (collagen in bones), provide defensive activity (immunoglobulins and antibodies), regulate secretions (insulin), provide nutrition and storage in the body (ovalbumin), generate and transmit nerve impulses, provide immune protection through antibodies, and control growth.

Bioapplications of proteins and peptides include the use of erythropoietin to stimulate red blood cell production, tissue plasminogen activator for treating heart attacks and strokes, oxytocin to manage labor pain, bradykinins to enhance peripheral circulation, somatostatin to reduce bleeding in gastric ulcers, gonadotropin to induce

ovulation, and insulin to regulate blood sugar levels. Although proteins and peptides offer many benefits, their delivery poses challenges due to their large size and instability. Their structures are maintained by weak noncovalent forces, making them susceptible to degradation under mild storage conditions and gastric juices [2–4].

2. Problems with protein and peptide

The primary challenges in delivering proteins and peptides are proteolysis by exo/endo proteases, small-size proteins getting filtered out by kidneys very easily, causing elimination of B and T cells, and may show unwanted allergic reactions (even toxicity), and less therapeutic activity of proteins due to insolubility/adsorption [5].

3. Barriers to protein and peptide delivery

3.1. Enzymatic barriers

Proteins and peptides can be degraded by enzymes in two distinct ways. One method involves the hydrolytic breaking of peptide bonds by enzymes that degrade insulin, convert angiotensin, and renin. Since proteolysis is an irreversible event, it may harm medications that include proteins and peptides. Others include chemical modification of proteins by oxidizing them using glucose oxidase or xanthine oxidase or phosphorylating them with kinases [6].

3.2. Intestinal epithelial barrier

It prevents protein medications from passing through the intestinal epithelium. Transport of protein and peptide medicines across the intestinal epithelium is accomplished by several processes, including paracellular movements, endocytosis, transcytosis, passive transport, and carrier-mediated transport. Dipeptides and tripeptides from the small intestine are extensively absorbed through active transport. Proteins and peptides that are too large to be absorbed through carrier-mediated transport can be taken up via endocytosis, which is one of the processes by which cellular internalization of proteins and peptides occurs that include pinocytosis (cell drinking) and phagocytosis (cell eating). Paracellular migration and persorption are the two mechanisms involved in drug absorption. The epithelial mucosa of the small intestine serves as a barrier against macromolecule penetration [7].

3.3. Capillary endothelial barrier

Proteins and peptides must either cross the endothelial cells themselves or move between the cells to pass through the capillary endothelium. Cytoplasmic enzymes can alter or metabolize solutes that pass through endothelial cell membranes. Therefore, the endothelial passage presents an enzymatic or metabolic barrier to the passage of the proteins and peptides [8].

3.4. Blood-brain barrier

One of the main barriers to protein delivery to the brain compartment is the blood-brain barrier (BBB). It is made up of the blood-cerebrospinal fluid barrier and the vascular BBB. BBB is made up of a monolayer of cells at both locations that are joined

by tight junctions and contain additional mechanisms to prevent or slow the flow of plasma into the central nervous system, permitting only the passage of uncharged, tiny, lipophilic molecules and gases. Proteins and other large molecules cannot cross the BBB [9].

Different barriers to protein and peptide drug delivery are illustrated in a visual format in **Figure 1**.

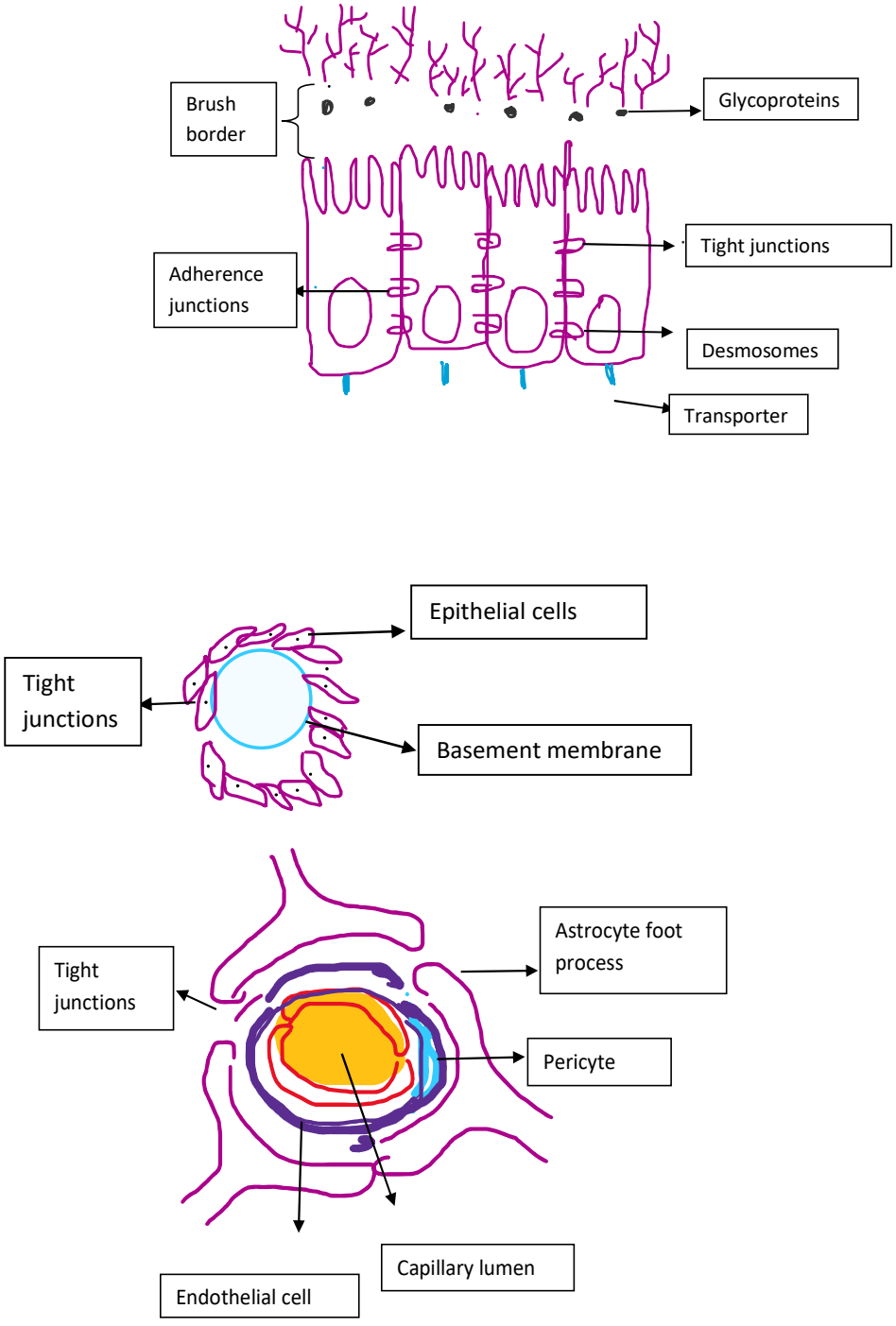

Figure 1. Intestinal barrier, capillary endothelial barrier and blood brain barrier for protein and peptide delivery.

4. Polymeric nanocarriers for protein and peptide delivery

Polymeric nanoparticles (NPs), which are solid colloidal carriers ranging from 10 to 100 nm in size, can be formulated with synthetic, semi-synthetic, or natural polymers. The choice of materials used to synthesize these NPs affects their drug delivery performance and therapeutic effects. Proteins can be chemically attached, adsorbed, or encapsulated on the surface of polymeric NPs [10,11].

4.1. Natural polymers

Therapeutic proteins can be delivered to specific sites for distinctive benefits with natural polymers. The natural polymers may be of plant, animal, and marine origin and are generally inexpensive. The existence of reactive sites in natural polymers is significant in drug delivery systems because it facilitates ligand conjugation, cross-linking, and other modifications that make the polymers perfect drug carriers for a variety of therapeutic proteins. However, natural polymers are more susceptible to processing parameters.

4.1.1. Polysaccharide-based natural polymers

Polysaccharides exhibit structural and functional diversity because of their vast number of reactive groups, wide range of molecular weights, and varied chemical composition. Being naturally occurring biomaterials, polysaccharides are safe, nontoxic, and biodegradable and exhibit remarkable stability in biological fluids. Polysaccharides are also known as mucoadhesive polymers because of their mucoadhesive qualities (polysaccharides, which are hydrophilic in nature, create non-covalent connections with biological tissues) due to the presence of many derivable groups on the molecular structure, such as hydroxyl, carboxyl, and amino groups. Mucoadhesive polysaccharide-based NPs have been demonstrated to improve the residence and absorbance time of integrated therapeutic protein. Various polysaccharides used for the transport of proteins and peptides are represented in **Table 1**.

Table 1. List of various polysaccharide polymers for proteins and peptide drug delivery.

Polysaccharide polymers	Properties	Role of polymers in transportation of Protein and Peptides	Reference
Chitosan	Biocompatible, Non-toxic	Aid in facilitating the intestinal epithelial mucosal absorption of therapeutic proteins with high molecular weight.	[12,13]
Cyclodextrins	Utilized as potential carriers in pharmaceutical biotechnology and non-toxic	Improve the bioavailability of the protein molecule by delivering it directly to the barrier membrane	[14,15]
Alginates	Excellent mucoadhesive qualities, Biocompatibility, biodegradability, High degree of flexibility and non-toxic	Helps in transport of therapeutic proteins that are heat sensitive. Alginate also transports labile proteins and peptides safely to the colon while shielding them from the stomach environment.	[16,17]
Pectins	Mucoadhesive activity Inert for physiological fluids and non-toxic	Stops integrated proteins from being broken down by gastric enzymes and greatly boosts the intestinal absorption by its unique mucoadhesive activity on the intestinal epithelium.	[18,19]
Xanthan Gum	Biocompatible, Non-toxic, biodegradable and bioadhesive properties	Preserves the integrity of the therapeutic protein and extends its sustained release	[20,21]

4.1.2. Protein-based polymers

Amidst natural polymers, protein-based polymers have garnered significant attention because of their attributes such as abundance, accessibility, minimal toxicity, modifiability due to their intricate heterogeneity, and adaptability in delivery methods as depicted in **Table 2**. There is still more work to be done to make protein-based polymers stable enough to be used as the perfect therapeutic polymers.

Table 2. List of various protein polymers for proteins and peptide drug delivery.

Protein polymers	Properties	Role of polymers in transportation of Protein and Peptides	Reference
Lectins	Sugar-binding proteins	Ability to improve the active transport of therapeutic proteins with large molecular weights from the intestinal epithelium	[22]
Albumin	Non-toxic, Biocompatible	Deliver therapeutic proteins across the blood-brain barrier and nuclear membrane	[23]
Collagen	Biocompatible Easily modifiable and available Synergic with bioactive components	Facilitates the delivery of therapeutic proteins and extends the sustained release of the incorporated proteins.	[24]
Gelatin	Biocompatible Biodegradable	Thermo-reversible properties are utilized for delivering therapeutic molecules through targeted drug delivery systems	[25]

4.2. Synthetic polymers

Formulators are focusing on synthetic polymers for the delivery of therapeutic proteins and peptides. It has been demonstrated that these polymers lengthen the pharmacokinetic and circulation periods of integrated medicinal compounds. Drug carriers made of synthetic polymers frequently serve a passive purpose as tabulated in **Tables 3** and **4**.

Table 3. List of various synthetic biodegradable polymers with their properties and transport of proteins and peptide drug delivery.

Synthetic polymers	Properties	Role in transportation of Protein and Peptides	Reference
Polyethylene Glycol	Non-immunogenic, Nontoxic, Highly soluble in water	PEGylation of therapeutic proteins protects them from enzymatic degradation and reduces immunogenicity, thereby extending their residence time in the body, enhancing stability, and modifying pharmacokinetics by altering various physicochemical properties	[26,27]
Polaxomers	Thermosensitive Inert and stable	Helps to sustain the stability of incorporated therapeutic proteins and peptides more effectively than other prolonged release drug delivery systems	[28,29]
Poly (lactic-co-glycolic acid)	Biocompatible and Biodegradable	Several types of PLGA-PEG block copolymers have been developed for the proteins and peptides sustained delivery	[30,31]
Pluronic F127	Non-irritant Biocompatibility Good mechanical strength Bioadhesive properties	Ensures greater stability of incorporated therapeutic proteins and peptides compared to other prolonged release drug delivery systems	[32]

Table 4. List of various synthetic non-biodegradable polymers with their properties and transport of proteins and peptide drug delivery.

Synthetic polymers	Properties	Role in transportation of Protein and Peptides	Reference
Silicons	High loading capacity, surface functionalization, biocompatibility, physicochemical and thermal stability	Protection of proteins from degradation, increase the half-life of protein, targetted delivery, ability to functionalize their surfaces	[33]
Polyacrylate	Versatile, non-toxic, mucoadhesive	Safe delivery of protein, controlled and sustained release of proteins and peptides	[34]

4.2.1. Synthetic biodegradable polymers

Biodegradable polymers offer significant advantages in biomedical applications, especially for drug delivery. Their capacity to break down into smaller, absorbable molecules eliminates the need for surgical removal, improving patient comfort and compliance. Moreover, their biocompatibility and non-toxicity make them ideal for use in a range of medical devices and treatments, reducing the risk of adverse reactions and promoting better integration within the body. This makes them a favored option for many cutting-edge therapeutic solutions [35,36].

4.2.2. Synthetic non-biodegradable polymers

Non-biodegradable polymers present considerable challenges in medical applications, as they require surgical removal once the medication, they deliver is exhausted. This limitation confines their use to cases where the implant can be easily retrieved. On the other hand, biodegradable polymers naturally break down within the body, providing a more convenient and less invasive alternative for drug delivery systems. This distinction highlights the importance of choosing the appropriate polymer type based on the specific medical requirements and desired treatment outcomes.

5. Method of preparation of nanocarriers

Typically, two primary approaches are utilized, which are the dispersion of prefabricated polymers or the polymerization of monomers. The lists of commonly used techniques are presented in **Figures 2** and **3**.

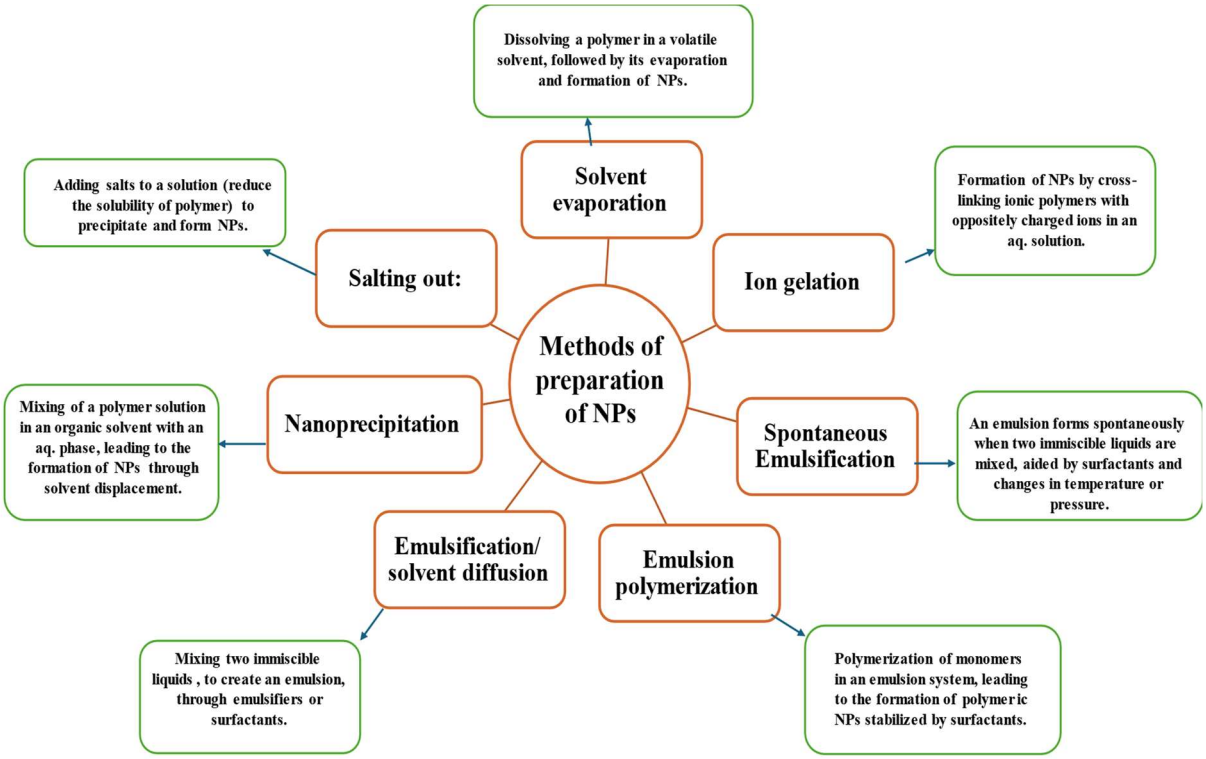

Figure 2. Methods of preparation of NPs.

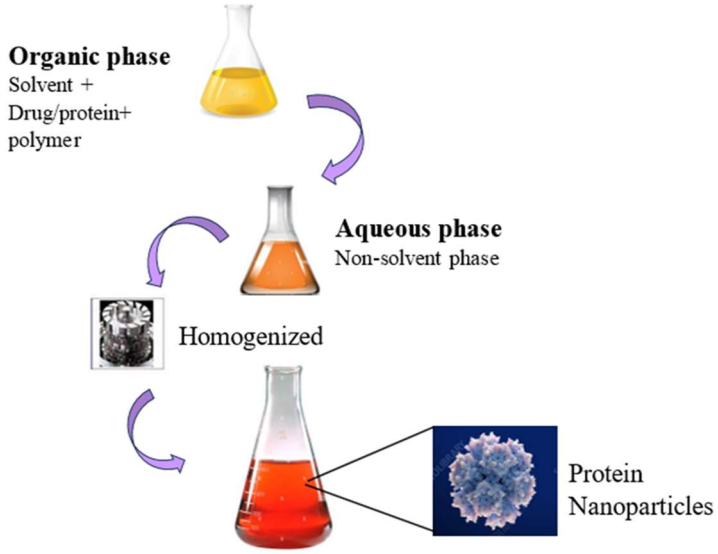

Figure 3. Preparation of NPs.

5.1. Solvent evaporation method

An emulsion is prepared by dissolving the polymer in an organic solvent like chloroform, acetone, or ethyl acetate using polyvinyl alcohol as a stabilizer. If the homogenization procedure is carried out for a long enough duration, it can help to evaporate the organic solvent [37]. Ultracentrifugation is used to gather the NPs at the end of the homogenization process. The desired particle size and other characteristics can be attained by modifying process variables, including the ratio of polymer to

organic solvent, the type of organic solvent, and the speed and duration of homogenization [38].

5.2. Spontaneous emulsification/solvent diffusion method

This method utilizes water-immiscible solvents such as dichloromethane or chloroform as the organic phase, while water-miscible solvents like methanol or acetone serve as the organic phase. NPs are formed when these two phases are combined, resulting in interfacial turbulence and leading to the formation of NPs. Despite producing nanosized particles, this approach has certain drawbacks, such as the existence of leftover organic solvent [39,40].

5.3. Salting out/emulsion diffusion method

In the salting-out process, the polymer is first dissolved in a water-miscible organic phase, such as acetone or tetrahydrofuran, and then added to the aqueous phase containing the emulsifier [39]. The fast addition of water to the emulsion, combined with gentle stirring, decreases the ionic strength, causing the water-soluble organic solvent to migrate to the aqueous phase and resulting in the formation of polymeric nanoparticles [41].

5.4. Phase separation method

This technique works with both lipophilic and hydrophilic medications. The hydrophilic medications are typically introduced to the organic phase after being dissolved in water. Conversely, the medications that are lipophilic are dissolved in polymer solutions. After the aqueous and organic phases are combined to form an emulsion, a second organic nonsolvent, such as silicone oil, is added while stirring vigorously. Silicone oil is miscible with the initial organic phase but does not dissolve the medication. Consequently, the first organic solvent is extracted, reducing the solubility of the polymer and causing phase separation, leading to the formation of a polymer coacervate. Drug-loaded nanoparticles are formed when this polymer coacervate adsorbs onto active pharmaceutical ingredients [42].

5.5. Emulsion polymerization method

Emulsion polymerization is a widely used technique for synthesizing polymeric NPs. In this process, the monomer is emulsified in a continuous phase of an immiscible liquid. A polymerization reaction occurs in situ, resulting in nanospheres. The choice between water-in-oil (w/o) or oil-in-water (o/w) emulsion systems depends on the hydrophobicity of the desired monomer. Surfactants play a crucial role in stabilizing emulsions and controlling particle size. High-speed mixing, homogenization, or ultrasound sonication are common methods for carrying out this process [43].

5.6. Nanoprecipitation

The nanoprecipitation technique utilizes two miscible solvents: One acts as a good solvent (usually an organic solvent such as acetone, isopropanol, or ethanol), while the other functions as a non-solvent for the polymer or lipid used to form the NPs. For instance, water can be a non-solvent. The procedure involves preparing an

organic phase and a non-solvent phase (often referred to as the aqueous phase). Both phases ensure complete solubility of all starting materials. The organic phase may include polymers, solid or liquid lipids, surfactants with low HLB values, and active compounds dissolved in organic solvents. The solubility of the active molecule in the solvent affects the drug loading capacity of the particles. Meanwhile, the non-solvent phase includes stabilizing agents dissolved in water, facilitating NP formation and ensuring system stability [44,45].

5.7. Ion gelation

Ionotropic gelation is a well-studied method for preparing nanocarrier systems due to its mild conditions and straightforward procedures. In this technique, polyelectrolytes (such as chitosan, alginate, hyaluronic acid, and carrageenan) are cross-linked in the presence of counterions. The cross-linking process involves the formation of a network through ionic bridges between macromolecular chains. Typically, a charged ionic entity with a defined molecular weight serves as the cross-linking agent. Researchers frequently use this procedure to prepare NPs [46,47].

6. Characterization of protein loaded NPs

6.1. Size, polydispersity index (PDI), and zeta potential

Particle size characterization of protein-loaded NPs involves morphological examination by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) [46]. Transmission electron microscopy (TEM) provides direct visualization of NP size and provides detailed images of NP morphology. Dynamic light scattering (DLS) is commonly used to measure the hydrodynamic diameter and PDI of NPs [48–50].

6.2. Stability and surface properties

Surface charge (zeta potential), hydrophilicity, and wettability are vital for determining the interactions between NPs and biological systems. Zeta potential measurements predict the stability of NP dispersions and can be measured by a zeta sizer. *This* measures the surface charge of NPs, which influences their stability in suspension. A higher absolute zeta potential value typically indicates better stability due to electrostatic repulsion between particles. NPs with zeta potential of more than 30 mV (+/−) have been reported to be stable in the deposit because the surface charge prevents the particles from clotting.

6.3. Encapsulation efficiency

The encapsulation efficiency is determined by measuring the amount of protein in the supernatant after centrifugation of the NPs. The protein content is quantified using methods like Lowry's assay or BCA assay. The encapsulation efficiency is calculated as:

$$(\text{Total protein} - \text{Protein in supernatant})/\text{Total protein} \times 100\%$$

Release Kinetics: The drug release behaviour of NPs is evaluated in simulated biological fluids to understand how they release their therapeutic load over time. This assessment is conducted using techniques like dialysis or sample-and-separate methods.

Biodegradability and degradation rate: Gel Permeation Chromatography (GPC) and Mass Spectrometry (MS) are used to track the degradation of polymeric NPs over time.

Cellular Uptake Studies: NPs internalization by cells is measured and visualized through techniques such as confocal microscopy and flow cytometry, which help assess how effectively the NPs are delivered to target cells.

Cytotoxicity: The compatibility of NPs with cells is tested using various assays like MTT, XTT, or LIVE/DEAD assays to evaluate their potential toxicity to healthy cells.

Haemolysis and Protein Binding: Haemolysis tests determine how NPs interact with red blood cells and are analysed through SDS-PAGE or mass spectrometry that investigates how serum proteins attach to NPs and potentially affect their behaviour.

In-vivo characterization: It focuses on how polymeric NPs perform inside living organisms, providing critical information for preclinical evaluation.

- Pharmacokinetics (PK): The absorption, distribution, metabolism, and excretion (ADME) of NPs are examined using techniques such as HPLC, LC-MS, and fluorescence imaging. Prolonged circulation times of NPs are often preferred for better bioavailability.
- Biodistribution: NPs distribution across different organs or tissues is analysed using radioactive labelling or fluorescent dyes in combination with imaging technologies like MRI, PET, or bioluminescence imaging to ensure that NPs reach their target, such as tumours.
- In-vivo toxicity: The safety of NPs is evaluated by examining major organs (e.g., liver, kidneys, lungs) using histopathological analysis, blood tests, and immune response monitoring. Both acute and chronic toxicity assessments are conducted to detect any adverse effects.
- In-vivo Efficacy: These studies evaluate whether the NPs effectively deliver their therapeutic payload in animal models. Endpoints like tumour shrinkage, inflammation reduction, or changes in specific biomarkers are monitored to assess therapeutic success.
- Immunogenicity and Immune Response: NPs should not trigger harmful immune reactions. The immune response is gauged by measuring levels of cytokines, antibodies, and other immune markers using ELISA or multiplex assays.
- Targeting Efficiency: The effectiveness of targeted delivery mechanisms (e.g., ligand-receptor binding or enhanced permeability and retention (EPR) effect) is studied. Imaging techniques or tissue analysis help determine the extent of NPs accumulation at the target site [51–57].

Each of these techniques provides insights into different aspects of protein-loaded NPs, helping to optimize their design for specific applications, such as drug delivery or therapeutic interventions (**Table 5**).

Table 5. Applications.

Polymer used	Type of polymeric NP /method of preparation	Route of administration	Drug/Active agent	Treatment	Reference
Chitosan	Ion crosslinking method	Nasal	Quercetin	Allergic rhinitis (AR)	[58]
Chitosan	CS-Au based on gold NPs and chitosan (CS)	Intravenous	Myricaria germania	Immunization	[59]
Poly (β-amino esters) (PBAEs)	Encapsulation of the synthetic mRNA encoding bevacizumab, an anti-VEGF antibody in NPs	Intravenous	mRNA encoding bevacizumab	Non-small cell lung cancers (NSCLCs)	[60]
Chitosan	MTX was entrapped in the Chitosan NPs	Topical application	Methotrexate (MTX)	Rheumatoid arthritis	[61]
Chitosan	Encapsulation of AMP NRC-07 in CS-NPs by ionotropic gelation	Intravenous	antimicrobial peptide NRC-07	Antibacterial and *in vitro* anticancer activities	[62]
Elastin-like peptides (ELPs)	Supramolecular NPs based on elastin-like peptides modified capsid protein	Intravenous	Doxorubicin	Murine melanoma and colorectal cancer.	[63]
Poly(N-isopropylacrylamide)	Temperature-responsive Polymer NPs	Intravenous	Paclitaxel	Anticancer activity	[64]
Albumin	Elastin-targeted NPs	Intravenous	Doxycycline	LPS-mediated lung inflammation	[65]
Polymeric and lipid-based	Levodopa-loaded NPs	Intravenous, transdermal delivery and intranasal administration	Levodopa	Parkinson's disease	[66]
Hydrogel	pH-responsive polymeric nanocarriers	Intravenous	IL-12	Immunotherapy of Cancer	[67]
Chitosan	Concanavalin A (ConA) coated chitosan (CS) nanocarrier	Intravenous	Short antimicrobial peptide (CM11)	Helicobacter pylori gastric infection	[68]
Glutenin	Glucose-conjugated glutenin NPs	Intravenous	Camptothecin	Breast cancer	[69]
Chitosan	Peptides in chitosan NPs Coated with Zein	Oral	Antihypertensive Peptides, Isoleucine-Proline-Proline and Leucine-Lysine-Proline	Antihypertensive activity	[70]
ROS-responsive polymer	Modified emulsion approach	Intravenous	Dexamethasone	Acute lung injury (ALI)	[71]
Chitosan	Hyaluronic acid coated chitosan NPs	Oral	Insulin	Hypoglycemic activity	[72]
Phenylboronic ester	Phenylboronic ester-modified polymeric NPs	Nano vaccine	TRP2 peptide antigen delivery	Cancer immunotherapy	[73]
Poly(lactic-co-glycolic) acid (PLGA)	Double emulsion technique	Oral	Capreomycin peptide	Impact of stress conditions on peptide degradation: Thermal, mechanical, chemical	[74]
Chitosan	Self-assembled chitosan NPs by ionic cross-linking technique	Intranasal	Recombinant protein interleukin-17 receptor C (IL-17RC)	Asthma	[75]
pH-sensitive polymer	D-melittin polymeric NPs	Intravenous	D-melittin	Anti-cancer treatment	[76]
Chitosan	Cross-linked NPs	Percutaneous delivery	Betamethason	Contact dermatitis	[77]

Table 5. (*Continued*).

Polymer used	Type of polymeric NP /method of preparation	Route of administration	Drug/Active agent	Treatment	Reference
Hypoxia-responsive polymer PLA	Targeted and non- targeted self-assembled polymeric NPs	Intravenous	Doxorubicin (DOX)	Hypoxic, triple-negative breast tumors	[78]
Chitosan	Self-gelation method	Oral	Insulin	Diabetes	[79]
Poly (lactic-co-glycolic acid) (PLGA)	Double emulsion modified method	Intravenous	Recombinant adrenomedullin-2	Angiogenesis	[80]
PLGA, PEG	S2P peptide-conjugated PLGA-Maleimide-PEG NPs, modified emulsion/solvent evaporation technique.	Intravenous	Imatinib	Atherosclerosis	[81]
Poly (lactide-co-glycolide)-b-poly (ethylene glycol) NPs	Polymeric NPs functionalized with muscle-homing peptides	Intravenous	Phosphatase and tension homology inhibitor to skeletal muscle	Duchenne muscular dystrophy (DMD)	[82]
Human serum albumin (HSA)	Synthetic protein NPs (SPNP), polymerized HSA equipped with iRGD	Intravenous	siRNA against Signal Transducer	Glioblastoma	[83]

7. Conclusions and future prospects

Peptides and proteins are essential for numerous biological reactions and play significant roles in various pathological conditions. However, their therapeutic application in treating life-threatening disorders encounters several challenges, including instability, poor absorption, enzymatic degradation, a short biological half-life, and rapid elimination. Polymeric NPs have shown considerable potential in enhancing the absorption of macromolecules. These polymers are typically inert, biocompatible with biological fluids, biodegradable, and can be removed from the body as inert biodegradable products. The choice of polymers used to deliver therapeutic proteins and peptides significantly impacts their therapeutic efficacy. Polymeric NPs hold promise for various delivery routes, including nasal, pulmonary, oral, and ocular delivery; nonviral gene delivery; and crossing the blood-brain barrier. A versatile system that can ensure the delivery and systemic stability of various proteins and peptides would be highly beneficial in the near future.

Author contributions: Conceptualization, NM and NS (Nawale Sneha); methodology, NM; software, NS (Neerude Sirisha); validation, NM, NS (Nawale Sneha) and NS (Neerude Sirisha); formal analysis, NM; investigation, NS (Nawale Sneha); resources, NS (Neerude Sirisha); data curation, NM and NS (Nawale Sneha); writing—original draft preparation, NS (Neerude Sirisha); writing—review and editing, NM and NS (Nawale Sneha); visualization, NS (Neerude Sirisha); supervision, NM; project administration, NM; funding acquisition, NS (Neerude Sirisha). All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

References

1. Srivastava S, Sharma V, Bhushan B, et al. Nanocarriers for protein and peptide delivery: Recent advances and progress. Journal of Research in Pharmacy. 2021; 25(2): 99–116. doi: 10.29228/jrp.1

2. Panta P, Kwon JS, Son AR, et al. Protein drug-loaded polymeric nanoparticles. Journal of Biomedical Science and Engineering. 2014;7(10): 825–832. doi:10.4236/jbise.2014.710082
3. Schwendeman SP, Michael C, Alexander K, et al. Stability of proteins and their delivery from biodegradable polymer microspheres. Microparticulate systems for the delivery of proteins and vaccines. CRC Press; 1996. pp.1–49.
4. Zhu Q, Chen Z, Paul PK, et al. Oral delivery of proteins and peptides: Challenges, status quo and future perspectives. Acta Pharmaceutica Sinica B. 2021; 11(8): 2416–2448. doi: 10.1016/j.apsb.2021.04.001.
5. Karolina W. Biological barriers, and the influence of protein binding on the passage of drugs across them. Molecular Biology Reports. 2020; 47(4): 3221–3231. doi:10.1007/s11033-020-05361-2
6. Wu J, Sahoo JK, Li Y, et al. Challenges in delivering therapeutic peptides and proteins: A silk-based solution. Journal of Controlled Release. 2022; 345: 76–189. doi: 10.1016/j.jconrel.2022.02.011
7. Pardridge WM. Blood-brain barrier and delivery of protein and gene therapeutics to brain. Frontiers in Aging Neuroscience. 2020; 11: 373.
8. Cao S, Xu S, Wang H, et al. Nanoparticles: oral delivery for protein and peptide drugs. AAPS PharmSciTech. 2019; 20: 190. doi:10.1208/s12249-019-1325-z
9. Stevens CA, Kaur K, Klok HM. Self-assembly of protein-polymer conjugates for drug delivery. Advanced Drug Delivery Reviews. 2021; 174: 447–460. doi: 10.1016/j.addr.2021.05.002
10. Moraru C, Mincea M, Menghiu G, et al. Understanding the factors influencing chitosan-based nanoparticles-protein corona interaction and drug delivery applications. Molecules. 2020; 25(20): 4758. doi: 10.3390/molecules25204758
11. Pudlarz A, Szemraj J. Nanoparticles as carriers of proteins, peptides and other therapeutic molecules. Open Life Sciences. 2018; 13(1): 285–298. doi: 10.1515/biol-2018-0035
12. Liu J, Ding X, Fu Y, et al. Cyclodextrins based delivery systems for macro biomolecules. European Journal of Medicinal Chemistry. 2021; 212: 113105. doi: 10.1016/j.ejmech.2020.113105
13. Abhishek P. Cyclodextrin-based nanoparticles for pharmaceutical applications. Environmental Chemistry Letters. 2021; 19(6): 4297–4310. doi: 10.1007/s10311-021-01275-y
14. Kabir II, Sorrell CC, Mofarah SS, et al. Alginate/polymer-based materials for fire retardancy: Synthesis, structure, properties, and applications. Polymer Reviews. 2021; 61(2): 357–414. doi: 10.1080/15583724.2020.1801726
15. Uyen NTT, Hamid ZAA, Tram NXT, et al. Fabrication of alginate microspheres for drug delivery: A review. International Journal of Biological Macromolecules. 2020; 15(153): 1035–1046. doi: 10.1016/j.ijbiomac.2019.10.233
16. Du Q, Zhou L, Lyu F, et al. The complex of whey protein and pectin: Interactions, functional properties and applications in food colloidal systems—A review, Colloids and Surfaces B. Biointerfaces. 2022; 210: 112253. doi: 10.1016/j.colsurfb.2021.112253
17. Li D, Xu F, Li J. Pectin-based micro-and nanomaterials in drug delivery in Micro-and Nanoengineered Gum-Based Biomaterials for Drug Delivery and Biomedical Applications. 2022; 97: 125. doi: 10.1016/b978-0-323-90986-0.00015-7
18. Mahmoud H. Elella A, Magdy W, et al. Antimicrobial pH-sensitive protein carrier based on modified xanthan gum. Journal of Drug Delivery Science and Technology. 2020; 57: 101673. doi: 10.1016/j.jddst.2020.101673
19. Aristeidis P, Aggeliki S. Xanthan-based polysaccharide/protein nanoparticles: Preparation, characterization, encapsulation and stabilization of curcumin. Carbohydrate Polymer Technologies and Applications. 2021; 2: 100075. doi: 10.1016/j.carpta.2021.100075
20. Xue Y, Li Y, Zhang D, et al. Calcium phosphate silicate microspheres with soybean lecithin as a sustained-release bone morphogenetic protein-delivery system for bone tissue regeneration. ACS Biomaterials Science & Engineering. 2023; 9(5): 2596–2607. doi: 10.1021/acsbiomaterials.2c01065
21. Radhika R, Xu Y, Nidhi J, Stenzel MH. Progress of albumin-polymer conjugates as efficient drug carriers. Pure and Applied Chemistry. 2022; 94(8): 983–997. doi: 10.1515/pac-2021-2006
22. Ashni A, Pratyusha M, Anindita L, et al. Collagen nanoparticles in drug delivery systems and tissue engineering. Applied Sciences. 2021; 11(23): 11369. doi: 10.3390/app112311369
23. Hong S, Choi DW, Kim HN, et al, Hee Ho Park, Protein-based nanoparticles as drug delivery systems. Pharmaceutics. 2020; 12(7): 604. doi: 10.3390/pharmaceutics12070604
24. Hsing-Wen S, Zi X, Shu F, Tu H. Nanomega Medical Corp National Tsing Hua University NTHU GP Medical. Assignee. Nanoparticles for protein drug delivery. U.S. Patent 8,283,317B1, 23 January 2012.

25. Xu T, He D, Jessica S, et al. The Regents of the University of California (Oakland, CA). Assignee. Bis-polymer lipid-peptide conjugates and nanoparticles thereof. U.S. Patent Application No. 10,806,702. 2018.
26. Muso-Cachumba JJ, Feng S, Belaid M, et al. Polymersomes for protein drug delivery across intestinal mucosa. International Journal of Pharmaceutics. 2023; 648: 123613. doi: 10.1016/j.ijpharm.2023.123613
27. Lee J, Yoo E, Choi SJ. Fabrication and characterization of nanoparticles with lecithin liposomes and poloxamer micelles: Impact of conformational structures of poloxamers. Food Chemistry. 2024; 435: 137613. doi: 10.1016/j.foodchem.2023.137613
28. Butreddy A, Gaddam RP, Kommineni N, et al. PLGA/PLA-based long-acting injectable depot microspheres in clinical use: production and characterization overview for protein/peptide delivery. International journal of molecular sciences. 2021; 22(16): 8884. doi: 10.3390/ijms22168884
29. Angkawinitwong U, Courtenay AJ, Rodgers AM, et al. A novel transdermal protein delivery strategy via electrohydrodynamic coating of PLGA microparticles onto microneedles. ACS applied materials & interfaces. 2020; 12(11): 12478–12488. doi: /10.1021/acsami.9b22425
30. Kadekar S, Nawale GN, Rangasami VK, et al. Redox responsive Pluronic micelle mediated delivery of functional siRNA: a modular nano-assembly for targeted delivery. Biomaterials Science. 2021; 9(11): 3939–3944. doi: 10.1039/D1BM00428J
31. Hosseinpour S, Walsh LJ, Xu C. Biomedical application of mesoporous silica nanoparticles as delivery systems: a biological safety perspective. Journal of Material Chemistry B. 2020; 8(43): 9863–9876. doi: 10.1039/d0tb01868f
32. Faruck MO, Zhao L, Hussein WM, et al. Polyacrylate-Peptide Antigen Conjugate as a Single-Dose Oral Vaccine against Group A Streptococcus. Vaccines (Basel). 2020; 8(1): 23. doi: 10.3390/vaccines8010023
33. Szczęch M, Szczepanowicz K. Polymeric core-shell nanoparticles prepared by spontaneous emulsification solvent evaporation and functionalized by the layer-by-layer method. Nanomaterials 2020; 10(3): 49. doi: 10.3390/nano10030496
34. Pulingam T, Foroozandeh P, Chuah JA, Sudesh K. Exploring various techniques for the chemical and biological synthesis of polymeric nanoparticles. Nanomaterials. 2022; 12(3): 576. doi: 10.3390/nano12030576
35. Saha-Shah A, Sun S, Kong J, et al. Design and study of PEG linkers that enable robust characterization of PEGylated proteins, ACS Pharmacology & Translational Science. 2021; 4(4): 1280–1286. doi: 10.1021/acsptsci.1c00112
36. Li M, Jiang S, Simon J, et al. Brush conformation of polyethylene glycol determines the stealth effect of nanocarriers in the low protein adsorption regime. Nano Letters. 2021; 21(4): 1591–1598. doi: 10.1021/acs.nanolett.0c03756
37. Souto EB, Souto SB, Campos JR, et al. Nanoparticle delivery systems in the treatment of diabetes complications. Molecules. 2019; 24: 4209. doi: 10.3390/molecules24234209
38. Duong VA, Nguyen TTL, Maeng HJ. Preparation of solid lipid nanoparticles and nanostructured lipid carriers for drug delivery and the effects of preparation parameters of solvent injection method. Molecules. 2020; 25(20): 4781. doi: 10.3390/molecules25204781
39. Ana L, Martínez L, Cristina P, et al. Protein-based nanoparticles for drug delivery purposes. International journal of pharmaceutics. 2020; 581: 119289. doi: 10.1016/j.ijpharm.2020.119289
40. Teleanu DM, Chircov C, Grumezescu AM, et al. Neuronanomedicine: An Up-to-Date Overview. Pharmaceutics. 2019; 11(3): 101. doi:10.3390/pharmaceutics11030101
41. Pulingam T, Foroozandeh P, Chuah JA, et al. Exploring various techniques for the chemical and biological synthesis of polymeric nanoparticles. Nanomaterials. 2022; 12(3): 576. doi: 10.3390/nano12030576
42. Sanchez-Lopez E, Egea MA, Davis BM, et al. Memantine-loaded pegylated biodegradable nanoparticles for the treatment of glaucoma. Small. 2018; 14(2): 14. doi: 10.1002/smll.201701808
43. Martinez Rivas CJ, Tarhini M, Badri W, et al. Nanoprecipitation process: From encapsulation to drug delivery. International Journal of Pharmaceutics. 2017; 532: 66–81. doi: 10.1016/j.ijpharm.2017.08.064
44. Hernández-Giottonini KY, Rodríguez-Córdova RJ, Gutiérrez-Valenzuela CA, et al. PLGA nanoparticle preparations by emulsification and nanoprecipitation techniques: Effects of formulation parameters. RSC Advances. 2020; 10 (8): 4218–4231. doi: 10.1039/c9ra10857b
45. Pedroso-Santana S, Fleitas-Salazar N. Ionotropic gelation method in the synthesis of nanoparticles/microparticles for biomedical purposes. Polymer International. 2020; 69(5): 443–447. doi: 10.1002/pi.5970
46. Algharib SA, Dawood A, Zhou K, et al. Preparation of chitosan nanoparticles by ionotropic gelation technique: Effects of formulation parameters and in vitro characterization. Journal of Molecular Structure. 2022; 1252: 132129. doi: 10.22159/ijap.2018v10i5.26375

47. Carvalho PM, Felício MR, Santos NC, et al. Application of light scattering techniques to nanoparticle characterization and development. Frontiers in Chemistry. 2018; 6: 237. doi: 10.3389/fchem.2018.00237
48. Mourdikoudis S, Pallares RM, Thanh NT. Characterization techniques for nanoparticles: Comparison and complementarity upon studying nanoparticle properties. Nanoscale. 2018; 10: 12871–12934. doi: 10.1039/C8NR02278J
49. Dazon C, Witschger O, Bau S, et al. Nanomaterial identification of powders: Comparing volume specific surface area, X-ray diffraction and scanning electron microscopy methods. Environmental Sciences: Nano. 2019; 6: 152–162. doi: 10.1039/C8EN00760H
50. Rasmussen MK, Pedersen JN, Marie R. Size and surface charge characterization of nanoparticles with a salt gradient. Nature communications. 2020; 11(1): 2337. doi: 10.1038/s41467-020-15889-3
51. Kaur P, Khanna A, Kaur N, et al. Synthesis and structural characterization of alumina nanoparticles. Phase Transitions. 2020; 93(6): 596–605. doi: 10.1080/01411594.2020.1765245
52. Zielińska A, Ferreira NR, Feliczak-Guzik A, et al. Loading, release profile and accelerated stability assessment of monoterpenes-loaded solid lipid nanoparticles (SLN). Pharmaceutical Development and Technology. 2020; 25:1–13. doi: 10.1080/10837450.2020.1744008
53. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry. 2019; 12(7): 908–931. doi: 10.1016/j.arabjc.2017.05.011
54. Zielińska A, Carreiró F, Oliveira AM, et al. Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology. Molecules. 2020; 25(16): 3731. doi:10.3390/molecules25163731
55. Ostolska I, Wiśniewska M. Application of the zeta potential measurements to explanation of colloidal Cr2O3 stability mechanism in the presence of the ionic polyamino acids. Colloid and Polymer Sciences. 2014; 292: 2453–246. doi: 10.1007/s00396-014-3276-y
56. Altammar KA. A review on nanoparticles: characteristics, synthesis, applications, and challenges. Frontiers in microbiology. 2023; 14: 1155622. doi: 10.3389/fmicb.2023.1155622
57. Sajid M, Plotka-Wasylka J, Nanoparticles: Synthesis, characteristics, and applications in analytical and other sciences. Microchemical Journal. 2020; 154: 104623. doi: 10.1016/j.microc.2020.104623
58. Mu D, Zhou L, Shi L, et al. Quercetin-crosslinked chitosan nanoparticles: a potential treatment for allergic rhinitis. Scientific Reports. 2024; 14(1): 4021. doi: 10.1038/s41598-024-54501-2
59. Wang Y, Qiu F, Zheng Q, et al. Preparation, characterization and immune response of chitosan-gold loaded Myricaria germanica polysaccharide. International Journal of Biological Macromolecules. 2024; 257 (2): 128670. doi: 10.1016/j.ijbiomac.2023.128670
60. Le ND, Nguyen BL, Patil BR, et al. Antiangiogenic therapeutic mRNA delivery using lung-selective polymeric nanomedicine for lung cancer treatment. ACS Nano. 2024; 18(11): 8392–8410. doi: 10.1021/acsnano.3c13039
61. Al-Nemrawi N, Wahsheh Y, Alzoubi KH. Transdermal delivery of methotrexate loaded in chitosan nanoparticles to treat rheumatoid arthritis. Current Drug Delivery. 2024; 21(3): 451–460. doi: 10.2174/1567201820666230428124346
62. Turky NO, Abdelmonem NA, Tammam SN, et al. Antibacterial and in vitro anticancer activities of the antimicrobial peptide NRC-07 encapsulated in chitosan nanoparticles. Journal of Peptide Science. 2024; 30(4): e3550. doi: 10.1002/psc.3550
63. Shen L, Zhou P, Wang YM, et al. Supramolecular nanoparticles based on elastin-like peptides modified capsid protein as drug delivery platform with enhanced cancer chemotherapy efficacy. International Journal of Biology and Macromolecules. 2024; 256 (Pt 2): 128107. doi: 10.1016/j.ijbiomac.2023.128107
64. Koide H, Yamaguchi K, Sato K, et al. Engineering temperature-responsive polymer nanoparticles that load and release paclitaxel, a low-molecular-weight anticancer drug. ACS Omega. 2023; 9(1): 1011–1019. doi: 10.1021/acsomega.3c07226
65. Arora S, Vyavahare N. Elastin-targeted nanoparticles delivering doxycycline mitigate cytokine storm and reduce immune cell infiltration in LPS-mediated lung inflammation. PLoS One. 2023; 18(6): e0286211. doi: 10.1371/journal.pone.0286211
66. Van Vliet EF, Knol MJ, Schiffelers RM, et al. Levodopa-loaded nanoparticles for the treatment of Parkinson's disease. Journal of Control Release. 2023; 360: 212–224. doi: 10.1016/j.jconrel.2023.06.026
67. Zhou S, Cheng F, Zhang Y, et al. Engineering and delivery of cGAS-STING immunomodulators for the immunotherapy of cancer and autoimmune diseases. Accounts of Chemical Research Journal. 2023; 56(21): 2933–2943. doi: 10.1021/acs.accounts.3c00394

68. Moghaddam MM, Bolouri S, Golmohammadi R, et al. Targeted delivery of a short antimicrobial peptide (CM11) against Helicobacter pylori gastric infection using concanavalin A-coated chitosan nanoparticles. Journal of Materials Science: Materials in Medicine. 2023; 34(9): 44. doi: 10.1007/s10856-023-06748-w
69. Rajeshkumar RR, Pavadai P, Panneerselvam T, et al. Glucose-conjugated glutenin nanoparticles for selective targeting and delivery of camptothecin into breast cancer cells. Naunyn Schmiedebergs Archives of Pharmacology. 2023; 396(10): 2571–2586. doi: 10.1007/s00210-023-02480-y
70. Khalid Danish M, Gleeson JP, Brayden DJ, et al. Formulation, characterisation and evaluation of the antihypertensive peptides, isoleucine-proline-proline and leucine-lysine-proline in chitosan nanoparticles coated with zein for oral drug delivery. International Journal of Molecular Sciences. 2022; 23(19): 11160. doi: 10.3390/ijms231911160
71. Muhammad W, Zhu J, Zhai Z, et al. ROS-responsive polymer nanoparticles with enhanced loading of dexamethasone effectively modulate the lung injury microenvironment. Acta Biomaterial. 2022; 148: 258–270. doi: 10.1016/j.actbio.2022.06.024
72. Wu H, Guo T, Nan J, et al. Hyaluronic-acid-coated chitosan nanoparticles for insulin oral delivery: fabrication, characterization, and hypoglycemic ability. Macromolecular Biosciences. 2022; 22(7): e2100493. doi: 10.1002/mabi.202100493
73. Wang Q, Dong Z, Lou F, et al. Phenylboronic ester-modified polymeric nanoparticles for promoting TRP2 peptide antigen delivery in cancer immunotherapy. Drug Delivery. 2022; 29(1): 2029–2043. doi: 10.1080/10717544.2022.2086941
74. Ahmed SMA, Ibrahim M, El-Bagory E, et al. Design of polymeric nanoparticles for oral delivery of capreomycin peptide using double emulsion technique: Impact of stress conditions. Journal of Drug Delivery Science and Technology. 2022; 71: 103326. doi: 10.1016/j.jddst.2022.103326
75. Lv Y, Zhang J, Wang C. Self-assembled chitosan nanoparticles for intranasal delivery of recombinant protein interleukin-17 receptor C (IL-17RC): preparation and evaluation in asthma mice. Bioengineered. 2021; 12(1): 3029–3039. doi: 10.1080/21655979.2021.1940622
76. Lv S, Sylvestre M, Song K, et al. Development of D-melittin polymeric nanoparticles for anti-cancer treatment. Biomaterials. 2021; 277: 121076. doi: 10.1016/j.biomaterials.2021.121076
77. Hudan-Tsilo I, Tokarskyy O, Shevchuk O, et al. Chitosan self-assembled polymeric nanoparticles for percutaneous delivery of betamethasone in contact dermatitis. Drug Development and Industrial Pharmacy. 2021; 47(8): 1310–1317. doi: 10.1080/03639045.2021.1989457
78. Mamnoon B, Loganathan J, Confeld MI, et al. Targeted polymeric nanoparticles for drug delivery to hypoxic, triple-negative breast tumors. ACS Applied Bio Materials. 2021; 4(2): 1450–1460. doi: 10.1021/acsabm.0c01336
79. Mumuni MA, Kenechukwu FC, Ofokansi KC, et al. Insulin-loaded mucoadhesive nanoparticles based on mucin-chitosan complexes for oral delivery and diabetes treatment. Carbohydrate Polymer. 2020; 229: 115506. doi: 10.1016/j.carbpol.2019.115506
80. Quadros HC, Santos LMF, Meira CS, et al. Development and in vitro characterization of polymeric nanoparticles containing recombinant adrenomedullin-2 intended for therapeutic angiogenesis. International Journal of Pharmaceutics. 2020; 576: 118997. doi: 10.1016/j.ijpharm.2019
81. Esfandyari-Manesh M, Abdi M, Talasaz AH, et al. S2P peptide-conjugated PLGA-Maleimide-PEG nanoparticles containing Imatinib for targeting drug delivery to atherosclerotic plaques. DARU Journal of Pharmaceutical Sciences. 2020; 28(1): 131–138. doi: 10.1007/s40199-019-00324-w
82. Huang D, Yue F, Qiu J, et al. Polymeric nanoparticles functionalized with muscle-homing peptides for targeted delivery of phosphatase and tensin homolog inhibitor to skeletal muscle. Acta Biomaterial. 2020; 118:196–206. doi: 10.1016/j.actbio.2020.10.009
83. Gregory JV, Kadiyala P, Doherty R, et al. Systemic brain tumor delivery of synthetic protein nanoparticles for glioblastoma therapy. Nature Communications. 2020; 11(1): 5687. doi: 10.1038/s41467-020-19225-7