



Review Article An Overview on Protein and Peptide Drug Delivery System: Advances and Strategies

Diwas Adhikari^{*}, Sharada Pokhrel

M. Pharm-Veer Madho Singh Bhandari Uttarakhand Technical University, Dehradun, India, Senior QC Officer- TIME Pharmaceuticals Limited, Nepal.

ARTICLE INFO

Article history:

Received: 07, October, 2024 Revised: 06 November, 2024 Accepted: 04 December, 2024 Published: 30 December, 2024

Keywords:

Peptide, Invasive, Non-Invasive, Hybridoma technology, Therapeutic efficacy **DOI:** 10.21590/jddhs.01.04.07

BACKGROUND

Chain of amino acids joined together by the covalent bond forms protein. Amino Acid goes extensive polymerization process with the help of peptide bonds and thus forms the structural framework of the protein (Nelson David et. al., 2005). Number of Amino acids determines the terminology that means over 50 amino acids is defined by term protein and less than 50 amino acids are defined by the term peptide. Elucidation of the protein structure is of vital significance in order to understand the protein drug delivery system and to deal with the various problems associated with the formulation (Satyanarayan U & Chakrapani U, 2008).Protein has different structure such as Primary, Secondary, tertiary and Quaternary based on

ABSTRACT

Being a novel concept in the delivery of drugs, Protein/Peptide based pharmaceuticals are considered as important parts of medical research. Protein plays an important role for life process integration. Different forms of delivery methods invasive or non-invasive are well adopted. Various pharmaceutical approaches are adopted for the drug development. All of them has their own significance which needs to be considered for the drug development. Parenteral Systemic delivery is the considered as efficient route of choice among others. Biological cells and organic molecules are majorly benefitted through these systems. As a therapeutic/diagnostic agent, they play a crucial role. It enhances the r-DNA technology and Hybridoma technology. Disadvantages associated with the existing delivery system can be addressed and better therapeutic efficacy can be achieved through such delivery systems. Development of these types of dosages form significantly boosts the energy among the Protein/Peptide Research Scientist.

numbers and structures of amino acids (Figure 1). We may face different types of difficulties during formulation of protein/peptide drugs due to unique physical &chemical properties (Degim et. al., 2007).We should keep the protein in the refrigerator to protect them from the degradation caused by heat/agitation and physiochemical degradation (Roberts, Bentley, & Harris,

Protein has different structural functions and dynamic functions. Structural functions maintain body strength whereas with dynamic functions they act as factors for clotting of blood, immunoglobulin, genetic control and muscle contraction etc.

Protein Drug Delivery follows the specialized transport mechanisms for their effective action. Size of the protein and

*Corresponding Author: Diwas Adhikari

Address: M. Pharm-Veer Madho Singh Bhandari Uttarakhand Technical University, Dehradun, India, Sr. Asst. QA Manager- TIME Pharmaceuticals Limited, Nepal.

2002).

Email : adhikaridiwas51@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2024 First Author *et al.* This is an open access article distributed under the terms of the Creative Commons Attribution- NonCommercial-ShareAlike 4.0 International License which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

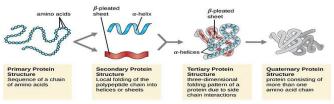


Figure 1: Structures of protein

High molecular weight affects the diffusion of the particular drugs through epithelial layers (McMartin & Colin et. al., 1987) (Donovan et. al., 1990). Different barriers create significant challenges for delivery of these dosage forms.

Significance of Protein/Peptide Drugs Delivery

Biological cells along with Organic molecules get majorly benefitted through these systems. As a therapeutic/ Diagnostic agent, they play a crucial role. It enhances the r-DNA technology and Hybridoma technology (Lee, 2002). Maintenance of blood sugar level through Insulin, Production of RBC through Erythropoietin, Increase of peripheral circulation through the Bradykinin, Decreasing of bleeding level in the gastric ulcer with Somatostatin, Prevention of heart attack and stroke through the use of Plasminogen activator, Relieving of pain through the use of β-endorphin, Treatment of diabetes insipidus through the use of Vasopressin, Enhancement of activity of killer cells through the use of Interferon's, Treatment of Dwarfism through the use of Human Growth Hormone, Reduction of secretion of gastric acid through the use of gastrin antagonist, Digestive Supplement through the use of Pancreatic enzymes etc. are being possible through these approaches (Soltero, 2005).

Different forms of delivery such as invasive or non-invasive are there for the Protein/Peptide Drug delivery. Direct injection, Intravenous, subcutaneous, intramuscular, intracerebral and depot system are the invasive routes for the protein drug delivery whereas Oral/Buccal, Pulmonary, Transdermal, Nasal, Buccal, Rectal/Vaginal etc. are noninvasive routes(Sinha & Trehan, 2003) (Figure 2).

General Considerations on Different Routes of Delivery (Jani, Manseta & Patel, 2012)

Parenteral Systemic Delivery

It is the most efficient and best routes of delivery among others (Okumu &Cleland, 2002).Intravenous route is the preferred choice among others. Various approaches such as Microspheres, Hydrogels, PEGylation, and Nanoparticles of Solid/Lipid are some approaches for parenteral systemic delivery of the protein.

Non-Parenteral Systemic Delivery

It is necessary to understand the physiochemical properties of proteins/peptides for the oral delivery system development. Various properties such as Molecular weight, Constant of ionization, Hydrophobicity, Stability

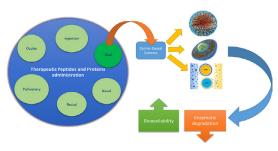


Figure 2: Routes of Administration

in pH, Enzyme degradation are necessary to understand (Clark and Shire, 2000).Various Approaches such as chemical modification with Amino acid substitution and Nobex Conjugated technology, Protease inhibitors, Use of penetration enhancers, Formulations as Emulsion, Microspheres, Liposomes, Nanoparticles are some of the approaches. Similarly, Viscosity modification, pH modification, increasing of nasal blood flow, Dissociation of aggregation, Membrane transport and enzyme inhibition etc. are some of the approaches for the Nasal Delivery. Likewise, Liposomes, Lipid based Micro particles, Microspheres, using of delivery devices are some of the approach of pulmonary delivery. As in other delivery system, Iontophoresis, Phonophoresis, Penetration enhancers, Transferosomes, Macroflux technology etc. is some of the approach in Transdermal Delivery. Development of self-adhesive buccal patch is adopted for Buccal Delivery.

Pharmaceutical Approaches

Chemical Modification:

This is also called the prodrug approach. When we closely observe the nature of the protein, they are highly liable for the degradation. Proteins undergo proteolytic cleavage. So chemical modification of the protein is essential as Prodrug. Various approaches can be used for the Prodrug formation such as substitution of olefin, substitution of d-amino group, substitution of dehydroamino group etc. These actions also improve the enzymatic stability and membrane penetration. Immunogenicity can also be minimized through this approach.

Chemical modification can be achieved either through the amino acid modification or through the hydrophobization.

Amino acid modification

Individual amino acid modification combined with the substitution of one or more L-amino acids with D-amino acids significantly alters the physiological properties. These types of modification enhance the penetration and increase the compatibility than the parent drugs.

Hydrophobization

Hydrophobization facilities mucosal transport of protein and stabilize the proteins to prevent the degradation from enzymes (Paula et. al., 2007).



Enzyme/Protease Inhibitors

Protease inhibitors are enzymatic approach for protein/ peptide drug delivery system. Proteolytic activities are supposed to be suppressed with the help of protease inhibitors with the alteration of the environment which supports the proteolytic activity. These enzymes are sufficiently found in the gastrointestinal tract and liver. Fragmentation of the proteins/peptides needs to be inhibited for their proper action. Phosphoramidon is a specific inhibitor for the enzyme metalloendoproteases, α 2-Macroglobulin is a specific inhibitor for the enzyme end protease, Arphanamine is a specific inhibitor for the enzyme Aminopeptidase B, and Bestain is a specific inhibitor for the enzyme Aminopeptidases. Some Protease inhibitors manipulate the pH of the site location thus inactivating the local digestive enzymes.

Penetration enhancers

Different group of chemicals such as surfactant such as Polysorbate, Sodium Lauryl Sulphate, Pluronic F68, Chelating agents such as EDTA, Mucoadhesive polymeric systems such as thiomers, cellulose derivatives etc. are used as Penetration enhancers.Some enhances the Transcellular transport of drugs whereas Paracellular transport of the Hydrophilic drugs is enhanced by the EDTA, which forms the complex with the calcium ions as a result, the tight junctions gets ruptured and thus the hydrophilic drugs gets easily passed through the channel. Some penetration enhancers enhance the penetration of the drugs without causing to mucosa of the intestine.

Formulation Vehicles

Emulsions

Emulsion is a heterogeneous system which contains the two immiscible liquid phases where the uniform fine droplets distributes in the another continuous phase (liquid) with the help of agitation mechanically. Mainly the emulsions are Oil in water or Water in oil emulsion. These techniques helps in the improving the stability of the drugs and thus helps in preventing the degradation caused by various enzymes found in the lumen. Also they help for the enhancement of the permeation through the mucosa of the intestine (Verissimo et. al., 2010).

Water-in-oil-in-water (w/o/w) emulsions also demonstrated significant delivery potential compared to simple aqueous solutions. To address the physical and chemical instability of emulsions, they are often formulated into dry emulsions. The use of micro-domains with varying polarities within a single-phase solution helps improve the solubility of hydrophilic or lipophilic peptides during formulation.

Microspheres

Microspheres are small, spherical particles used in oral drug delivery systems for protein or peptide drugs. They ensure a uniform distribution of the drug, which can be present either in solution or in a microcrystalline form within the microspheres.

Various biodegradable polymers are utilized in microspheres for the delivery of micro-molecules, macromolecules, and proteins. These are capable for releasing of the drug for a period of 1 to 3 months following intramuscular/subcutaneous injection in animals.

Microspheres offer more physical and chemical stability than liposomes, making them an important delivery vehicle for protein-based drug delivery systems (DDS), particularly in pulmonary delivery.

Microspheres are created through various polymerization and encapsulation processes. Analyzing the particle size distribution, surface morphology, and zeta potential of the microspheres is a critical aspect of developing this dosage form (Cleland, 1997).

Hydrogels

These are three-dimensional, hydrophilic polymer networks which are capable for absorption of large amounts of water (Peppas, et. al., 2000). Hydrogel provides the protein friendly environment and also they are more biocompatible as compared. For the controlled release of the pharmaceuticals, dextran based hydrogels are used. Gels are formed through the cross linking process that may be physically/chemically (Langer & Folkman, 1976).

Liposomes

They are Novel system with bilayers of phospholipids chains and they encapsulate the proteins/peptides within the core of the lipid. They prolong the circulation time, High amount of drug loading and ligands can be targeted. Currently various Liposomal formulations have been approved such as liposomes of amphotericin, liposomal formulation of doxorubicin. They prevent and protect the drugs from the oxidative process and the deamidation process (Yau, Lee & Chen, 2021).

Particulate Carriers

Protein/Peptide drugs can be delivered as Nanoparticles or micro particles. We should understand the properties of the surface and composition (chemical) of the carrier. Different anticancer drugs and proteins have been formulated in nanoparticles drugs. They prevent the drugs from degradation due to enzymes and increase the absorption through the epithelium of the intestine (Rezaei, Safavi & Shojaosadati, 2019).

Mucoadhesive Polymeric systems

These systems are very useful as they prevent the problems encountered with the presystemic metabolism and the first pass metabolism. Also they maintain the therapeutic efficacy. They are important for the site specific delivery and helps for the permeation enhancement through the membrane. Properties of bio adhesiveness between the surface of mucosa and polymeric materials are used for these systems. When the polymers get contact with the water, it gets adhered to the mucosa which improves the time of residence as a result of which concentration of drug molecules gets increased. Derivatives of Cellulose, Derivatives of Polyacrylic acid and thiomers are mainly used for these types approaches (Shaikh, et. al., 2011) (Renukuntla et.al. 2013).

Incorporation into matrix for drug delivery

It involves the different methodologies such as Emulsification, Extrusion & Spray Drying and Polymerization. It is suggested to avoid the extreme stress, high temperature, and Heat and cross linking agents for the purpose of maintaining the stability. Lyophilization and Spray drying techniques are widely used (Wang & Selomulya, 2020).

Protein Engineering

It is used for the improvement of stability and specificity of the endogenous protein. Selectivity is enhanced and delivery rate will get prolonged at the active sites. Deletion of the mutants, cloning of the genes is widely applicable approach. Hybrid Protein approach is another concept in the Protein engineering (Tobin & Richards, 2014)(Leisola & Turunen, 2007).

Advanced PEGylation

PEG can be used in Foods, Pharmaceuticals or cosmetics. Polymers of the PEG may be linear in shape or they may be branched. PEGylated compounds when binds with water acts more vigorously than the soluble protein of identical molecular weight. These molecules are found more mobile and protect the drugs from degradation by the enzymes and prevents the filtration through renal channel by getting size increased (Ryan, et. al., 2008).

Bioavailability of the drug gets enhanced, Frequency of the dosing gets decreased, Degradation by the metabolic enzymes gets decreased, Residence time of the drugs gets enhanced, Pharmacokinetics of the drugs gets enhanced, Efficacy of the drug will get increased, Safety profile will be improved, Immunogenicity of the protein is reduced, Solubility behavior of the drug will be enhanced, Stability of the drug will be increased using the Advanced PEGylation technique (Veronese & Pasut, 2005).

Evaluation of Protein and Peptide Drug Formulations

Stability Testing

It determines how capable the formulation is to remain unaffected in the particular closure system with the maintenance of different physical, toxicological, chemical, microbiological specifications. For the stability testing, we assess the impact of environmental factors of the drug substances quality thus helps for the determination of the shelf life and the appropriate storage condition of the drug.

Bioassay

It is done to identify how potent the formulation is. It can be done In-vivo and In-Vitro both. Cell's response to growth factor and hormone is monitored in the In-Vitro Bioassays whereas animal's pharmacological response to the protein is monitored in the In-Vivo Bioassays. (Jorgensen et. al.,2006)

UV Spectroscopy

It can be used for the determination of the content of the protein/peptides in the particular formulation such as phenyl alanine, tryptophan, tyrosine etc. It is an analytical approach.

Bradford Assay

The principle in this technique is that absorption maximum of the dye changes due to the availability of protein in the acidic medium. Dye used is Coomassie brilliant blue G-250. In the absence of binding protein, solution remains brown whereas the blue colored solution is formed if there is presence of protein and in this dye forms a complex carboxyl end of the available protein. Finally, the colored solution's intensity is measured for determination of concentration of protein in the sample (Kruger, 2009).

CONCLUSION

Advancement of the Pharmaceutical Biotechnology has paved the way for the enhanced and better delivery of the Protein and Peptides utilizing the various approaches. It is widely considered as the future of the medicines. Disadvantages associated with the existing delivery system can be addressed and better therapeutic efficacy can be achieved through such delivery systems.

AUTHORS CONTRIBUTION

Both Authors have contributed for gathering the information and preparation of the review article and finalization for the publication.

CONFLICT OF INTEREST

None.

REFERENCES

- Clark, A. R., & Shire, S. J. (2000). Protein formulation and delivery. Drugs and the Pharmaceutical Science, 99, 201-12.
- Cleland, J. (1997). Protein delivery from biodegradable microspheres. Protein delivery: Physical systems, 1-43.
- David, L. N., & Michael, M. C. (2021). Lehninger Principles of Biochemistry. wh freeman.
- De Paula, D., Bentley, M. V. L., & Mahato, R. I. (2007). Hydrophobization and bioconjugation for enhanced siRNA delivery and targeting. Rna, 13(4), 431-456.
- Donovan, M. D., Flynn, G. L., & Amidon, G. L. (1990). Absorption of polyethylene glycols 600 through 2000: the molecular weight dependence of gastrointestinal and nasal absorption. Pharmaceutical research, 7, 863-868.
- H. Tobin, P., H. Richards, D., A. Callender, R., & J. Wilson, C. (2014). Protein engineering: a new frontier for biological therapeutics. *Current drug metabolism, 15(7), 743-756*.



- Jani, P., Manseta, P., & Patel, S. (2012). Pharmaceutical approaches related to systemic delivery of protein and peptide drugs: an overview. Int. J. Pharm. Sci. Rev. Res, 12, 42-52. Okumu, F. W., & Cleland, J. L. (2002). Implants and injectables. In Modified-Release Drug Delivery Technology (pp. 657-662). CRC Press.
- Jorgensen, L., Moeller, E. H., Van de Weert, M., Nielsen, H. M., & Frokjaer, S. (2006). Preparing and evaluating delivery systems for proteins. European journal of pharmaceutical sciences, 29(3-4), 174-182.
- Kruger, N. J. (2009). The Bradford method for protein quantitation. The protein protocols handbook, 17-24.
- Langer, R., & Folkman, J. (1976). Polymers for the sustained release of proteins and other macromolecules. Nature, 263(5580), 797-800.
- Lee, H. J. (2002). Protein drug oral delivery: the recent progress. Archives of pharmacal research, 25, 572-584.
- Leisola, M., & Turunen, O. (2007). Protein engineering: opportunities and challenges. Applied Microbiology and Biotechnology, 75(6), 1225-1232.
- Maminov, A. D. (2022). Short-length peptides contact map prediction using Convolution Neural Networks. In 6th International Workshop on Deep Learning in Computational Physics (p. 16).
- McMartin, C., Hutchinson, L. E., Hyde, R., & Peters, G. E. (1987). Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. Journal of pharmaceutical sciences, 76(7), 535-540.
- Peppas, N. A., Bures, P., Leobandung, W. S., & Ichikawa, H. (2000). Hydrogels in pharmaceutical formulations. European journal of pharmaceutics and biopharmaceutics, 50(1), 27-46.
- Raeisi Estabragh, M. A., Bami, M. S., Ohadi, M., Banat, I. M., & Dehghannoudeh, G. (2021). Carrier-Based Systems as Strategies for Oral Delivery of Therapeutic Peptides and Proteins: A Mini-Review. International Journal of Peptide Research and Therapeutics, 27, 1589-1596.
- Renukuntla, J., Vadlapudi, A. D., Patel, A., Boddu, S. H., & Mitra, A. K. (2013).

Approaches for enhancing oral bioavailability of peptides and proteins. International journal of *pharmaceutics*, 447(1-2), 75-93.

- Rezaei, L., Safavi, M. S., & Shojaosadati, S. A. (2019). Protein nanocarriers for targeted drug delivery. Characterization and biology of nanomaterials for drug delivery, 199-218.
- Roberts, M. J., Bentley, M. D., & Harris, J. M. (2002). Chemistry for peptide and protein PEGylation. Advanced drug delivery reviews, 54(4), 459-476.
- Ryan, S. M., Mantovani, G., Wang, X., Haddleton, D. M., & Brayden, D. J. (2008). Advances in PEGylation of important biotech molecules: delivery aspects. Expert opinion on drug delivery, 5(4), 371-383.
- Satyanarayan U, Chakrapani U, (2008) Biochemistry, 3rd Ed., Books and allied (p) Ltd., Kolkata, 2008, 43-44.
- Shaikh, R., Singh, T. R. R., Garland, M. J., Woolfson, A. D., & Donnelly, R. F. (2011). Mucoadhesive drug delivery systems. Journal of pharmacy and Bioallied Sciences, 3(1), 89-100.
- Sinha, V. R., & Trehan, A. (2003). Biodegradable microspheres for protein delivery. Journal of controlled release, 90(3), 261-280.
- Soltero, R. (2005). Oral protein and peptide drug *delivery*. *Drug Delivery*: *Pr*inciples and Applications, 189-200.
- Tuncer Degim, I., & Celebi, N. (2007). Controlled delivery of peptides and proteins. Current pharmaceutical design, 13(1), 99-117.
- Verissimo, L. M., Agnez Lima, L. F., Monte Egito, L. C., de Oliveira, A. G., & do Egito, E. S. T. (2010). Pharmaceutical emulsions: a new approach for gene therapy. Journal of drug targeting, 18(5), 333-342.
- Veronese, F. M., & Pasut, G. (2005). PEGylation, successful approach to *drug delivery. Drug discovery* today, 10(21), 1451-1458.
- Wang, Y., & Selomulya, C. (2020). Spray drying strategy for encapsulation of bioactive peptide powders for food applications. Advanced Powder Technology, 31(1), 409-415.
- Yau, A., Lee, J., & Chen, Y. (2021). Nanomaterials for Protein Delivery in Anticancer Applications. Pharmaceutics 2021, 13, 155.

HOW TO CITE THIS ARTICLE: Adhikari, D., Pokhrel, S. An Overview on Protein and Peptide Drug Delivery System: Advances and Strategies. J. of Drug Disc. and Health Sci. 2024;1(4):239-243. DOI: 10.21590/jddhs.01.04.07