

<https://doi.org/10.56770/jcp2025919>**RETROSPECTIVE REVIEW ON ORAL DELIVERY OF PROTEINS AND PEPTIDE: BARRIERS, STRATEGIES AND CLINICAL APPLICATIONS****Nabila Abdus Salaam¹, Shaheen Kausar^{2*}** ¹Faculty of Pharmacy, Government College University Faisalabad, Punjab, Pakistan.²Department of Biosciences, COMSATS University Islamabad, Sahiwal Campus Pakistan.

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ABSTRACT

Proteins and peptides have gained the attention of researcher in recent era for the cure of many disorders. Proteins possess complex architecture as they formed from amino acid subunits. Challenges associated with the biotherapeutics delivery like higher molecular weight, enzymatic susceptibility, less permeability across the biological membranes and complex architecture are crucial which make them less selected candidate for research and therapy through noninvasive route but these challenges could be overcome by using the different type approaches either formulation based or novel nanocarrier based technology. Review summarized the various types of barriers, challenges associated with biotherapeutics delivery as well as approaches to overcome these barriers for oral delivery of biotherapeutics.

Keywords: Retrospective Review, Oral Delivery, Proteins and Peptides, Challenges.***Corresponding Author.** E-mail: nshaheen1992@hotmail.com**INTRODUCTION**

From last three decades proteins and peptides have diverted the attention of researchers towards their use as a therapeutic for the treatment of various kinds of disorders which are associated due their deficiency in human being. With the recent advancement in technology and science use of proteins and peptide as therapeutic remedy has been extended.

Using the wide variety of techniques one can synthesize and purify protein of interest. Recombinant technology has promising result regarding proteins synthesis and purification at a low cost which is being further used for the treatment of different kind of diseases. Proteins and peptides are used for different types of acute or chronic disorder for example cardiovascular, hormonal, metabolic, autoimmune, hepatic and central nervous system [1]. Economic forecast has suggested that these biopharmaceuticals will occupy the major area of pharmaceutical industry [2]. Currently over 160 proteins based products are in market and hundreds are in different phases of clinical trials by researchers. Protein drug market makes up more than 30 billion with expected rise of 10 percent every year [3].

STRUCTURE OF PROTEINS

Proteins are the basic building block of our body

which are composed of amino acid, that join together to form long chain peptide consisting of 50 amino acids [4]. Proteins are macromolecules with complex architecture. Proteins and peptides are seldom linear or folded together to form secondary, tertiary and quaternary structure or conformation [5].

Primary structure involves linear chain of amino acids, in secondary structure amino acids are arranged to form alpha or beta sheet/helix. Tertiary structure consist of folded chains of amino acids to form three dimensional structure while in quaternary structure three dimensional pattern is well organized in a complex manner [6].

Hydrogen bonding, disulphide linkage covalent bonding and non-covalent link ensure the stability of proteins structure. Proteins are specific in their function which depends upon its structures. Any disruption in structure mean disturbance in its function; structural defects in proteins causes various types of diseases for example neurodegenerative disease such as Alzheimer's disease Parkinsonism and Cow disease. If there is correct knowledge of protein folding then one can identify as well as design a drug delivery system for that disease. Peptide based synthetic agent now a days in research used as chemotherapeutic agent due to their



biological and antitumor efficacy [7,8].

Classification of Protein

Proteins are classified by the function e.g. enzymes, hormones, antibodies and receptors, by location into living cells e.g. membrane proteins, internal proteins, and external proteins and by post translational modifications e.g. native or glyco proteins. On the basis of conformation proteins are classified as a fibrous protein and non-fibrous or globular proteins. In fibrous proteins polypeptides chains are arranged into parallel direction to form the sheets or fibers. They are water insoluble e.g. elastin, collagen and keratin. Globular proteins have tightly packed chains of polypeptide to form globular or spherical shape. They are water soluble and used as pharmaceuticals [9].

Stability of Proteins

Proteins and peptides are highly instable susceptible molecules that undergoes to the physical and chemical degradation due to the changes in environmental or storage conditions like temperature, pH, buffer, salt type or cosolvent. Instability may be physical or chemical. Physical instability involves absorption, precipitation, denaturation and aggregation. Chemical instability involves redox reaction, disulphide link exchange, racemization and proteolysis [6]. Protein and peptide portfolio has attracted attention of many researchers as therapeutic agent for the cure of different types of disease.

ROUTES FOR PROTEIN AND PEPTIDE DELIVERY

Parenteral Route

Owing to short half-life proteins are mostly delivered by parenteral route as they are susceptible to degradation by gastric enzymes and pH. Parenteral route includes intramuscular, intravenous, and subcutaneous. Major complication of parenteral route include thrombophlebitis, tissue necrosis and in some case immunogenicity. Parenteral route lack the facility of self-administration as it requires a trained person for the administration of drug. This situation is worsened when patient is on protein and peptide therapy in case of chronic disease [10]. Parenterally administered protein and peptide include insulin, vasopressin, octreotide, growth hormone and calcitonin.

Oral Route

Oral route is more preferable route for the delivery of drugs as it is non-invasive. Moreover patient compliance is more for drug therapy in case of oral route. It has advantage of self-administration, no pain and thrombophlebitis unlike the parenteral route. Most of the proteins and peptides has a problem of being degraded by enzymes in gastrointestinal tract and decreased permeability through the intestinal mucosa as it allow only selective molecules to pass

so drug stability is reduced when administered by oral route as compared to parenteral route in which drug bypass all the barriers of gastrointestinal tract [11]. GIT is the mainly responsible for the digestion, uptake of nutrients, fluids and electrolyte and protection from various kind of toxin, pathogens and antigens thus enhances immunity. Drug administered through oral route have to face the barrier function of GIT before their availability at site of action. Barrier property of GIT is may be physical or biochemical by nature that reduces the bioavailability of proteins and peptide drugs, a major limiting factor in designing oral drug delivery system for proteins and peptides. Focus of recent research is on designing the oral drug delivery system for proteins and peptides with improved bioavailability.

BARRIER TO ORAL DELIVERY OF PROTEINS AND PEPTIDES

Physical Barrier

Unstirred Water Layer

Human intestine is covered of columnar epithelial cells which are joined at the apical surfaces to form tight junctions [12]. Whole epithelial layer is covered by thin layer of mucus, water and glycocalyx. Mucus itself is composed of glycoprotein known as mucin, salt, water, and surfactant. Mucus acts as barrier for the orally administered macromolecules i.e. proteins and peptides due to interaction of mucin with other component of mucus and unstirred water layer.

Intestinal Epithelial Cell Membrane

Intestinal epithelium composed of single layer of columnar epithelial cells which further includes enterocytes, goblet, paneth and endocrine cells. Drug will be passed through either passive diffusion, carrier mediated transport or vesicular transport mechanism. Plasma membrane is semipermeable membrane of lipid bilayer allows the selective molecules to pass through it. Lipophilic molecules can easily pass the plasma membrane via passive diffusion while restricting the movement of charged large size and hydrophilic molecules also of proteins (Hochman and Artursson, 1994). In general plasma membrane restrict passage of large size proteins and peptides but there are some proteins that passes through the plasma membrane like cyclosporine and desmopressin after their oral administration [13].

Tight Junction

Paracellular pathway is entry restricted pathway because of tight junction which lacks the proteolytic activity thus gaining the attention of researcher for delivery of proteins and peptides [14]. Tight junction is occluding junction made of transmembrane and cytosolic protein which interact with membrane, cytoskeleton, and each other. Tight junction allows transport of the selective molecules like certain ions,

drugs, nutrients due to presence of fenestrae/pore thus acting as both gate and fence [15]. The gate function of the tight junction is responsible for the controls diffusion of solutes through the paracellular route, whereas the fence function is to maintains polar distributions of the plasma membrane. Apical and basolateral membrane separation ensure the unidirectional transport of molecules across the membrane.

Efflux Pump

Other than tight junction, stirrer water layer efflux pump located in cell membrane also contribute to barrier property for protein and peptide delivery. P-glycoprotein (Pg) is efflux pump located inside the cell membrane and expel out the proteins and peptides thus reduces their bioavailability. Originally it was discovered as membrane bounded multi drug resistance transporter expressed in cancer cell but is also expressed in normal tissues and cell like liver, intestine, kidney and placenta. Different agent with diverse structure and function acts as substrate, inducers and inhibitor of Pg. It is also believed that Pg increases the access of CYP3A enhancing the drug metabolism [16].

Biochemical Aspects

Acidic environment, various types of metabolic enzymes and microbial flora leads to instability and degradation of drug. Enzymatic barrier is the major obstacle to protein and peptide delivery through oral route as they have specific features and characteristics. For example proteolytic enzymes are responsible for the degradation of proteins and peptides in food or even in drugs form. They degrade the proteins and peptide at more than one peptide linkage [17]. Microflora in GIT especially in colon secretes enzymes that cause the degradation of drug as they are capable of reaction such as glucuronidation, oxidation, reduction, esterification, decarboxylation and amide hydrolysis. Enzyme e.g. xanthine oxidase kinase are responsible for the proteolysis of dietary protein into small subunit via hydrolytic cleavage. Proteolysis process starts from stomach; continue throughout the whole GIT. Pepsin enzyme starts the proteolysis. Pancreatic proteases such as serine endopeptidases trypsin, α -chymotrypsin, elastase, and the exopeptidases carboxypeptidase A and B leads to luminal degradation after their release from pancreas to luminal region of intestine[18]. Variability in pH leads to pH dependent hydrolysis of proteins and peptides as in stomach environment is highly acidic while in intestine pH is basic.

CHALLENGES FOR ORAL DELIVERY OF PROTEINS AND PEPTIDES

As far as physiochemical properties of proteins and

peptides are different which lead to development of different type of drug delivery system such as oral, nasal, ocular, transdermal, pulmonary, parenteral and vaginal. Following are the challenges which draw the attention of researchers for delivery of proteins and peptides as a therapeutic agent.

1. Proteins and peptides are biomolecules with the higher molecular weight, size and have both hydrophilic and hydrophilic domain in a single molecule which render their partition across the various biological membrane and intestinal mucosa. log P for proteins and peptides is less than 5 indicating these are hydrophilic by nature.
2. Most of the therapeutic proteins and peptides tertiary structure which get deactivated with the change in physical and chemical conditions results in loss of specific biological activity of protein and peptide making it unstable.
3. Proteins and peptides possess short half-life due to degradation or deactivation by proteolytic enzymes and pH.
4. Various drug delivery systems have been designed to minimize the proteolytic degradation and to increase their stability into intestine such enteric coated formulation delaying the absorption of drug from formulations. These may result in altered microflora in G.I.T that produces many useful products such as vitamin K performs which leads to blood clotting.
5. Proteins and peptides give rise to immunogenic reaction. Some of the proteins and peptides after their administration in body give rise to immunological response which causes neutralization of respective protein and peptide and in some cases severe reaction in recipient. Recombinant technology has solved this issue.
6. Pharmacoeconomics of proteins and peptides production bears a high cost value due to use of various advance and intermediate technologies required for their production [19].

APPROACHES FOR ORAL DELIVERY OF PROTEINS AND PEPTIDES

For effective oral delivery of proteins and peptides various approaches are under consideration which may include either chemical modification such as prodrug approach, structural modification, peptidomimetics and targeting the receptors, tissues and transporters or formulation strategies such as absorption enhancers, enzyme inhibitors, targeted delivery and carrier system. Basic purpose of effective oral protein and peptide drug delivery system is to increase the bioavailability for cure of an alignment and basic aim of these approaches is to improve the pharmacokinetic and pharmacodynamics of delivered protein and peptide drug.

Chemical Modification

Prodrug Approach

Prodrug is therapeutically inactive compound that after oral administration into body after biotransformation converted into active metabolite capable of initiating therapeutic response. It will increase the bioavailability, permeability, solubility and also targeting of drug with a limitation of structural complexity, stability and technologies required [20, 21]. A drug could be conjugated with another molecule that will convert it into prodrug e.g. biotinylated acyclovir is more bioavailable on MDCK-MDR1 and Caco-2 cells as compared to non-biotinylated acyclovir. Size control is major drawback of this lipid raft system [22]. Enkephaline (Leu5) is chemically modified using to form prodrug that showed greater bioavailability due to increased permeability in Caco-2 cell line. Likewise prodrug of cyclic kyotrophin exhibit increased permeability in rat small intestine [23, 24].

Structural Modification

Another approach is to modify the structure of concerned protein or peptide. It may involve the modification by attaching polyethylene glycol, acylation, analogue formation; this PEGylation approach enhances the oral bioavailability of proteins and peptide. PEGylation not only increases the solubility of drug but also protect the drug from enzymatic degradation [25]. Some of the chemical modification involves exploiting the carbohydrate component (glycoprotein) attached with protein or side chain of protein. PEGylated insulin showed enhanced bioavailability as compared to non-PEGylated insulin [26].

Strategies for Formulation Development

Formulation based Strategies aimed to improve bioavailability as well as stability of protein and peptide drug including the absorption or permeation enhancers, mucoadhesive polymeric system and use of enzyme inhibitors. Permeation enhancers increase the permeability of drug across the biological membranes, mucoadhesive system swell up due to changes in environmental factors like pH and enzyme inhibitors provide against the proteolytic degradation in GIT thus resulting in increased stability of drug candidate [27, 28].

Enzyme Inhibitors

Proteins being macromolecules are not absorbed in biological membranes owing to their large size, so they are subjected to breakdown process by proteolytic enzymes which after proteolysis convert these macromolecules to simple and small sized molecules that are easily absorbed. Either endopeptidase or exopeptidase causes the cleavage of amide bond in amino acid chain. Enzymes are specific in action. Various enzyme inhibitors inhibit

the enzymes responsible for the degradation of proteins and peptide after oral administration; thus providing ultimate protection against proteolytic degradation to proteins and peptide drug [29].

Trypsin or α -chymotrypsin inhibitors, such as pancreatic inhibitor soybean trypsin inhibitor, FK-448, camostatmesylate and but administration of such types of protease inhibitors for long duration results in the deficiency of these enzymes in humans. Polymethacrylate based microparticles of insulin for oral delivery were prepared by coprecipitation method. Dissolution studies were performed in the presence of new class of enzyme inhibitors chicken and duck ovomucoids in the presence of trypsin and chymotrypsin. It was observed that this new class of enzyme provides 100% protection against the added trypsin and chymotrypsin (Yamamoto et al., 1994). A drug-carrier matrix has been developed which protects embedded insulin from degradation by the lumenally secreted serine-proteases trypsin, chymotrypsin and elastase in vitro. Mucoadhesive polymer like sodium carboxymethylcellulose was added in formulation. Inhibitory effect of enzyme inhibitors was studied and observed that even after four hour of incubation, 33% of insulin was stable. One drawback of protease inhibitors is that they induce severe toxic effect if administered for long time and also alter absorption of other protein.

Permeation Enhancer or Absorption Enhancers

As the name indicates they enhance the permeation of an agent across the biological membrane. In case of protein and peptides absorption enhancers increase the permeability by increasing the permeability of tight junction in reversible manner. Detergents, surfactants, chelating (calcium) agent, fatty acids, chitosan and its derivatives and bile salt comes under the categories of permeation enhancers [30, 31].

A novel class of toxins name Zonula Occludens toxins (ZOT) are produced from *Vibrio cholera* that causes residual diarrhea in human. These toxins also increase the membrane permeability of small intestine by altering the structure of intracellular tight junction. In vivo studies using IEC6 cell line were conducted using ZOT. Intestinal epithelium is major barrier for absorption of for protein and peptides when administered through oral route as it limit their absorption due to complex biochemical structure. ZOT alter the permeability of tight junction and allows the passage of proteins and peptide drugs through paracellular pathway. The insulin and immunoglobulin co administered with ZOT showed more increase in permeability of rabbit intestine for insulin and immunoglobulin. ZOT alter rabbit intestinal permeability in reversible way. Bioavailability of insulin was enough to control level of glucose in diabetic rats. This study provided

innovative strategy drug delivery system for oral insulin delivery.

Thiolated polymers, Chitosan and its derivatives and Pz-peptide have also studied as penetration enhancers for oral insulin delivery, showed promising results in reducing glucose level. Bioadhesive chitosan-EDTA conjugate protects leucine enkephalin from degradation by aminopeptidase. *N*-[8-(2-hydroxy-4-methoxy)benzoyl]amino caprylic acid (4-MOAC) act as absorption enhancer of parathyroid hormone followed by oral delivery for treatment of osteoporosis. 4-MOAC acts as delivery agent resulting in increased absorption of other macromolecules like proteins and peptides. Studies claimed that such molecules that alter the conformation of proteins in a reversible manner and provide their transport across mucosa [32].

Common but major drawback of permeation enhancers is that they damage or dissolve the plasma and nuclear membranes leading to inflammation if used for long time period [33]. Surfactant are agent which lowers the interfacial tension between two phases but when they are incorporated into formulation they tend to disrupt lipid bilayer of plasma membrane and increase the absorption of drugs through transcellular pathway. Chelating agent rupture tight junction after forming complex with calcium ions thus promoting paracellular transport while fatty acid facilitate paracellular transport of molecules due to transient opening of tight junction.

Mucoadhesive Polymeric Systems

Mucoadhesive polymers exhibit swelling behavior due to changes in network structure in response to

external stimuli like pH, ionic strength, temperature and electric field. Basically they increases the residence time of drug in GIT which in turn increase the absorption of drug due to prolong contact time with intestinal epithelium. Mucoadhesive polymers increase the intestinal absorption of proteins e.g. carbomer and chitosan increases the transepithelial absorption of peptide drug through paracellular transport mechanism [33, 34]. Carbomer 934P and chitosan HCl are promising excipients as they have ability to increase per oral absorption of peptide drug buserelin; gonadotropin-releasing hormone agonist in rats.

Mucoadhesive conjugated system has been designed which ensured the controlled release of both drug and inhibitor. This system provides efficient protection to drug. For insulin most common approach is to encapsulate the drug using mucoadhesive polymer like chitosan, PLGA, thiolated polymer and alginate which have been extensively studied. Chitosan is biodegradable and biocompatible polymer of natural origin has been used to form oral gel of TGF- β in buccal mucosa. Chitosan increase the permeability of drug upto 6-7 fold in buccal mucosa for peptide drug. Insulin loaded mucoadhesive microparticles having poly methacrylic acid and poly ethylene glycol exhibiting mucoadhesive and pH dependent behavior. Small sized microparticles showed greater hypoglycemic effect due to burst release of insulin followed by rapid absorption as compared to large size microparticles and more mucoadhesive capacity to jejunum and ileum [35].

Table 1: In-vitro studies for permeation enhancer effects.

Agent	Model	Mechanism of absorption enhancement	Toxicity
Na lauryl sulfate	Caco-2	-	Morphology changes
Dioctylsodium sulfosuccinate	Caco-2	-	Morphology changes
Na caprate	Caco-2	Paracellular	Decreased Trypan blue exclusion, increased protein release & DNA staining
Deoxycholate	Caco-2	Paracellular Transcellular	Decreased Trypan blue exclusion, increased protein release & DNA staining
Glycocholate Taurodi hydrofusidate	Caco-2	Paracellular	Decreased Trypan blue exclusion
Saponin, DS-1	Caco-2	Transcellular	-
Palmitoylcarnitine	Caco-2	Paracellular	Increased DNA staining
Palmitoylcarnitine	Rat colon	Paracellular	Minor morphology changes

Table 2: Carrier systems for delivery of proteins and peptide

Drug	Carrier	Model
Insulin	Nanocubicals	STZ induced diabetic rats
Insulin, calcitonin, HGF (Granulocyte colony stimulating factors)	Nanocapsules	-
Salmon calcitonin	PLGA nanopartilces	Rats in vivo
Insulin	Acrylic based copolymers nanoparticles	Streptozocin induced diabetic rats
Cyclosporine	Lipid microemulsion	Rats in vivo
Leucine encephalin	Sugar coupling with cellobiose and gentiose	-
Insulin	Chitosan nanopartilces	Alloxan induced diabetic in rats
desglycinamide-(Arg8)-vasopressin	Niosomes	Rats in vivo
Adamantyltripeptides	Liposomes	-
Leishmania antigen -	Liposomes	Mice
Nerve growth factor	Liposomes	blood-brain barrier model
Bovine serum albumin	Liposomes	-
Human recombinant EPGb	Solidlipid nanocarrier	-
Thymopentin	Solidlipid nanocarrier	-
Superoxide dismutase	Liposomes	-

STZ –streptozocin, DGAVP desglycinamide-(Arg8)-vasopressin

Thiolated polymers have thiol group being immobilized on their surfaces exhibit strong mucoadhesive property due to covalent linkage with mucin glycoprotein through thiol disulphide reaction which provide more drug concentration at absorption site due to strong adhesion and prolonged residence time. Besides, the permeation enhancing effect of thiomers is also ascribed to the inhibition of protein tyrosine phosphatase and intestinal P-glycoprotein. Insulin loaded trimethyl chitosan cysteine conjugated nanoparticles showed more mucoadhesion and greater insulin permeation across the rat intestinal mucosa after the oral delivery administration. Encapsulation increase the stability of proteins and peptide by providing protection against proteolytic enzymes [36]. Enzyme inhibitors have more efficient protection against the proteolytic degradation as compared to encapsulation. Currently, only two peptide- and protein-based drugs (Interferon- α and human growth hormone (hGH) that can be given orally are known to be in clinical development [37].

NOVEL APPROACH

Novel approach for peptide and protein delivery includes the vesicular or carrier system which is extensively studied for oral delivery of proteins and peptides. Carrier system provides protection from proteolytic degradation in GIT, acidic environment

and also provides site specific, controlled release and enhance delivery of drug.

Lipophilic surfactant coated insulin solid in oil in water (S/O/W) was prepared in order to protect and increase bioavailability of insulin. The major drawback of this system was physical and chemical instability due to lack of proper storage conditions but this drawback could be overcome by using other method of emulsion preparation like freeze drying, spray drying and evaporation [38, 39].

A dry emulsion formulation in which the surfactant–insulin was entrapped in the oil phase of a solid formulation was further enteric-coated with a pH-sensitive polymer being responsive under the pH change in GIT. Novel iron and polysaccharide conjugated insulin loaded microcapsules developed for the continuous release of insulin. Insulin-loaded microcapsules were prepared via layer-by-layer deposition of oppositely charged Fe^{3+} and dextran sulfate (Jan et al.) onto the surface of insulin microparticles. These microcapsules provide the possibility of delivering the insulin and other peptides without degradation [40].

Coated insulin loaded liposomes were prepared using mucin and polyethylene glycol as coating component. It was observed that coating of liposomes not only lowers the drug degradation in intestinal fluid but

Table 3. Clinical applications of novel technologies for proteins and peptide development.

Product name	Products	Technology and dosage form	Possible outcomes	Clinical trial
Oraldel	Insulin, TNF blockers	Nanoparticles and tablets		Clinical phase Ib
Eligen	Calcitonin, insulin, PTH, heparin, calcitonin, enzymes (lipases, esterases, proteases)	Penetration enhancers	Increase membrane permeability	Clinical phase II
HIM2, ORMD-0801, ORMD-0901	Insulin, enkephalin, calcitonin, PTH, Insulin/Exenatide	Pegylation + PE Salts of EDTA (enteric coated + PE	Protect proteins from acidic degradation and Increase membrane permeability	Abandoned Phase I
Hepatic-directed vesicles-insulin (HDV-1	Liposomal insulin	Insulin		Phase II,III
Capsulin	Penetration enhancer	Insulin	Increase membrane permeability	Phase II
Intesulin	Nanoparticles encapsulation	Insulin		Preclinical
Vetsulin	PE (sodium caprate {C10}	Insulin and GLP-1 analogue	Increase membrane permeability	Phase I
Octreolin	PE(sodium caprate-C8	Octreotide	Increase membrane permeability	Phase III
Peptelligence™	PE (Citric acid + acyl carnitine)	Salmon calcitonin	Increase membrane permeability	Phase III
CLEC	Protein crystallization	Calcitonin and other polypeptides	Stable against proteolysis and self-digestion	Trial and error approach
Oral-Lyn	PE Spray devices and aerosol particles	Insulin, Macrotonin	Penetrate the buccal epithelium	Phase IV
Macrulin™	Lipid based microemulsion	Insulin, Salmon calcitonin	Protect proteins from proteolysis or acidic degradation, and enhance the protein absorption in GIT	Phase II
BioOral™	Calcium phosphate nanoparticles	Insulin and vaccines	Protect proteins from acidic degradation and improve membrane permeability	Preclinical

also provide effective hypoglycemic effect in diabetic rat compared to uncoated liposomes [41]. Liposomes with phosphatidylethanol as a carrier for oral delivery of insulin were prepared. Three different kind of lipids

(dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylethanol, palmitoyl–stearoyl sucrose and natural phosphatidylcholine and phosphatidylinositol) were included in liposome and *In-vivo* studies in the rat showed that these liposomal preparation provide effective hyperinsulinemia which in turn leads to hypoglycemia while Phosphatidylinositol based liposomes did not show any correlation with glucose level.

P(MAA-g-EG) based insulin loaded microsphere were prepared. P(MAA-g-EG) is an ideal candidate for oral delivery of drug. At acidic pH polymer form interpolymer complexes which suppress the drug degradation. At high pH these interpolymer complexes break up and release insulin from microsphere. Particle size also affects the drug loading in microspheres.

Calcitonin loaded self-assembling pectin–liposome nanocomplexes were prepared by simple mixing of cationic liposomes with pectin; a mucoadhesive polymer thus prongs the retention time of drug in intestinal mucosa leading to increased drug absorption. *In vivo* studies showed that Calcitonin loaded nanocomplexes prolonged reduction in calcium level; strong pharmacological activity [42].

Multilamellar coated mucoadhesive liposome using chitosan and carbapol were prepared and loaded with calcitonin. These liposome were evaluated and compared for their mucoadhesive and coating property with uncoated liposomes in rat intestine. Mucoadhesive property was decreased with increase in pH for carbapol. Effectiveness of calcitonin loaded liposomes in lowering the calcium level evaluated in rats and observed that this formulation provide prolonged suppress calcium level in rats [43].

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Recently different carrier systems like nanocube and nano crystals are being investigated for their efficiency for protein and peptide delivery.

CONCLUSION

Oral delivery of proteins and peptides presents a promising alternative to invasive methods and is an exciting area of research. However, developing oral biotherapeutics has long posed challenges for researchers due to the high molecular weight of these compounds, their susceptibility to chemical and enzymatic degradation, and their inability to permeate the intestinal mucosa. Recent advancements in biotechnology have enabled cost-effective, pilot-scale production of proteins and peptides suitable for oral delivery, with several products currently in various stages of clinical trials. Notable oral biotherapeutics developed recently include Oral Recosulin, Octreolin, and Sandimmune.

Nanotechnology provides a range of efficient carriers for protein delivery, including solid lipid nanoparticles, nanostructured lipid carriers, liposomes, niosomes, cubosomes, and nanoparticles. Various formulation development approaches for oral biotherapeutics have been discussed, which can be implemented for large-scale production. However, challenges such as maintaining protein stability during formulation and managing product development costs must be addressed at all levels of research and development to unlock the potential of this innovative technology for biotherapeutics delivery.

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