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Review Article

Biotechnology for Prospective Medical Science

Protein and Peptide Based Drug Delivery- A Review

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Abstract: Biologics include vaccines, recombinant proteins, genes, synthetic tissues and viruses, which have emerged from molecular and cellular biology, with respect to unmet clinical needs and expanding indications. Hence it is important at this stage to outline the essential events that have led to the current level of interest in protein and peptide drug delivery systems. Polymers consisting of amino acids that are covalently linked by peptide bonds are known as Proteins and inturn peptides are small proteins composed of few dozen amino acids. Due to large molecular sizes of the proteins ranging from thousands to several millions atomic masses sizes; absorption through the epithelial barriers in the gastrointestinal tract is low. Moreover, proteins are rapidly degraded by digestive enzymes. As the bioavailability by oral route is poor they can be administered by other routes mainly by parenteral (IV, SC, IM) injections. The biological activity of proteins is strongly dependent on their molecular structure i.e 1^0 structure; the amino acid sequence, which ultimately dictates the non-covalent interactions and the 2^0i.e alpha helices and beta helices; the periodic spatial arrangement of the polypeptide chain backbone and 3^0; the 3-dimensional conformation of the whole molecule, including the positions of all amino acid side chains. Some proteins may consist of multiple peptide chains grouped together by non-covalent intermolecular interactions. The arrangement of these subunits relative to each other constitutes the 4^0structure. An alteration at any level of molecular structure leads to a change or loss of biological activity. The present review focuses on the development of various routes of drug administration, formulation as well as stability aspects of protein and peptide drug delivery system.

Keywords: Invasive route; Prodrug approach; Formulation; Degradation; PEGylation.

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1. INTRODUCTION

Proteins are defined as the chains of amino acids which are joined to its neighbor by a specific type of covalent bond. The polymerization of amino acids by peptide bonds forms the structural framework of proteins. The term protein and peptide is used for molecules composed of over 50 amino acids and less than 50 amino acids respectively[1]. Specialized mechanisms for transport across biological membranes are to be considered for the delivery of Proteins and Peptides[2]. Peptide and protein structure is essential to have an idea about the structure in order to deal with various problems encountered while developing drug delivery system[3].

1.1 Need of protein and peptide drug delivery system [4-7].

1. They are very important in biological cells and organic molecules. Proteins can be delivered by invasive and non-invasive routes (Table 1).
2. In the absence of proteins and peptides, disease like Diabetes mellitus is caused due to the lack of protein called INSULIN).
3. In protein and peptide based pharmaceuticals, r-DNA technology and hybridoma techniques are used.
4. An essential role is played by peptides in biotechnological applications as therapeutic and diagnostic agents.

1.2 *Advantages of protein and peptide drug delivery systems* [8-11]

1. For production of RBC, Erythropoietin is used.
2. For heart attack and stroke Tissue Plasminogen Activator (TPA) is used.
3. In the management of labor pain oxytocin is used
4. Bradykinin increases the peripheral circulation
5. Somatostatin decrease bleeding in gastric ulcer
6. Gonadotropin induces ovulation
7. Insulin maintains blood sugar level.
8. Peptides achieved resounding success in drug delivery and in nanomedicine smart applications.

Table 1. Protein and peptide delivery[11]

S.No	Route	Formulation requirements	Commercial products
1.	**Invasive**		
	Direct injection	Liquids or reconstituted solids, syringes.	
	Intravenous (i.v.)		Activase®
	Subcutaneous(s.q.)		Nutropin®
	Intramuscular (i.m.)		RecombiVax®
	Intra cerebral vein (i.c.v)		
	Depot system	Biodegradable polymers, liposomes permeable polymers (not degradable), microspheres, implants.	
2.	**Non invasive**		
	Pulmonary	Liquids or powders formulations, nebulizers, metered dose inhalers, dry powder inhaler.	Pulmozyme®
	Oral	Solids, emulsions, micro particulates, absorption enhancers.	
	Nasal	Liquids usually require permeation enhancers.	Synarel®
	Topical	Emulsions, creams or pastes (liposomes).	
	Transdermal	Electrophoretic (iontophoresis), chemical permeation enhancers, pro drug, ultrasonic.	
	Buccal, Rectal, Vaginal	Gel, suppositories, Bio adhesives.	

1.3 Pharmaceutical approaches in protein peptide delivery system. [11,12,13,14]

The following are the various pharmaceutical approaches

1. Chemical modification
2. Enzyme inhibitors
3. Penetration enhancers
4. Formulation vehicle
5. Mucoadhesive polymeric system

1.3.1 Chemical modification (Prodrug approach) [11]

Due to susceptibility of the peptide backbone to proteolytic cleavage due to their molecular size and complex structures, proteins are labile. A chemical modification of peptide and protein drugs like prodrug formation include olefenic substitution, d-amino acid substitution, dehydro amino acid substitution, carboxyl reduction, retro inversion modification, polyethylene glycol (PEG) attachment to amino group and thio-methylene modification also improves their enzymatic

stability and/or membrane penetration of peptides and proteins. It can also be used for minimizing immunogenicity. Protein modification can be done either by direct modification of exposed side-chain amino acid groups of proteins or through the carbohydrate part of glycoproteins and glycoenzymes[11]

Types of chemical modifications

1. Amino acid modification.
2. Hydrophobization.

1.3.1.1 Amino Acid modification[12]

Individual amino acids modification combined with the substitution of one more L-amino acid with D-amino acids significantly alters the physiological properties. This was demonstrated by vasopressin analogs 1-deamino-8-D-arginine vasopressin (DDAVP) and [Val4, D-Arg8] called desmopressin and arginine-vasopressin (dVDAVP), called deamino vasopressin. Desmopressin involves deamination of the first amino acid and replacement of the last L-arginine with D-arginine, the latter also has the fourth amino acid changed to valine..In Nobex insulin (hexyl-insulin-monoconjugate 2 or "HIM2") a single amphiphilic oligomer is covalently linked to the free amino group on the Lys-29 residue of recombinant human insulin via an amide bond[12], that is intended, on delivery by mouth, to resist degradation by enzymes of the stomach and intestine and to be efficiently absorbed into the bloodstream, also with increased penetration and increased compatibility than parent drug was observed.

1.3.1.2 Hydrophobization[13]

Insulin was conjugated to 1,3-dilpalmitoylglycerol at the free amino groups of glycine, phenylalanine, and lysine to form mono and dipalmitoyl insulin[13] which has facilitated the transfer of insulin across the mucosal membranes of the large intestine and improved its stability against intestinal enzymatic degradation.

1.3.2. Enzyme inhibitors (Protease) [14]

The choice of protease inhibitors depends on the structure of these therapeutic drugs, and the information on the specificity of proteases is essential to guarantee the stability of the drugs in the GI tract[14]. The quantity of co-administered Inhibitor (s) is essential for the intestinal permeability of a peptide or protein drug. The enzyme (protease) inhibitors are the enzymatic approach of the protein and peptide drug delivery systems. GIT and liver play an important role in metabolization of protein and peptides into smaller fragments of the two to ten amino acids with the help of the variety of proteolytic enzymes. These protease inhibitors are co-administered with protein and peptide to alter the environment for the enzyme stability to suppress the proteolytic activity [15]. Protease inhibitors are divided into four types. Aspartic proteases (Pepsin, Rennin), Cystinyl Proteases (Papain, Endopeptidase), Serinyl Proteases (Thrombin, Trypsin), and MetalloProteases (Carboxypeptidase). For example, enzyme degradation of insulin is known to be mediated by the serine proteases trypsin, α-chymotrypsin and thiol metalloproteinase insulin degrading enzymes. The stability of insulin has been evaluated in the presence of excipients that inhibit these enzymes. Representative inhibitors of trypsin and α-chymotrypsin include pancreatic inhibitor and soybean trypsin inhibitor, FK-448, Camostatmesylate and aprotinin[16].Thiomers are promising candidates as enzyme inhibitors in strong reduction of albumin degradation by a mixture of proteases in the presence of carbopol 934P[17]. A subsequent study showed that polycarbophil and carbopol 934P were potent inhibitors of the proteolytic enzymes trypsin, α-chymotrypsin and carboxypeptidase A. As a result of the covalent attachment of cysteine to polycarbophil, the inhibitory effect of the polymer towards carboxypeptidase A, carboxypeptidase B and chymotrypsin could be significantly improved. Another approach to enzyme inhibition is to manipulate the pH to inactivate local digestive enzymes. A sufficient amount of a pH-lowering buffer that lowers local intestinal pH to values below 4.5 can deactivate trypsin, chymotrypsin and elastase[18]. Some of the enzymes and their specific inhibitors are listed in Table 2.

Table 2.Enzymes and their specific inhibitors[13]

Enzymes	Specific inhibitors
Acid protease (e.g.Pepsin, Renin, CathepsinD, Chymosin)	Diazoacetyl DL-norleucine methyl ester;1,2-epoxy-3(Q-nitrophenoxy)propane;Pestatine
Aminopeptidases	Bestain
Aminopeptidase B	Arphanamine
Ca^{2+} activated neural protease	Protease inhibitor
Calpains I and II	Acetyl-leucyl-leucylnorleucinal
Chymotrypsin	Chymostatin;N-Tosyl-L-phenylalaninechloromethyl ketone
Endoprotease	α2-Macroglobulin
Metalloendoproteases	Phosphoramidon

Metalloprotease	Ethylenediamine tetra-acetic acid
Serine proteases (e.g. Elastase;Cathepsin; Trypsin; Thrombin;Kallikrein)	4-Amidinophenyl-methanesulfonyl fluoride Aprotinin 3 4-Dichloroisocoumarine Leupeptin Phenylmethanesulfonyl fluoride
Thiolproteases (e.g.Plasmin;Cathepsin B; Cathepsin L)	Cystain (N-[N(L-3-trans-carboxy-oxiran-2-carbonyl]-L-leucyl-1[-agmatine] Leupeptin
Trypsin	Nα-Q-Tosyl-L-lysine chloromethylketone;Trypsin inhibitor

1.3.3 Penetration enhancers [16]

Penetration enhancers are one of the most important components responsible for the disruption of the mucosal barriers and applicable to improve the membrane permeation of large macromolecular substances like proteins and peptides. Several classes of compounds are mainly used as penetration enhancers such as surfactant (Polysorbate, SLS, Pluronic F-68), Ca^{2+} chelating agent (EDTA), fatty acids (Sodium caprate), mucoadhesive polymeric systems (thiomers, cellulose derivatives), phospholipids (PC). The basic mechanism of penetration enhancers is to increase the transcellular transport of drugs by disrupting the structure of the lipid bilayer rendering the cell membrane more permeable and/or by increasing the solubility of insoluble drugs. The chelators are believed to exert their action by complex formation with calcium ions, thus rupturing the tight junctions (TJs) and facilitate paracellular transport of hydrophilic drugs. Often Ca^{2+} depletion induces global changes in the cells, including disruption of actin filaments, disruption of adherens junctions, and diminished cell adhesion. Some enhancers, including fatty acid sodium caprate and long chain acyl carnitines, have been shown to improve absorption without obvious harmful effects to the intestinal mucosa. Transient opening of TJs would seem less damaging than disruption of cell membrane structure. Several studies on sodium dodecyl sulfate, sodium caprate, and long-chain acylcarnitines shows increased permeability through the paracellular pathways. Example: The mechanism of paracellular transport enhancement by sodium caprate was via phospholipase C activation and upregulation of intracellular Ca^{2+}, leading to contraction of calmodulin dependent actin-myosin filaments and opening of TJs. Dodecylphosphocholine and quillajasaponin, dipotassiumglycyrrhizinate, 18-glycyrrhetinic acid, sodium caprate, and taurine also increases the permeability of hydrophilic compounds across Caco-2 cells.

1.3.4 Formulation vehicles[18]

To overcome acid and luminal proteases as barriers, several formulation strategies have been investigated (Table 3)

1. Lipid vesicles and emulsions
2. Microspheres
3. Liposomes
4. Particulate carriers
5. Aquasomes
6. Mucoadhesive polymeric systems

1.3.4.1 Lipid vesicles and emulsions[18]

These dosage forms have shown great potential in the delivery of proteins and peptides. Before a drug can exert its therapeutic effects, its penetration through the plasma membrane (a lipoid bilayer) or its uptake through carrier systems is a mandatory step. The use of lipid vesicular carrier systems and emulsions have paved the way to circumvent membrane barriers and thereby promoting the uptake of this difficult class of therapeutics. Solid nanospheres and fat emulsions have also been suggested as lymphotrophs to traffic "problem" molecules effectively through lymphatic circulation utilizing typical endogenous lipid digestion and assimilation system especially the chylomicrons. Insulin delivered in liposomes produced better hypoglycaemic effects than the free insulin following oral administration. Water-in-oil-water (w/o/w) emulsion also exhibited significant delivery potential when compared with the plain aqueous solutions. To overcome the physical-chemical instability of emulsions, it is formulated into dry emulsion. Micro-domain of different polarity within the same single phase solution can facilitate solubility of hydrophilic or lipophilic peptide is mainly considered in formulation.

1.3.4.2 Microspheres[17]

Microspheres are discrete spherical particles ranging from 1 to 50 microns that contain dispersed drug in either solution or microcrystalline form and are prepared by various polymerization and encapsulation processes. Biodegradable microspheres of 1:1 copolymer of lactic acid and glycolic acid containing ACTH, poly(d,l-lactide-co-glycolide) microspheres of LHRH,poly (lactide-co-glycolide) microspheres of human serum albumin and poly (d,l-lactide) microspheres of insulin have been tested successfully with promising results. Similar results were obtained with growth hormone and for vaccines based on entrapped antigens and immunomodulators in crystallized carbohydrate spheres. In a recent study, an alginate-chitosan microsphere was developed by membrane emulsification using calcium ion and polymer solidification. The particle size distribution, surface morphology and the zeta potential of the microsphere were studied in detail. While in the basic and neutral environments of the intestine, the complexes dissociated which resulted in rapid microspheres swelling and insulin release. Microspheres can be targeted to a particular organ, a specific part of the organ or to a selective intracellular site. Passive targeting can be achieved by occlusion, cellular uptake or local injection. Eg.Targeting of microspheres to the RES (1-7 μm particles) and to the lung capillaries (7-12μm particles).The microspheres conjugating receptor specific moieties, such as monoclonal antibodies (immunomicrospheres), or incorporating magnetic particles (magnetic immunomicrospheres), or based on a combination of the two.

1.3.4.3 Liposomes[20]

Liposomes consist of phospholipid bilayers (lamellas) which help to encapsulate the proteins or peptides within its lipid core. Liposomes are classified into large unilamellar, small

unilamellar and multilamellar. Some of the advantages of liposomes include long circulation time, high drug loading and can be equipped with targeting ligands. Some of the clinically approved liposomal-based drugs are liposomal amphotericin, liposomal doxorubicin[19]. Liposomes are reported to protect the encapsulated drug/ peptide from oxidation and deamidation. Moreover, liposomes encapsulating drug/peptide are known to be taken by the lymphatic system which drains them directly into the systemic circulation thereby by-passing first pass metabolism[20].

1.3.4.4 Particulate carriers[21]

Nano and microparticles can be employed as oral carriers for peptide and protein delivery. Intact uptake of particles up to 10µm from intestinal wall has been reported. Native surface properties and chemical composition of the carrier nanoparticles are crucial in determining the extent of uptake. Eg: Luteinizing hormone releasing hormone (LHRH) was prepared by conjugating it with vinylacetic acid which was then co-polymerized with n-butylcyanoacrylate (n-BCA) and a radiolabel. The reaction conditions were manipulated to exploit the particle forming properties of n-BCA.

1.3.4.5 Aquasomes[23]

Aquasomes are self-assembling nanoconstructs comprising of a solid ceramic core and a glassy polyhydroxyoligomeric surface coating. Water is a vital requirement for maintaining structural conformation of proteins and their biological activities. A variety of environment changes such as pH, temperature, tonicity and solvents can cause protein inactivation when in aqueous state leading to irreversible protein inactivation. Aquasomes appear to enable them to preserve biological molecules as well as to act as delivery vehicle. Proteins are more stable in the solid state, however, dehydration, the loss of water molecules is critical in maintaining the molecular shape and activity. It follows that in order to maintain structural integrity as well as the activity awell balanced mini environment should be contrived so that even on drying a minimum required aqueous domain in immediately vicinity is maintained. Aquasomes are special systems which while dry and in the solid state, exhibit water-like properties that enables the molecules to stabilize the 'aqueous conformation'. Since they are based on ceramic materials coated with polyhydroxyl-oligometric substances with inherent aqueous properties (bound water) can successfully be utilized for immobilization of susceptible protein and peptide molecules into their well defined interior or through adsorption phenomenon.

1.3.5 Mucoadhesive polymeric systems[23]

Mucoadhesive polymeric systems are important to prevent the problem associated with presystemic metabolism or first pass metabolism and maintain its therapeutic efficacy[22]. Eg: Thiomers, polyacrylic acid derivatives and cellulose derivatives. The stronger mucoadhesive properties of thiomers are believed to be based on covalent bonds between thiol groups of the thiomer and cysteine- rich domains of mucus glycoproteins. (Higher amount of thiol groups is responsible for the stronger mucoadhesive properties).

Table 3.Various pharmaceutical approaches and their outcomes[20]

S.No	Approaches	Outcomes
1.	Chemical modification	
a.	Amino acid modification	Improves enzymatic stability.
b.	Hydrophobization	Improves membrane penetration.
2.	Enzyme inhibitors (Protease)	Resist degradation by enzymes, present in stomach and intestine.
3.	Absorption enhancers	Increases membrane permeability.
4.	Formulation vehicles	
a.	Emulsions	Protects drug from acid & luminal proteases in the GIT, enhance permeation through intestinal mucosa.
b.	Microspheres	Prevents proteolytic degradation in the stomach & upper portion of the small intestine. Restricts release of drugs to favorable areas of GIT.
c.	Liposomes	Improves physical stability, increases membrane permeability.
d.	Nanoparticles	Prevents enzymatic degradation, increases intestinal epithelial absorption.
5.	Mucoadhesive polymeric system	Achieve site –specific drug delivery, improves membrane permeation.

1.4 Incorporation into drug delivery matrix[23 24,25,26]

Incorporation of drug in the protein and peptide drug delivery system undergoes three methods such as

1. Emulsification
2. Extrusion and spray drying
3. Polymerization.

In all these processes, it is highly emphasized that high stress, high temperature, heat and crosslinking agents must be avoided (or minimized), to ensure the stability during the formulation. As proteins are more stable in solid state than in liquid, its incorporation in solid form in the delivery matrix is advantageous. Spray drying and lyophilization are widely used for formulation of protein and peptide delivery systems.

1.5 Site-specific protein modification (protein engineering)[25]

This approach is being used to improve the stability and specificity of endogenous proteins, in addition, they improve the selectivity and have a prolonged delivery at the active site. One such approach is deletion mutants which is being extensively studied by genetic engineering and cloning of tPA gene and its subsequent expression in eukaryotic cells. Another approach towards site-specific protein modification is the hybrid proteins. Hybrid proteins have the combination or re-ordered features of one or more proteins as well as their effector functions, protection and recognition properties. These site-specific hybrid proteins can be produced by synthetically linking protein fragments or by using ligated gene fusion process. A list of hybrid proteins is given in Table 4.

Table 4 Hybrid protein delivery systems[27]

Fragments of recognition portion	Effector portion
Gene Fusion Products	
Interleukin 2	Diphtheria toxin
Growth factor	Toxin (e.g. ricin A)
Cell-specific polypeptide (α/β MSH; substrate P)	Restructured diphtheria toxin
Antitumor Fab immunoglobulin	Fragment A of diphtheria toxin
CD4	Pseudomonas exotoxin
	γ-interferon and β-tumor necrosis factor
Chemical Linkage Of Fragments/Proteins	
Human placental lactogen hormone	Diphtheria toxin A chain
β chain of h chorionic gonadotropin hormone	Ricin A chain
Insulin	Diphtheria A
Epidermal growth factor	Ricin A
Antibody fragments	Deglycosylated ricin A
IgG (2a) fragments	Gelonin
	Diphtheria toxin
	Pseudomonas toxin
Anti-collagen antibody	Toxin
HIV-specific Ab	Ricin A
AntifibrinAb	Tissue Plasminogen activator
Anti-T-cell antibody	Ricin A chain
Antibody fragments	Gelonin toxin
Anti-endothelial IgG	Glucose oxidase
Anti-epithelial Ig/fragments	Ricin and other toxins
Anti-sIgM-IgM	Saporin-6

1.6 AdvancedPEGylation[27]

Poly Ethylene Glycol (PEG) is non-toxic and has been approved by the FDA for use in foods, cosmetics, and pharmaceuticals. PEG polymers can be linear or branched in shape, and can be engineered in a variety of molecular weights. Studies on PEG solution show that each ethylene glycol subunit is tightly associated with two or three water molecules. A binding process with water makes PEGylated compounds function as though they were 5 to 10 times larger than a corresponding soluble protein of similar molecular weight. Further, the PEG polymer with associated water molecules is very mobile, and acts like a shield to protect the attached drug from enzyme degradation and interactions with cell surface proteins, and provides increased size to prevent rapid renal filtration and clearance[27].Advanced PEGylation, which involves modification of protein, peptide, or non-peptide (drug or therapeutic protein) by attaching with specific PEG polymer chains, is a proven method for enhancing the potentials of peptides and proteins as therapeutic agents. The advantages of advanced PEGylation for therapeutic molecules includes enhanced bioavailability, decreased dosing frequency, due to prolonged residence in the body, a decreased degradation by metabolic enzymes,

optimized pharmacokinetics, increased efficacy, improved safety profile, a reduction or elimination of protein immunogenicity, improved drug solubility, and stability to hydrophobic drugs and proteins[28]. Prodrugs are also prepared by this advanced PEGylation technique. During biotransformation, active drugs are released by degradation of more complex molecules (prodrugs) under suitable physiological conditions, providing an efficient method of drug delivery. The advanced PEGylation technology also offered new opportunities for creating viable peptides and protein drugs by site-specific PEGylation[29].Eg: Coupling certain PEG reagents to protein thiol groups on cystines may offer advantages, as cystines are typically less abundant in proteins than other polymer attachment sites, such as amino groups, resulting in more selective PEGylation of the target protein. Greater selectivity allows greater control over the resulting PEG-conjugate in both the number of attachment sites and the position of the attachment, by reducing the likelihood of protein deactivation upon conjugation. In addition to minimizing loss of biological activity, site-specific PEGylation can also reduce immunogenicity. Thiol groups may be naturally occurring or the biomolecule may be modified or engineered to contain a thiol group suitable for conjugation.

1.7 Applications[28]

1. CVS acing drugs protein and peptides
2. Angiotensin 2 antagonists, Bradykinin, Captopril
3. CNS active protein and peptides.
4. Cholecystokinin, B-endorphin, GI-active protein and peptides
5. Gastrin antagonist, pancreatic enzymes
6. Immunomodulation of the protein and peptides
7. Bursin, Cyclosporine, and Interferon
8. Metabolism modulating protein and peptides
9. Insulin, Vasopressin.

Some of the marketed products of proteins and peptides are listed in Table 5.

Table 5.Marketed products of proteins and peptides[28]

S.No	Drug	Brand	Route	Use
1.	Insulin	Humulin®, Novolin®	Parenteral	Diabetes mellitus
2.	Captopril	Capoten	Oral	Anti hypertensive
3.	Enfurvitide	Fuzeon	Parenteral	Anti viral
4.	Streptokinase	Streptase	Parenteral	Thromboembolism
5.	Oxytocin	Pitocin	Parenteral	Induction of labor

2. CONCLUSION

Advances in Pharmaceutical Biotechnology have led to numerous and large scale production of therapeutic agents among which proteins and peptides. Due to enzymatic degradation of GIT and first –pass hepatic metabolism, these proteins and peptides i.e active biopharmaceuticals are mainly administered by various routes. Hence even though a rapid progress in molecular biology has occurred, there has not been matched by progress in the formulation and development of protein and peptide drug delivery systems. This can be due to various properties including physical, chemical, and biological of proteins and peptides on routes of delivery as well as on delivery system and formulation. Innovative technologies have been developed over the past few years for promoting absorption across GIT.

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4. AUTHORS CONTRIBUTION STATEMENT

Dr.P.V.Kamala Kumari gathered details of manuscript and contributed to writing the manuscript regarding this work. Dr. S. Satya Lakshmi gave the detailed information regarding the applications of the present review. Ms M.Yamini contributed for the collection of information from various books. Dr.Y.Srinivasa Rao analyzed these data and necessary inputs were given towards the designing of the manuscript.

5. CONFLICT OF INTEREST

Conflict of interest declared none.

6. REFERENCES

1. Ratnaparkhi MP, Chaudhari SP, Pandya VA. Peptides and proteins in pharmaceuticals. Int J Curr Pharm Res. 2011;3(2):1-9.
2. Gilad Y, Firer M, Gellerman G. Recent innovations in peptide based targeted drug delivery to cancer cells. Biomedicines.2016;4(2):11. doi:10.3390/biomedicines4020011, PMID 28536378.
3. Bruno BJ, Miller GD, Lim CS. Basics and recent advances in peptide and protein drug delivery. TherDeliv.2013;4(11):1443-67. doi: 10.4155/tde.13.104, PMID 24228993.

4. Maloney CM, Kesner RK, Klein G, Bockenstette J. The rectal administration of MS Contin: clinical implications of use in end stage cancer. AmJHosp Care.1989;6(4):34-5.
doi: 10.1177/104990918900600409, PMID 2604981.
5. Babul N, Darke AC, Anslow JA, Krishnamurthy TN. Pharmacokinetics of two novel rectal controlled-release morphine formulations. J Pain SymptomManag.1992;7(7):400-5.
doi: 10.1016/0885-3924(92)90019-e, PMID 1484193.
6. Warren DE. Practical use of rectal medications in palliative care.J Pain SymptomManag.1996;11(6):378-87.
doi: 10.1016/0885-3924(96)00012-7, PMID 8935142.
7. Tesauro D, Accardo A, Diaferia C, Milano V, Guillon J, Ronga L, Rossi F. Peptide-based drug-delivery systems in biotechnological applications: recent advances and perspectives. Molecules.2019; 24(2):351.
DOI: 10.3390/molecules24020351
8. Schaafsma G. The protein digestibility–corrected amino acid score. J Nutr.2000;130(7):1865S-7S.
doi: 10.1093/jn/130.7.1865S, PMID 10867064.
9. Sellmeyer DE, Stone KL, Sebastian A, Cummings SR. A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Study of Osteoporotic Fractures Research Group.AmJ ClinNutr.2001;73(1):118-22.
doi: 10.1093/ajcn/73.1.118, PMID 11124760.
10. Di Vincenzo R. 5 Nutritional therapies. Complementary therapies and the management of diabetes and vasculardisease: Amatter of balance. 2006;77-124.
11. Wang J, Hu X, Xiang D. Nanoparticle drug delivery systems: an excellent carrier for tumor peptide vaccines. Drug Delivery. 2018;25(1):1319-27.
Doi: 10.1080/10717544.2018.1477857
12. Kipnes M, Dandona P, Tripathy D, Still JG, Kosutic G. Control of postprandial plasma glucose by an oral insulin product (HIM2) in patients with type 2 diabetes. Diabetes Care. 2003;26(2):421-6.
doi: 10.2337/diacare.26.2.421, PMID 12547873, Google Scholar.
13. Hashimoto M, Takada K, Kiso Y, Muranishi S. Synthesis of palmitoyl derivatives of insulin and their biological activities. Pharm Res. 1989;6(2):171-6.
doi: 10.1023/a:1015992828666, PMID 2668914, Google Scholar.
14. Bernkop-Schnürch A. The use of inhibitory agents to overcome the enzymatic barrier to perorally administered therapeutic peptides and proteins. J ControlRelease.1998;52(1-2):1-16.
doi: 10.1016/s0168-3659(97)00204-6, PMID 9685931, Google Scholar.
15. Kim IS, Lee SK, Park YM, Lee YB, Shin SC, Lee KC, Oh IJ. Physicochemical characterization of poly (L-lactic acid) and poly (D, L-lactide-co-glycolide) nanoparticles with polyethylenimine as gene delivery carrier.IntJ Pharm. 2005Juil14;298(1):255-62.
doi: 10.1016/j.ijpharm.2005.04.017, PMID 15941631.
16. Soltero R, Soltero R. Oral protein and peptide drug delivery. Drug DelivPrinc Appl. 2005:189-200.
17. Hutton DA, Pearson JP, Allen A, Foster SN. Mucolysis of the colonic mucus barrier by faecal proteinases: inhibition by interacting polyacrylate. ClinSci (Lond). 1990;78(3):265-71.
doi: 10.1042/cs0780265, PMID 2156646.
18. Bernkop-Schnürch A, Krauland AH, Leitner VM, Palmberger T. Thiomers: potential excipients for non-invasive peptide delivery systems. EurJ Pharm Biopharm. 2004;58(2):253-63.
doi: 10.1016/j.ejpb.2004.03.032, PMID 15296953.
19. Zhang Y, Wei W, Lv P, Wang L, Ma G. Preparation and evaluation of alginate–chitosan microspheres for oral delivery of insulin. EurJ Pharm Biopharm. 2011;77(1):11-9.
doi: 10.1016/j.ejpb.2010.09.016, PMID 20933083.
20. Sharma A. Liposomes in drug delivery: progress and limitations. IntJ Pharm. 1997;154(2):123-40.
doi: 10.1016/S0378-5173(97)00135-X.
21. PorterCJH, Charman WN. Uptake of drugs into the intestinal lymphatics after oral administration.AdvDrugDelivRev.1997;25(1):71-89.
doi: 10.1016/S0169-409X(96)00492-9.
22. Hussain N, Florence AT. Utilizing bacterial mechanisms of epithelial cell entry: invasin-induced oral uptake of latex nanoparticles.PharmRes.1998;15(1):153-6.
doi: 10.1023/a:1011981610840, PMID 9487564.
23. Savale SK. Protein and peptide drug delivery system. World J Pharm PharmSci.2016;5(4):1-9.
24. Chen H, Chang X, Du D, Liu W, Liu J, Weng T, Yang Y, Xu H, Yang X. Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. J ControlRelease.2006;110(2):296-306.
doi: 10.1016/j.jconrel.2005.09.052, PMID 16325954.
25. Rolland A, Wagner N, Chatelus A, Shroot B, Schaefer H. Site-specific drug delivery to pilosebaceous structures using polymeric microspheres. PharmRes.1993;10(12):1738-44.
doi: 10.1023/a:1018922114398, PMID 8302759.
26. Vogt A, Combadiere B, Hadam S, Stieler KM, Lademann J, Schaefer H, Autran B, Sterry W,

Blume-Peytavi U. 40 nm, but not 750 or 1,500 nm, nanoparticles enter epidermal CD1a+ cells after transcutaneous application on human skin. J InvestDermatol.2006;126(6):1316-22.
doi: 10.1038/sj.jid.5700226, PMID 16614727.

27. Goodson RJ, Katre NV. Site-directed pegylation of recombinant interleukin-2 at its glycosylation site.Biotechnology (N Y).1990;8(4):343-6.
doi: 10.1038/nbt0490-343, PMID 1366535.

28. Cho K, Wang XU, Nie S, Chen ZG, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. ClinCancerRes.2008;14(5):1310-6.
doi: 10.1158/1078-0432.CCR-07-1441, PMID 18316549Ihttps://doi.org/10.1038/aps.2013.27.

29. Calceti P, Salmaso S, Walker G, Bernkop-Schnürch A. Development and in vivo evaluation of an oral insulin–PEG delivery system. EurJPharmSci.2004;22(4):315-23.
doi:10.1016/j.ejps.2004.03.015, PMID 15196588.