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Review

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A review on the strategies for oral delivery of protein and peptides and their clinical perspectives

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Abstract

In the modern world, a number of therapeutic proteins such as vaccines, antigens, hormones are being developed utilizing different sophisticated biotechnological techniques like recombinant DNA technology and protein purification. However, the major glitches in the optimal utilization of therapeutic proteins and peptides by oral route are their extensive hepatic first-pass metabolism, degradation in the gastrointestinal tract (presence of enzymes and pH-dependent factors), large molecular size and poor permeation. These problems can be overcome by adopting techniques such as chemical transformation of protein structures, enzyme inhibitors, mucoadhesive polymers and permeation enhancers. Being invasive, parenteral route is inconvenient for the administration of protein and peptides, several research endeavours have been undertaken to formulate a better delivery system for proteins and peptides with major emphasis on non-invasive routes such as oral, transdermal, vaginal, rectal, pulmonary and intrauterine. This review article emphasizes on the recent advancements made in the delivery of protein and peptides by non-invasive (*peroral*) route into the body.

Keywords*:* proteins, peptides, insulin, permeability, enzyme inhibitor, *peroral*

1. Introduction

Proteins and peptides are the building blocks of life and are now evolving as a very promising brand of therapeutic entities. Once a rarely used subset of medical treatments, therapeutic proteins have increased dramatically in number and frequency of use since the introduction of first recombinant protein therapeutic viz. human insulin, 25 years ago. Therapeutic proteins and peptides hold a significant role in almost every field of medicine, but this role is still only in its infancy. The foundation for the popularity of protein therapeutics was laid down with the regulatory approval of recombinant insulin by US Food and Drug Administration (FDA) in 1982, which became the first commercially-available recombinant protein and a source of major therapy for patients suffering from diabetes mellitus (Leader et al., 2008). Three decades have passed since the inauguration of approval of first recombinant protein i.e. insulin by the FDA, and its clinical success has inspired the field of therapeutic proteins into wider horizon ever since, with more than 130 different proteins or peptides already approved for clinical use by the FDA till 2008 alone, and many more in development pipeline.

A better understanding of molecular biology and biochemistry behind the macromolecular endogenous proteins, peptides and peptidergic molecules, and their role in various body functions and pathological conditions has led to the realization of the enormous therapeutic potential of proteins and peptides in the last few decades. Consequently, a variety of new therapeutic proteins have been developed showing therapeutic benefits in the treatment of ailments like diabetes, cancer which offer several advantages over the conventional small-molecule drugs. Firstly, proteins often serve a highly specific and complex set of functions in the body that cannot be mimicked by simple chemical compounds. Secondly, since the action of proteins is highly specific, there is often less potential for therapeutic protein to interfere with normal biological processes and cause adverse effects. Thirdly, because the body

naturally produces many of the proteins that are used for therapeutic purpose, these agents are often well-tolerated and are less likely to elicit immune responses. Fourthly, for diseases in which a gene is mutated or deleted, protein therapeutics can provide an effective replacement for the treatment without the need for gene therapy, which is not currently available for most genetic disorders. Fifthly, the clinical development and FDA approval time of protein therapeutics may be faster than that of small-molecule drugs. A study published in 2003 showed that the average clinical development and approval time was more than one year faster for 33 protein therapeutics approved between 1980 and 2002 than for 294 small-molecule drugs approved during the same time period. Lastly, because proteins are unique in form and function, companies are able to obtain far-reaching patent protection for protein therapeutics. The last two advantages make proteins an attractive alternative from a financial perspective compared with small-molecule drugs (Leader et al., 2008).

As a result of intensive research efforts in both academic and industrial laboratories, recombinant DNA, protein and peptides engineering and tissue culture techniques can now be used to obtain proteins and peptides for therapeutic use on a commercial scale which resemble an endogenous molecule and thus provoke fewer or minimal immunological responses. Though the initial problems related to obtaining non-immunogenic protein therapeutics in purer form at commercial scales have been overcome to quite some extent, their formulation and optimum delivery still remains the biggest challenge to pharmaceutical scientists. There are now many examples (Octreolin®, Sandimmune®, AI-401, HDV-I, Capsulin™, Oraldel™, IN-105, Oral-Lyn™, CLEC®, ORMD-0801, Eligen® etc.) in which proteins have been used successfully for therapeutic purposes (mentioned in detail later in this review under clinical applications). Nonetheless, potential protein therapies that have failed so far outnumber the successes, in part owing to a number of challenges that are faced in the development and use of protein therapeutics.

Route of administration is a critical factor in any therapeutic intervention which governs both the pharmacokinetics and efficacy of the drug. For protein and peptides therapeutics, an interplay of poor permeability characteristics, luminal, brush border, and cytosolic metabolism, and hepatic clearance mechanisms result in their poor bioavailability from oral and non-oral mucosal routes. Hence, at present these drugs are usually administered by parenteral route. However, inherent short half-lives of penetrating peptides (PP) and almost warranted chronic therapy requirements in a majority of cases make their repetitive dosing a necessity. Frequent injections, oscillating blood drug concentrations and low patient acceptability make even the simple parenteral administration of these drugs problematic. This has prompted researchers to develop new delivery systems capable of delivering such a class of drugs in a more effective manner. Although there have been reports of successful delivery of various PP therapeutics across non-*peroral* mucosal routes, *peroral* route continues to be the most intensively investigated route for PP administration. This interest in the *peroral* route, despite enormous barriers to drug delivery that exist in the gastrointestinal tract (GIT), can be very well appreciated from obvious advantages such as ease of administration, large patient acceptability, etc. Potential cost savings to the health care industry further augment the advantages of *peroral* systems in terms of patient compliance and acceptability, since *peroral* formulations do not require sophisticated sterile manufacturing facilities or the direct involvement of health care professionals.

There is a need to design an approach which not only protects the protein/peptide from enzymatic degradation but also aids in enhancing its absorption without altering its biological activity (Gupta et al., 2013). Although the oral delivery of proteins and peptides remains an attractive option, but to reach its true potential the challenges must be met. Oral delivery of proteins and peptides has long been hailed as the 'Holy Grail' of drug delivery by showing great potential but also presenting problems in their development (Shen, 2003).

The current article deals with the possibilities being explored in the oral delivery of protein and peptide therapeutics, the challenges in their development and the current and future prospects, with focus on technology trends in the market to improve the bioavailability of proteins and peptides and effect of different forms of therapeutic proteins by oral routes.

2. Peroral route: promises and pitfalls

Oral delivery is the most sought after route of administration for most of the drugs and pharmaceutical products, which depends on the drug's molecular structure or weight (Elsayed et al., 2009). Bioavailability is dependent upon the molecular mass of drugs if molecular mass increases above 500-700 Da, bioavailability of drugs decreases sharply whereas bioavailability is essentially independent of molecular mass for drugs of less than 500-700 Da(Donovan et al., 2000). Proteins have important therapeutic roles, such as insulin which is a major therapeutic agent for the management of insulin-dependent diabetes mellitus (Type 1) and for many patients with non-insulin dependent diabetes mellitus (Type 2) (El-Sayed et al., 2007; Khan, 2003). Intestinal mucosa is considered as a very complex structure. On the basis of adhesion in gastrointestinal tract, there are two main targeting areas, i.e. mucosal tissue and mucus gel layer. It may be due to adhesive interaction with mucoadhesive polymers either through non-specific (Van der waal and hydrophobic interaction) or specific interaction between complementary structures. On the other hand, regular renewal of mucosal surface by a turnover process restricts muco-adhesive drug delivery system (Ponchel and Irache,1998). Currently, pharmaceutical strategies aim to increase the bioavailability, overcome the enzymatic degradation, enhance the permeability and develop safe, efficacious and highly-potent proteinous drugs (Hamman et al., 2005; Shah et al., 2002) Proteins have been transported (actively) through the epithelial lining of the small intestine in membrane-bound vesicles after binding to the cell-surface receptor. Very few portions are released at the baso-

lateral membranes and then secreted in the intact form in the intestinal space. (Strous and Dekker, 1992). Drug absorption depends upon the age, diet and disease state (Morishita and Peppas, 2006). Mucus covers the epithelial cell surface, hence hampering the diffusion of peptide drugs. The globlet cells continuously secrete highly viscous gel whose viscosity enhances strongly towards the cell surface (Camenisch et al., 1998). Protein and peptides most commonly follow the paracellular route as compared to transport through the lipophilic cell membrane. Metabolic barriers consist of brush border peptidases and luminal proteases such as trypsin, α-chymotrypsin, elastase and carboxypeptidase. These enzymes easily degrade the therapeutic proteins and peptides administered through oral routes. Recently, there are only two oral proteins and peptides, e.g. Interferon-α and Human growth hormone (HGH) in clinical developmental stage (Orive et al., 2004) FDA has approved three drugs which augment glucagon like peptide-1 (GLP-1) production, on the basis of incretin based therapy for potential treatment in Type 2 Diabetes mellitus (Peters, 2010). It was reported that the intestinal uptake of therapeutic protein through biodegradable nanoparticles was enhanced by particle size reduction (enhanced dissolution) (Bakhru et al., 2013).

Insulin is released from pancreatic β-cells into the hepatic portal vein and releases into the liver which is the primary site of action. Whereas, parental route and other delivery system (buccal, pulmonary, nasal) delivers the drug directly into the systemic circulation. In this delivery system, drug reaches the systemic circulation bypassing the first-pass metabolism, but in case of oral delivery, the insulin first reaches the liver (20% of drug dose is available in liver) and then to the peripherals tissue. Oral route of administration is closer to the natural physiological route of insulin (Rekha and Sharma, 2013).

2.1 Transport mechanism of macromolecules

Large numbers of mechanisms are responsible for penetration such as simple diffusion (paracellular and transcellular), carrier-mediated transport, active transport and pinocytosis or endocytosis (Salamat-Miller et al., 2005). Proteins and peptides have very low log P (<0) value. Those drugs have lack of lipophilicity, no passive absorption can take place and absorbed through paracellular pathways (restricted to small molecules, less than 100-200 Da) (Camenisch et al., 1998). The paracellular space lies between 10 and 30-50^{o}A, therefore paracellular route is not feasible for large macromolecules. But in case of insulin, it is adsorbed on the apical membrane and is internalized by specific types of endocytosis processes (Agarwal and Khan, 2001). Few numbers of protein and peptides show practically active transport by binding to cell surface receptor or binding sites in the epithelial lining of the small intestine (membrane bound vesicles) (Bastian et al., 1999). Most commonly used transport mechanism is passive diffusion with two ways of transport: first, paracellular (transport of drug through the intercellular space between the cells) and second, transcellular (involves passage into or across the cells), is showed in Figure 1A. Transportation of drugs depends on overall molecular geometry, lipophilicity and charge of the transport pathway across the oral mucosa (Brayden and Mrsny, 2011). A minimum level of lipophilicity is essential in drugs to partition into epithelial membrane and absorbed through transcellular passive diffusion (Camenisch et al., 1998). Transport of therapeutic molecules from gastrointestinal tract into systemic circulation is through the mucosal layer then through the areolar layer. Other two intestinal layer (areolar or submucosal) connects together the mucus and muscular layers (Blanchette et al., 2004). Muscular and mucus layers are the strongest layer of the intestine which consists of the loose, filamentous areolar tissue containing lymphatic, nerves and blood vessels (Rekha and Sharma, 2013).

Membrane perturbing in order to increase transcellular permeation, showed on human Caco-2 epithelial cell monolayers when exposed at maximum concentration and demostrated

tolerance *in vitro*, but the best way is to attach any ligand on molecules that opens the tight junctions (Brayden and Mrsny, 2011; Aungst, 2000)

2.2Challenges associated with oral protein delivery

The unfriendly physiochemical properties of proteins and peptides have created great challenges for the formulation scientists, and have therefore resulted in a need to develop other routes of administration, such as oral, nasal, buccal, pulmonary, transdermal, rectal and ocular (Park et al., 2011). Use of proteins and peptides as therapeutic agents is limited due to lack of an effective route and method of delivery. Various critical issues associated with therapeutic protein and peptides delivery, that have drawn the attention of formulation scientists include the following:

(i) Proteins and peptides are high molecular weight biopolymers which serve various functions, such as enzymes, structural elements, hormones or immunoglobulins, and are involved in several biological activities. However, large molecular weight, size and presence of both hydrophilic and hydrophobic appendages in their structure, render proteins difficult to enter into cells and other body compartments, and thus impart poor permeability characteristics through various mucosal surfaces and biological membranes. Commonly, therapeutic proteins and peptides are hydrophilic with a log P <0 (Camenisch et al., 1998).

(ii) Many therapeutic proteins and peptides are efficacious in large part because of their tertiary structure, which can be lost under various physical and chemical environments, resulting in their denaturation or degradation with a consequent loss of biological activity, thereby making these molecules inherently unstable.

(iii)Many proteins and peptides have very short biological half-lives *in vivo* due to their rapid clearance in liver and other body tissues by proteolytic enzymes, protein-modifying chemicals or through other clearance mechanisms.

(iv) The protein and peptide degradation is highest in the stomach and duodenum and is significantly decreased in the ileum and colon. Various delivery systems have been developed to target absorption from the colon and ileum as a result, minimize exposure of drug to proteolytic enzyme. Thick enteric coating formulation has been used to target both the ileum and colon due to delay the release of drug for a sufficient period of time. However there is additional drawback such as potential changes in colon microflora, delay drug absorption and risk of absorption, along with drugs with endotoxins and other potentially harmful compounds residing in this intestinal region (Rubinstein, 2005; Van den and Kinget, 1995).

(v) As proteins and peptides deliver specific actions and are highly potent, a precise clinical dosing is of utmost importance.

(vi) The body may mount an immune response against the therapeutic protein and peptide. In some cases, this immune response may neutralize the protein and even cause a harmful reaction in the recipient. Recombinant technology and other advances have allowed the development of various antibody products that are less likely to provoke an immune response than unmodified murine antibodies, because in humanized antibodies, portions of the antibody that are not critical for antigen-binding specificity are replaced with human Ig sequences that confer stability and biological activity on the protein, but do not provoke an anti-antibody response. Exclusive human antibodies can be produced using transgenic animals or phage display technologies.

(vii) For a protein to be physiologically active there is a need for some post-translational modifications, such as glycosylation, phosphorylation and proteolytic cleavage. These requirements may dictate the use of specific cell types that are

capable of expressing and modifying the proteins appropriately. Thus, recombinant proteins can be synthesized in a genetically-engineered cell type for large-scale production.

(viii) The costs involved in developing therapeutic proteins and peptides are high due to the expensive intermediate technologies involved in their designing (Leader et al., 2008, Mahato et al., 2003).

Penetration of drug through oral mucosa into systemic circulation is a major hindrance in their absorption. A hydrophilic large molecular weight drug such as protein and peptides are easily degraded by oral route, as a result they are not or very less available in the systemic circulation (Mahato et al., 2003; Antunes et al., 2013). Aoki et al., (2005) demonstrated through his *in vitro* studies that mucus layer plays a critical role in the absorption of insulin across the small intestinal. In these studies mucus layers are removed from the intestinal segments using hyaluronidase without affecting the integrity of epithelial part of intestine. The transportation of therapeutic protein through hyaluronidase-treated small intestine was found to be significantly higher in comparison to the control group treated with phosphate buffered saline, PBS (Aoki et al., 2005).

3. Formulation approaches for oral delivery of proteins and peptides

The two important approaches for formulation of protein and peptides by oral route include: use of absorption enhancers and enzymatic inhibitor. Being charged, large in size and hydrophilic, proteins and peptides are notoriously poor permeators (and thus exhibit poor oral bioavailability per se). The former approach offers an opportunity to counter balance this permeation problem of therapeutic proteins. The latter approach is an answer to the instability exhibited by proteins on account of a plethora of proteolytic enzymes present in the GIT which have inherent dietary protein-digesting function. Various strategies for the development of oral protein and peptides are given below.

3.1 Enzyme inhibitors (protease inhibitors)

Macromolecules, such as proteins and carbohydrates, are broken down in the digestive system into simpler molecules, viz. amino acids and sugars, respectively, which are easily absorbed because intact protein absorption is typically minimal (<1%) (Iyer et al., 2010). Various types of enzymes (endopeptidases and exopeptidases) are responsible for the cleavage of amino acid chains, (e.g. trypsin, chymotrypsin, elastase, pepsin and carboxypeptidases etc). Each type of enzyme is specific for the cleavage of particular links of amino acids and different targeted inhibitors (Lueben et al., 1996; Bernkop-Schnurch et al., 1997; Gamboa and Leong, 2013). First approach is the use of enzyme inhibitor such as aprotinin and soybean trypsin inhibitor, camostat mesilate and chromostatin, but administration of such types of protease inhibitors for long duration results in the deficiency of these enzymes in humans (Figure 1C) (Yamamoto et al., 1994; Tozaki et al., 1997). A novel class of enzyme inhibitor, chicken and duck ovomucoids has been recently reported, and a formulation has been developed wherein the insulin and duck ovomucoids offered 100% protection against the action of trypsin and α-chymotrypsin (Agarwal and Khan, 2001). In another case study, polymer inhibitor conjugates such as carboxymethyl cellulose-Elastinal (CMC-Ela) have showed *in vitro* protection against enzymes (trypsin, α-chymotrypsin and Elastase. After 4 h of incubation, nearly 33% of the therapeutic protein was found to be active against the elastase (Park et al., 2011; Marschutz and Bernkop-Schnurch, 2000).

Serpin (Serine protease inhibitor) forms covalent complexes with the target protease and in such a way, the protein is protected from the protease enzymes. On the basis of structural studies, it has been demonstrated that inhibitory members of the group undergo conformational changes, known as stressed and relaxed transition, and conformational change which is the critical step in the mechanism of inhibition of a targeted protease (Egelund et al., 1998).

3.2 Absorption enhancers (permeation enhancers)

Penetration enhancers (PEs) directly transport protein molecules through the epithelium without major effects on their solubility (Brayden and Mrsny, 2011). PEs are commonly classified as either tight junction (TJ) selective, in order to increase paracellular permeability through slight modification of TJ functional properties or in order to increase transcellular permeation (membrane perturbing). These mechanisms ascertained using human Caco-2 epithelial cell monolayer at the maximum concentration in which the systems can tolerate *in vitro* conditions. In early 1990s, there was some consensus that the smarter strategies for poorly permeable drugs were to opt for specific agents, that opened tight junction of epithelial cell membrane, but the latter strategies suggested that membrane perturbation was considered potentially toxic (Maher et al., 2009). Enhancers have been studied for oral insulin delivery, such as fatty acids and bile salts, which enhance the permeability across the mucosal walls (Obata et al., 2000). They open up the tight junctions reversibly and improve the permeability of insulin and several other proteins (Figure 1B). A novel absorption enhancer, viz. Zonula occludens toxin (ZOT) (Salama et al., 2006), chitosan (Prego et al., 2005), thiolated polymers (Bernkop-Schnurch., 2005) and Pz-peptide have all been studied as penetration enhancers for oral insulin delivery, and have resulted in effective reduction of glucose levels in the body (Fasano and Uzzau, 1997). Sachdeva et al. (1997) reported that proteases (pancreatic enzymes) are less active against small peptides, such as cyclosporine and vasopressin analogues (Sachdeva et al., 1997). Leone-bay et al. (2001) described a new class of molecules that alter the conformation of proteins reversibly and provide facility for their transport across mucosa (Leone-Bay et al., 2001). The most common drawback of penetration enhancers in case of long-term usage is that they may damage or even dissolve biomembrane, leading to local inflammation (Iyer et al., 2010).

Surfactants also enhance the transcellular transport by disrupting the lipid bilayer and make it more permeable for drugs (Lecluyse and Sutton, 1997), a mechanism very similar to that of chelating agents which form complex with calcium ions and rupture the tight junctions and facilitate the transport of proteins (Aungst, 2000; Park et al., 2011). When proteins and peptides are given with lipophilic carriers, they enhance their absorption (Sood and Panchagnula, 2001) such as insulin, human growth hormone (HGH), calcitonin and recombinant parathyroid hormone (Lee et al., 2005; Kidron et al., 2004). The carrier alters the lipid solubility and then makes access to pore of the integral membrane (Leone-Bay et al., 2001). Merrion Pharmaceuticals (Dublin, Ireland) produced a novel formulation of alendronate with paracellular penetration enhancer known as Almerol^{TM} formally known as MER-103. Almerol^{TM} was found to have better bioavailability and fewer side effects as compared to alendronate for the treatment of osteoporosis (Walsh et al., 2011; Frost, 2008).

3.3 Mucoadhesive polymeric systems

They have a changing swelling behaviour in response to the environmental factors, such as ionic strength, electric field, light, temperature and pH (Park et al., 2011). The most common approach for the encapsulation of oral insulin is using mucoadhesive polymers, such as chitosan (Mathiowitz et al., 1997), poly [lactic-co-glycolic acid] (PLGA) (Damge et al., 1988), thiolated polymer and alginate, which have been studied extensively (Takka and Acarturk, 1999). Chitosan is a natural non-toxic, biocompatible and biodegradable polymer (Hejazi and Amiji, 2003). When a peptide (transforming growth factor [TGF-β]) was delivered with chitosan, as a result, a 6-7 fold enhancement of permeability of TGF-β with chitosan was attained. This resulted in the healing of the oral mucosa by arresting epithelial cell division and thus destruction of the cells from the effects of anticancer therapy (Senel et al., 2000). Mucoadhesive polymer adheres to the mucus and increases the drug concentration gradient. When insulin was encapsulated with Poly (methacrylic acid-g-ethylene

glycol)[P(MAA-g-EG)], [P(MAA-g-EG)] being a pH sensitive mucoadhesive polymer, showed pH-dependent swelling behaviour, as a result of formation or dissociation of inter-polymer complex [MAA-g-EG] polymer and it showed ~10% bioavailability of orally-administered insulin encapsulated with pH sensitive mucoadhesive polymer as compared to insulin (Lowman and Peppas, 1997; Peppas and Klier, 1991). Thiolated polymers (thiols side chains) have strong mucoadhesive properties due to covalent bonding with cysteine-rich subdomains of mucus glycoprotein (Leitner et al., 2003). Alone, protein encapsulated in polymer did not show efficient absorption as compared to polymer with enzyme inhibitor or protease inhibitor. Encapsulation leads to successful protection of the protein formulations from enzymatic degradation and also gets successful result. 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 (Renukuntla et al., 2013).

3.4 Novel carrier systems

A large number of carriers for proteins and peptides delivery, such as emulsions, nanoparticles, microspheres and liposomes, have been used to protect the protein formulation against the harsh environment of the GI tract (acidic medium and enzymes). Emulsion developed by using lipophilic surfactant-coated insulin decreased its degradation and increased its permeation. The critical drawback of emulsions is its physiochemical stability (Toorisaka et al., 2003). Stability problem of emulsions may be overcome by dry emulsion formulations, which are prepared by spray drying, lyophilisation or evaporation (Dollo et al., 2003). Liposomes have also been exploited to improve the bioavailability of proteins from the intestinal tract (Park et al., 2011). Liposomal system containing insulin and sodium taurocholate markedly reduced the blood glucose levels after oral administration and showed a high *in vitro/in vivo* correlation in the Caco-2 cell model (Degim et al., 2004). Langer and

his colleagues developed polymerized liposomes with covalent double bonds to improve the stability of biomolecules against the harsh environments (Langer, 1998).

Carrier nanoparticles consisting of lipophilic polystyrene, mucoadhesive chitosan and PLA-PEG were detected in both epithelial and Peyer's patches after inter-duodenal administration of drug molecules (Sakuma et al., 2001). Peyer's patches are the follicles of lymphoid tissue which contain M-cells. M-cells have an important role in particle uptake. Particle size and surface charge are important factors related to the uptake of particulates by M-cells (Shakweh et al., 2005; Brayden et al., 2005). Polymeric nanoparticles can be used to easily entrap and encapsulate therapeutic proteins and peptides and lead to targeted area. It can be smoothly functionalized toward off opsonisation, and therefore has shown reduced toxicity towards the non-target areas (peripheral tissues) (Chan et al., 2010). Kafka et al. (2011) investigated the *in vitro* and *in vivo* studies of gonadotropin releasing hormone-loaded nanoparticles. Different *in vitro* conditions (artificial gastric juice, simulated intestinal fluid and brushtail possum plasma) were studied, and it was found that less than 5% of the hormone was released over 6 h in artificial gastric juice and simulated intestinal fluid, and 60% of it was released in brushtail possum tail plasma over 1 h. *In vivo* study showed that sufficient therapeutic levels of these proteins were achieved from drug-loaded nanoparticles in the systemic circulation.

It was investigated that mucoadhesive nanoparticles increased the residence time of drug moiety because it allows the attachment of drug molecules into the mucous membrane of GIT. The concepts behind these nanocarriers can reduce clearance through alimentary canal and lead to increased bioavailability of therapeutic protein (Carvalho et al., 2010). Makhlof et al. (2010) revealed the permeation-enhancing properties of the mucoadhesive nanoparticles. Fluorescein isothiocyanate dextran (FITC dextran) -loaded polyelectrolyte complexes were prepared by interaction of spermine, polyacrylic acid and FITC dextran. Confocal

384 microscopy has been investigated for prolonged penetration using fluorescein isothiocyanate
385 dextran for *in vitro* and *in vivo* conditions. It was concluded that the drug loaded
386 mucoadhesive nanoparticles showed prolonged penetration (5-5.56 fold) as compared to free
387 FITC dextran through confocal microscopy.

388 **3.5 Derivatization or chemical modification of proteins** and peptides

389 Another approach is the derivatization of proteins and peptides by using polyethylene glycol
390 in order to protect the protein from enzymatic degradation and also to improve the solubility
391 (Clement et al., 2002). Lipidization, which is the covalent interaction of hydrophobic moiety
392 or non-covalent conjugation with hydrophobic moiety, results in the increase in the
393 hydrophobicity of proteins and peptides (Goldberg and Gomez-Orellana, 2003). This
394 approach has been used in clinic and has provided multiple drug candidates. Some others are
395 the formation of an inclusion complex with leucine encephalin, protect the peptides against
396 enzymatic degradation and also enhance absorption (Basu et al., 2006). Chemical
397 modification can be done by exploiting the carbohydrates moiety (glycoproteins) attached to
398 protein or side chain of protein (Calceti et al., 2004). The deamination of first amino acid and
399 substitution of last L-Arginine with D-Arginine along with simultaneous substitution of
400 fourth amino acid with valine forms1-Deamino-8-D-ArginineVasopressin (DDAVP). Such
401 derivative forms of vasopressin are two-times more potent than simple vasopressin (Shaji
402 and Patole, 2008). Transport of proteins and peptides have been studied with and without
403 absorption enhancers (Morishita and Peppas, 2006) through buccal epithelia, for example,
404 TRH (Thyrotropin-releasing hormone) and the LHRH (luteinizing hormone-releasing
405 hormone) analogue buserelin, a lauroyl tripeptide, the vasopressin fragment DGAVP, and
406 insulin resulted in increased bioavailability of protein molecules (Jana, et al., 2010).

407 **3.6 Prodrug strategies**

The prodrug is actually an active pharmacological moiety which has been converted into inactive form through chemical modification, and when administrated changes into the active form by enzymatic or non-enzymatic reactions (Figure 1D). It is complete bioreversible cyclization (Jana, et al., 2010). These approaches enhance the solubility, permeability and targeting of small molecules but it faces challenges, such as limitation in methodology, stability of proteins and structural complexity (Hsieh et al., 2009).

Drug + Carrier = "Prodrug" = After enzymatic degradation give free drug and carrier

A recent approach has enhanced the hydrophobicity and targeting through a lipid raft which has been conjugated with protein moiety, as well as attached specific transporter in the parent drug (Renukuntla et al., 2013). Prodrug approach may help in the absorption of various biomolecules such as RNA, DNA, oligonucleotides and proteins (enzymes, proteinous drugs and hormones) (Vadlapudi et al., 2012). (Lue5)-enkephaline was chemically-modified by phenyl propionic acid into a prodrug, which was found to improve their permeability across the Caco-2 1680-fold than the parent moiety (Cronauer et al., 2003).

3.7 Novel approaches

Novel vesicular delivery systems containing bile salts are known as "bilosome", which act as penetration enhancers and improve bioavailability (Sizer, 1997). Sadeghi et al. (2009) developed a gas-empowered delivery system for carbon dioxide-forced transport of the protein to the surface of small intestine. Insulin, together with a mucoadhesive polymer, trimethyl chitosan (a permeation enhancer) and polyethylene oxide, was delivered with carbon dioxide gas to the surface of the small intestine. This model enhanced the bioavailability of insulin upto seven-folds (Sadeghi et al., 2009).

A novel conjugation of iron and polysaccharide multi-layered microcapsules was developed for the continuous release of insulin (known as controlled delivery system). Multi-layered insulin-loaded microcapsules were prepared through layer-by-layer deposition of dextran sulphate and oppositely-charged Fe^{+3}(ferric ion) onto the surface of insulin microcapsules. In this model, two oppositely-charged substances (dextran acts as negatively-charged moiety and ferric ions act as the positive moiety) adhere on the insulin and result in the formulation of multi-layered insulin microcapsules (Zheng et al., 2009).

3.8. Novel functionality to macromolecules

3.8.1. Endogenous cell carrier systems

The endogenous carrier mechanisms are receptor-mediated endocytosis and membrane transporters. In some cases, when a drug is conjugated to a dipeptide, it gets detected by a peptide influx transporter, which in turn enhances its oral absorption (Morishita and Peppas, 2006). Efflux transport systems such as P-glycoproteins lead to inefficient bioavailability of proteins and peptides, and therefore, certain P-gp inhibitors are used with proteins and peptides to increase the bioavailability (Varma et al., 2003). The membrane transport is possible for small drug molecules; whereas receptor-mediated endocytic system does not have any limitation regarding the size of the drugs (Morishita and Peppas, 2006). Receptor-detectable ligands, such as vitamin B_{12}, transferrin, invasins, viral haemoaggulitinin, toxin and lectin, can be bound to the protein molecules to enhance the intercellular delivery to target cells (Russell-Jones., 2004, Lim and Shen, 2005). In cases of oral delivery system of proteins and peptides such as insulin and granulocyte colony-stimulating factors (G-CSF), they are conjugated with transferrin carrier to improve the bioavailability (Bai et al., 2005). There is a broad scope of use of recombinant fusion protein technology, and it may be useful for the future development of oral and buccal delivery systems for proteins and peptides.

3.8.2. Cell-penetrating peptides (CPPs)

Cell-penetrating peptides (CPPs), also known as protein transduction domains (PTD), are made up of 3-30 protein residues (Munyendo et al., 2012). CPPs consist of two groups, one is HIV-1 Tat peptide (cationic peptide) and artificial oligoarginine, and the other group is penetratin derived from *Drosophila antennapedia* homeoprotein (amhiphilic peptides) (Nakase et al., 2008, Derossi et al., 1996). They are employed to enhance the internalization of various biomolecules such as DNA, RNA, oligonucleotides, proteins and peptides (De Coupade et al., 2005). A group of small peptides such as TAT, oligoarginine and penetratin have been used to internalize different protein and peptide formulation into cells. The peptide enabled the delivery of the macromolecules, microparticles, liposomes and nanoparticles into cells or tissues by hybridizing with the target molecules. With regard to the harmful effects of the peptides, TAT has been shown to cause practically no toxic effects to membranes and in most of the *in vivo* application, no undesirable effect has been detected (Zorko and Langel, 2005). It has been identified that penetration occurred in cell membrane and they can cause small disturbance in membrane leading to enhanced absorption of proteins and peptides through the oral route. Peptide strategy is based on a non-specific delivery system, whereas it is proposed for the enhanced bioavailability and targeting of proteins and peptides through the oral route (Morishita and Peppas, 2006). Enhancement of safety and efficacy, and reduction in toxic effects are mandatory for the development of this delivery system for proteins and peptides. By co-administering the typical CPP with the insulin, enhanced intestinal bioavailability of insulin upto 30% was observed (Noriyasu et al., 2013).

4. Clinical application of oral proteins and peptides

Oral delivery systems for proteins and peptides are still in development stages. Oral delivery, being non-invasive, is the most favoured route of drug administration. This is illustrated by

the fact that oral delivery represents approximately US$ 25 billion worldwide (Werle et al., 2007). Various techniques for proteins and peptides delivery used by industries, to be highlighted in this section (Table 3).

4.1. Eligen®: Emisphere Technologies (USA)

This technology improves the transport of drugs through intestinal epithelium when a small carrier, (N-(8-(2-hydroxybenzoyl) amino) caprylic acid), is attached non-covalently with biomolecules, but the complex formation does not affect the chemical properties of biomolecules and the interaction is reversible. The drug-carrier complex is able to cross the epithelial membrane and break the non-covalent bond between drug and carrier, because it occurs spontaneously by simple diffusion on entering the blood circulation (Grosz et al., 2000; Wu and Robinson, 1999). These techniques play an important role in protection from digestive enzymes, as well as impart enhanced hydrophobic character to the macromolecules. Mostly, the molecular size is in the range from 500 to 1,500 Da (Walsh et al., 2011). In pharmacokinetic studies it was found that C_{max} for insulin was reached after ~20 min from the time of administration, and insulin level returned to the baseline within 80-120 min. Two most recently developed acylated entities are N-(8-(2-hydroxybenzoyl) amino) caprylic acid (SNAC) and N-(5-cholorosalicyloyl)-8-aminocaprylic acid (5-CNAC).SNAC was found to decrease transepithelial electrical resistance in Caco-2 monolayers, as well as improve the release of lactate dehydrogenase (LDH), suggesting that transcellular transport enhancement can also be a part of its mechanism (Hess et al., 2005). *In vitro* studies represented cytotoxicity in cell lines, but in animal models did not show pathological changes. An oral enteric-coated formulation for sCT (salmon calcitonin) has been found to possess higher efficacy than the nasal route of drug. In 2011, oral 5-CNAC/ sCT failed in the phase III of clinical trials (Karsdal et al., 2011). If higher doses of insulin are given to volunteers then they showed meaningful drop in HbA1c only after 3 months of studies. The high dose makes

the therapy cost-effective and ensures the commercial viability of oral proteins and peptides in the marketplace. At persent, no clinical efficacy of such system has been represented till date (*Emisphere Technologies, Inc*., 2006).

4.2. ORMD-0801: Oramed Company (Jerusalem, Israel)

The technology came with enteric-coated oral capsules wherein the protein part is released in the intestine with the help of penetration enhancers (Craik et al., 2013). Effect of oral insulin was determined by studies in eight volunteers in the fasted condition and demonstrated reduced glucose levels (7-35%) and also decline in the C-peptide level (13-87%) in all formulations. When the studies were conducted on fed volunteers, release of insulin was found to be adversely affected by meal and GIT motility. The onset and duration of action from time of administration was found to be 2 h and 5-6 h, respectively (Walsh et al., 2011).

4.3. CLEC®: Altus (USA)

Cross-linked enzyme crystal (CLEC) method mostly comprises of two steps including, first, batch crystallization of enzymes and second, crosslinking of enzyme microparticle (1-100µm) with cross-linking agents, such as glutaraldehyde. These above two steps must be optimized in order to ensure efficacy and safety (Judge et al., 1998). Altus has produced different CLEC® enzyme products, such as lipases, esterase and protease, but they have certain risks. Crystallization of proteins is not an easy step, therefore sometimes crystalline state may be inactive. Crystallization of biomolecules have several advantages, viz. higher solubility of crystalline form over amorphous form, easy purification of protein and concentrated protein crystals being beneficial for certain cases which require high doses at the site of action (Margolin, 1996).

4.4. Oral-Lyn™: Generex Biotechnology Corp. (Canada)

Oral-Lyn™ is delivered to the oral cavity through Rapidmist™ device (aerosol-type device containing non-chloroflurocarbon propellant, penetration enhancers and stabilizers) to the oral cavity which permeates across the buccal epithelium and reaches the blood circulation (Bernstein, 2006). Oral-Lyn™ delivery system has sufficiently large micellar size (larger than 7µm), therefore, it does not enter the respiratory system. A study was carried out to claim that Oral-Lyn is a safe formulation in which Oral-Lyn™ without insulin formulation were administrated into 40 dogs or nearly 1,000 patients and did not show any abnormalities in the buccal mucosa. These formulations were found to be also effective in type-2 diabetes, whose patients were resistant to diet, exercise, metformin, sulphonylureas and thiazolidenes. After the approval of Oral Lyn™ in India for the purpose of import, commercialization, marketing and sales for both types of diabetes, it has been issued the license, where the product has been renamed as Oral Recosulin (Shreya Life Sciences Pvt. Limited). Generex Biotechnology has claimed that it is close to completing the Indian clinical study needed to secure commercialization approval from the Central Drugs Standard Control Organization (CDSCO), Directorate General of Health Services, Ministry of Health and Family Welfare, and is awaiting advice from Shreya Life Sciences as to the anticipated timing of these initiatives. Generex Biotechnology Corp. has recently launched the Oral Recosulin for the treatment of Type-1 and 2 diabetes since 2009 (Generex biotechnology corporation, 2009).

4.4. IN-105: Nobex and Biocon (India)

Nobex technology (HIM2) is used in an oral delivery system which has been developed by Biocon. In this technique, enhancement of the hydrophobic character of proteins is achieved by chemical modification of insulin with a small PEG and penetration enhancers. New modified analogue called IN-105, which is advanced new generation molecules to HIM2 (hexyl-insulin mono-conjugate 2) was prepared (Wajberg et al., 2004). Introducing hydrophobicity to proteins by simple chemical linkage of the primary amine group of the

552 Lys-29 residue in the beta chain of insulin and amphiphilic oligomer resulted in enhanced
553 transcellular transportation, increased protein stability and resistance to enzymatic
554 degradation when administrated as oral semisolid hard gelatin capsules (Clement et al., 2002,
555 Kipnes et al., 2003). A study was conducted on 20 patients with T2DM (Type-2 diabetes
556 mellitus) poorly-controlled on metformin. The doses given were as follows: 10, 15, 20, 30 mg
557 of IN-105 and were compared with placebo control arm. The study concluded that the onset
558 of action occurred 10 min after administration of IN-105 and duration of action was near
559 about 1.5-2 h. Biocon did phase IV trials for IN-105 and marketed it as Insugen in India
560 (Kumar, 2009).

561 **4.5. Oraldel™: Apollo Life Sciences (Australia)**

562 Studies on Oraldel™ delivery system showed that it protects and transports biomolecules
563 (insulin), which are encapsulated inside them. The nanoparticles composed of carbohydrates-
564 based sugar (Rieux et al., 2005), protected polymer coated with cyanocobalamin (Vitamin
565 B_{12}) (Petrus et al., 2007). These formulations have the ability to entrap 100% protein with
566 vitamin B_{12}, and as a result they protect proteins from enzymatic degradation, as well as
567 enhance the transportation of proteins (Park et al., 2011). Various sizes of insulin
568 nanoparticles are delivered by Apollo Life Sciences. Other categories of drugs, such as TNF
569 blockers for the treatment of rheumatoid arthritis, are under development stages. The global
570 market of anti-TNF was almost US $ one trillion in 2006, growing at over 30% per year
571 (Craik et al., 2013, Apollo life sciences, 2010).

572 **4.6. Capsulin™: Diabetology (Jersey, UK)**

573 In UK, Capsulin™ is under clinical trials by Diabetology, which shows the onset of action
574 within 30 min and duration of action up to 4-6 h. During the fasting condition, higher doses
575 (300 I.U.), given to healthy volunteer with T1DM (Type-1 diabetes mellitus), showed sudden

fall in blood glucose level (1.6 mmol/l) and minimum doses (150 I.U.) which represented lowering of blood glucose levels (0.02 mmol/l). On the basis of clinical trial data, it was found that Capsulin™ has the ability to control the progression of diabetic conditions (Schwartz et al., 2008).

4.7. HDV-1: Diasome Pharmaceuticals (USA)

The concept of liposomal (vesicular) delivery system is growing by Diasome Pharmaceuticals. It is available in non-invasive (oral) and invasive (subcutaneous) forms. The study of 6 volunteers (with T2DM- Type-2 diabetes mellitus), which was based on comparison between placebo and doses in the ranging trial of oral HDV-1, represented significantly lowered mean and increased PPG area curve as determined over a period of 14 h as compared with placebo, which demonstrated non-linearity. The position of this drug is not clear due to insufficient data of pharmacokinetics. If HDV-1 is used for long duration, it becomes tough to control over-glycemic level due to the development of resistance (Skyler et al., 2005)

4.8. AI-401: Eli-Lily (USA)

Eli-Lily is still developing AI-401 for oral delivery of proteins (recombinant product of human insulin). Besides Oral-Lyn and HIM2, AI-401 is used for prevention and treatment of Type-1 diabetes. This technique uses the concept of oral-tolerance therapy. The data of Type-1 diabetes is organised by oral insulin arm of NIH-sponsored diabetes prevention and is advantageous for type-1 diabetes patients (www.accessdata.fda.gov., 2003, http://www.autoimmuneinc.com).

4.9. Sandimmune®: Novartis Pharmaceuticals (USA)

598 Sandimmune® is brand of Novartis, which consists of small hydrophobic cyclic polypeptide
599 of 11 amino acids called cyclosporine, and available in the form of a capsule. It is used as an
600 immunosuppressant for organ transplant rejection in kidney, liver and heart, as well as for the
601 treatment of auto-immune diseases (psoriasis and rheumatoid arthritis) (Holt et al., 1995). It
602 has a specific chemical structure of cyclosporine, therefore absolute bioavailability is about
603 30%. The uptake of cyclosporine is easy from intestine, and they are protected from
604 enzymatic action due to its lipophilicity and unique structure of the molecules. When
605 cyclosporine contacts with the aqueous environment it immediately forms micro-emulsion.
606 (Salama et al., 2010).

607 **4.10. Octreolin®: Chiasma (Israel)**

608 Transient permeability enhancer (TPE) system is an enteric-coated formulation which
609 facilitates intestinal absorbance of drug molecules with limited intestinal bioavailability. It is
610 formulated from sodium caprylate (C8) in hydrophobic microparticles and agitated with
611 castor oils or medium-chain glycerides, yielding emulsions (oily suspension)
612 (www.chiasmapharma.com). The FDA has approved the orphan status for the Octreotide
613 formulation, Octreolin®. During the phase III trials it (Octreolin®) showed no side effects in
614 all the 12 individuals. Most effective molecular weight of biomolecules that enhanced the
615 permeation of (TPE) is 4-10 KDa (Carino et al., 2000). C10 and C12 have more promoting
616 action than C8, in emulsion as an additive and its combination, to give TPE
617 (www.chiasmapharma.com).

618 **5. Conclusion and future prospects**

619 Oral delivery of proteins and peptides is most efficient way to replace the invasive route as
620 well as very interesting and promising area for research. The strategy for development of oral
621 biomolecules has always been challenged for the researchers due to their high molecular

weight, chemical or enzymatic degradation, and impermeability through the intestinal mucosa. The growing field of biotechnology has allowed cost-effective and pilot-scale production of proteins and peptides and it is used for oral delivery. In recent times, large numbers of proteins are invented through oral route such as Oral Recosulin, Octreolin® and Sandimmune® etc., in which a few are in clinical stage of development. As discussed in review, nanotechnology offers various efficient carriers for the delivery of proteins such as solid lipid nanoparticles, nanostructured lipid carrier, liposomes, niosomes, cubosomes and nanoparticles, etc. Various efficient approaches were discussed for formulation development of oral delivery of therapeutic proteins and it can be implemented in large-scale production. Protein stability during formulation, and the product development costs remain major challenges in pilot scale-up of these novel products which need to be addressed at all levels of research and development for this novel technology to be successfully transferred from the bench to the bedside.

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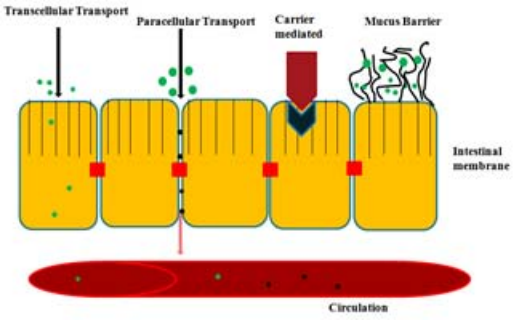
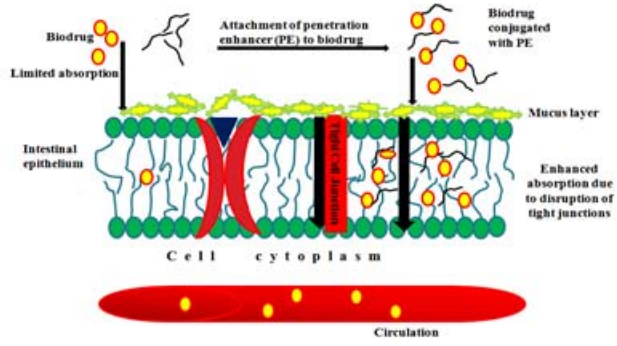
Figure Captions:

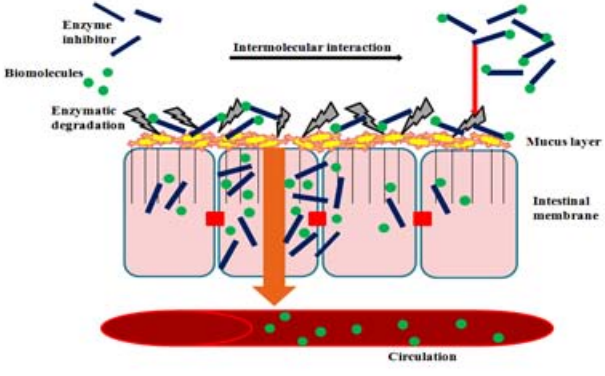
982 Figure: (A) Transport mechanism of biodrug through intestinal epithelium membrane, (B)
983 Probable mechanism of penetration enhancer, and (C) enzyme inhibitors, (D) Representative
984 mechanism of prodrug absorption and its activation
985


Transcellular Transport
Paracellular Transport
Carrier mediated
Mucus Barrier
Intestinal membrane
Circulation

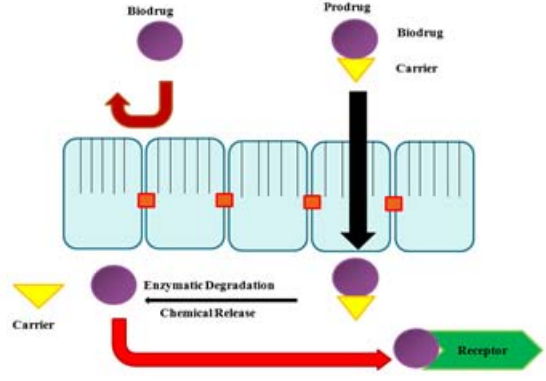
A


Biodrug
Limited absorption
Attachment of penetration enhancer (PE) to biodrug
Biodrug conjugated with PE
Mucus layer
Intestinal epithelium
Tight cell junction
Enhanced absorption due to disruption of tight junctions
Cell cytoplasm
Circulation

B


Enzyme inhibitor
Intermolecular interaction
Biomolecules
Enzymatic degradation
Mucus layer
Intestinal membrane
Circulation

C


Biodrug
Prodrug
Biodrug
Carrier
Enzymatic Degradation
Chemical Release
Carrier
Receptor

D

986 **Table 1** Various approaches for oral delivery of therapeutic proteins

Approach	Examples	Effects on bioavailability	Drawbacks	Reference
Absorption enhancers	Bile salts, fatty acids, Surfactants (anionic, cationic, nonanionic) chelators, Zonular OT, esters, cyclodextrin, dextran sulphate, azone, crown ethers, EDTA, sucrose esters, phosphotidyl choline	Enhanced bioavailability by increased membrane permeation	Available transport systems of both proteins/ peptides and undesirable molecules in GIT	Brayden and Mrsny, 2011
Enzyme inhibitors (protection against enzymes)	Sodium glycocholate, camostate mesilate, bacitracin soyabean, trypsin inhibitor, CROVM, DKOVM, polymer inhibitor conjugates, carbomers, polycarbophil, bestatin, aprotinin, streptozocin	Resisted enzymes degradation in stomach and intestines	Produced severe side effects in the treatment of chronic diseases such as diabetes, etc.	Park et al., 2011; Iyer et al., 2011
Mucoadhesive polymers	P(MAA-g-EG) hydrogel microparticles, lectin–conjugated alginate microparticles, thiolated polymer, natural oligosaccharides gum, drum dried waxy maize starch, carbopol 974P, chitosan derivatives, sea curve 240, scleroglucan, HE-starch, hydroxyl propyl cellulose, celloulose derivatives, pectin, xanthan gum, polycarbophil, amino dextran, DEAE-dextran	Site –specific delivery and improved membrane permeation	Limitation due to the mucus turnover in absorption sites (intestine)	Senel et al., 2000
Formulation vehicles	-Emulsion- *s/o/w *o/w * Enteric coated o/w	Protection against acids and enzymes	Physiochemical instability in case of long term storage	Park et al., 2011; Toorisaka et al., 2003
	-Liposomes *Double liposomes * Fusogenic liposomes * Cross-linked liposomes	Improve physical stability	Low loading efficiency of hydrophobic drugs	
	-Microsphere *Endragil-S100 microspheres	Restrict release of protein to	Difficulty of	

	***pH-sensitive P(MAA-EG)** **-Nanoparticle-** ***PMAA/Chitosan nanoparticle** ***Polystyrene/chitosan/PLGA-PEG nanoparticles**	**favourable area of GIT Increase membrane permeation Increase intestinal epithelial absorption**	**precise control - Avoidance of particle aggregation**	
Derivatization of proteins	**Polyethylene glycol**	**Protected against enzymatic degradation as well as enhanced the solubility**	**Non-specific pegylation**	**Clement et al ., 2002**
Endogenous cell carrier system	**Vitamin B_{12}, transferrin, invasins, viral haemoaggulitinin, toxin, and lectin**	**To enhance the intercellular delivery system to target cells, enhanced oral absorption**	**Limited to transporting of small drugs.**	**Bai et al., 2005; Morishita and Peppas, 2006**
Cell penetrating peptides	**Proteins were enabled to be delivered into cells or tissues by hybridizing with target molecules**	**Enhanced bioavailability and targeting of proteins**	**Toxic effect**	**Morishita and Peppas, 2006**
Prodrug approach	**Phenyl propionic acid**	**Prodrug permeability improved 1608fold than parent drug**	**Lack of methodology, structural complexity, stability problem of protein**	**Renukuntla et al., 2013; Hsieh et al., 2009**

987 Abbreviations- CROVM, Chicken ovomucoid; DEAE, Diethylaminoethyl cellulose; DKOVM, Duck ovomucoid; EDTA,
988 Ethylenediaminetetraacetic acid; PLGA-PEG, *Poly(lactic-co-glycolic) acid-Polyethylene glycol*; PMAA, Poly(methyl
989 methacrylate); P(MAA-g-EG), Poly(methacrylic acid-g-ethylene glycol; S/O/W, Solid-in-oil-in-water.

990

991
992 **Table 2** Different nanocarrier systems and models for oral delivery of proteins

Proteins	Carrier system	Models	Reference
Insulin	**Nano-cubicles**	**STZ-induced diabetic Rat**	**Chung et al., 2002**
Insulin, calcitonin, HGF (Human granulocyte colony stimulating factors)	**Nanocapsules**	_	**Oppenheim et al., 1982**
Salmon calcitonin	**PLGA-nanoparticle**	**Rat *in vivo***	**Sang and Park, 2004**
Insulin	**Acrylic-based co-polymer nanoparticles**	**STZ-induced diabetes in rat**	**Foss et al., 2004**
Cyclosporine	**Lipid microemulsions**	**Rat *in vivo***	**Constantinides, 1995**
Leucine encephalin	**Sugar coupling with cellobiose and gentiobiose**	_	**Mizuma et al., 1986**
Insulin	**Chitosan nanoparticles**	**Alloxan–induced diabetic rat**	**Pan et al., 2002**
HIV Protease (CGP57813)	**pH sensitive nanoparticles**	**Rat *in vivo***	**Leroux et al., 1996**
DGAVP	**Niosomes**	_	**Yoshida et al., 1992**

993 Abbreviations- DGAVP, desglycinamide-(Arg8)-vasopressin; HIV Protease *(CGP 57813),* is a peptidomimetic inhibitor
994 of *human immunodeficiency virus* type 1 (*HIV*-1) *protease; STZ, streptozocin*

995

996
997 **Table 3** Technologies for oral delivery of proteins under clinical development by companies

Company	Product name	Technology	Formulation	Development phase	Product	References
Apollo Life Science	Oraldel™	Nanoparticles	Tablet	Clinical phase I b	Insulin, TNF-blocker	http://apollolifesciences.com
Emisphere	Eligen	Penetration enhancers-Salcaprozate sodium	Tablet	Phase II	Calcitonin, insulin, PTH, heparin, calcitonin, enzymes (lipases, esterases, proteases)	http://emisphere.com
Nobex / Biocon	HIM2	Pegylation + PE	Liquid	Abandoned	Insulin, enkephalin, calcitonin, PTH	Wajberg et al., 2004
Oramed	ORMD-0801 ORMD-0901	Salts of EDTA (enteric coated +PE)	Capsule	Phase I	Insulin/ Exenatide	Kidron et al., 2004
Diasome pharmaceuticals	Hepatic-directed vesicles-insulin (HDV-1)	Liposomal insulin	Tablet	Phase II/III	Insulin	Schwartz et al., 2008
Diabetology	Capsulin	PE	Capsule	Phase II	Insulin	Whitelaw et al., 2005
Coremed	Intesulin	Nanoparticle encapsulation	Capsule	Preclinical	Insulin	Carino et al., 2000
Merrion pharma (Ireland) with Novo-Nordisk (Denmark)	Vetsulin	PE (sodium caprate {C10})	Matrix tablet	Phase I	Insulin and GLP-1 analogues	Walsh et al., 2011
Chiasma (Israel)	Octreolin	PE (sodium caprylate{C8})	Suspension	Phase I (phase I completed, phase III enrolling	Octreotide	http://chiasmapharma.com
Unigene/Tarsa (USA)	Peptelligence™	PE (Citric acid+acyl carnitine)	pH-dependent coated dosage form	2011, Phase III completed	Salmon calcitonin	http://tarsatherapeutics.com
Altus	CLEC®	Protein crystallization	Tablet	Trial and error approach	Calcitonin and other polypeptides	Margolin, 1996
Generex	Oral–Lyn™	PE	Spray devices and aerosol particles	Phase IV	Insulin, Macrotonin	http://www.generex.com
Endorex	Orasome™	Polymerized liposome	-	Phase II	Insulin, growth hormones,	Okada et al., 1998

					vaccines	
Provalis PLC	**Macrulin^{TM}**	**Lipid based microemulsion**	**Emulsion**	**Phase II**	**Insulin, Salmom calcitonin**	**Cilek et al., 2006**
Eli –lily	**AI-401**	**Enzyme inhibitor**	**Oral formulation**	**Phase II**	**Insulin**	**http:// autoimmuneinc.com**

Abbreviations: EDTA, Ethylene diaminetetraacetic acid; PE, Penetration enhancers; PTH, Parathyroid hormone; TNF, Tumour necrosis factor.