ABSTRACT
Advent of recombinant technology in protein synthesis has given birth to a new range of biopharmaceuticals. These therapeutic peptides and proteins are now emerging as an imperative part of various treatment protocols especially in the cancer therapeutics. Despite extensive research efforts, oral delivery of therapeutic peptide or protein is still a challenge for pharmaceutical industries and researchers. Number of factors including high proteolytic activity and low pH conditions of gastrointestinal tract act as major barriers in the successful delivery of intact protein/peptide to the targeted site. Low permeability of protein/peptide across the intestinal barrier is also a factor adding to the low bioavailability. Therefore, because of the short circulatory half-life exhibited by peptides in vivo, they need to be administered frequently resulting in increased cost of treatment and low patient compliance. Nano-carrier-based delivery presents an appropriate choice of drug carriers owing to their property to protect proteins from degradation by the low pH conditions in stomach or by the proteolytic enzymes in the gastrointestinal tract. This review focuses on recent aspects and patents on oral delivery of therapeutic proteins and peptides with special emphasis on nano-carrier-based approach.

KEYWORDS: Bioavailability, biopharmaceuticals, half-life, nano carrier, Oral route.

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 the 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. 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 a 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 the 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.

As a result of intensive research efforts in both academic and industrial laboratories, recombinant DNA, protein and peptide 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™, Oraldelem™, 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 peptide 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. 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.

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.

Different routes for delivery of Protein Peptide drugs

Different routes include
1. Oral route
2. Buccal route
3. Nasal route
4. Transdermal route
5. Pulmonary route
6. Rectal route

Barriers to oral delivery

Oral delivery is the preferred route of drug administration, as the majority of patients see it as the most convenient way to take their drugs. Drugs taken by the oral route have the highest level of patient compliance due to the ease and simplicity of taking medications. Despite the large number of protein therapeutics being discovered each year, oral delivery continues to be a barrier. As a whole, protein and peptide drugs have low bioavailability when administered
orally due to problematic barriers including gastrointestinal proteases, the epithelial barrier and efflux pumps. Common routes of administration for the systemic delivery of peptide and protein therapeutics are summarized in Figure 1, Table 2 provides an overview of the delivery enhancers discussed in this paper with regards to where they act.

Common routes of administration for systemic delivery of peptides and proteins.

Proteins are degraded via enzymes and hydrolysis in the acidic environment in the stomach and in the GI tract by a number of proteases and peptidases. The human degradome, a complete list of proteases in human cells, consists of at least 569 proteases. There are five broad classes of proteases, including serine, cysteine, threonine, aspartic and metallo proteinases. These proteases play roles in DNA replication, transcription, cell proliferation, fertility, stem cell mobilization, hemostasis, inflammation, senescence, apoptosis and many other vital cellular and regulatory processes. Trypsin, carboxypeptidase and chymotrypsin are secreted from the pancreas into the small intestine, mostly in the duodenum, where they are present in gram quantities. These enzymes are responsible for 20% of the enzymatic degradation of ingested proteins and peptides. The causes of the remaining 80% of enzymatic degradation are discussed below.

While peptide degradation is one obstacle to oral protein therapeutic delivery, the epithelial barrier of the small intestine poses an even greater challenge. This barrier consists of a single layer of columnar epithelial cells supported by lamina propria and muscularis mucosa. Molecules can cross the epithelium by either transcellular or paracellular routes as depicted in Figure 2. Apical to the epithelial cell barrier is the mucosal layer, which contains glycocalyx, a layer of sulfated mucopolysaccharides, glyco-proteins, enzymes, electrolytes
and water. Additionally, most mucosal surfaces are coated by a hydrated gel consisting of mucins, which are high MW, heavily glycosylated proteins. Bulk flow to the epithelial cells is limited, creating an unstirred layer near the epithelial surface. This unstirred layer is protected from convective mixing forces, slowing the absorption of small molecules and ions. Once a molecule passes the mucosal layer, however, the unstirred layer may act as an absorption enhancer by allowing the particle more time exposed to the epithelial barrier.

![Figure 2.](image)

**Mucosa:** It is the innermost, mucus secreting layer which contains many projections (villi) responsible for absorption of food and drug substances. This layer is further divided into epithelium, lamina propria and muscularis mucosa. These cells mainly secrete pepsinogen, hydrochloric acid and gastric lipase.

**Submucosa:** It consists of a connective tissue with large blood vessels, lymphatics and nerves branching into the mucosa and muscularis externa.

**Muscularis externa:** It is made up of longitudinal and circular muscle fibers. The longitudinal fibers shorten the tract, while the circular fibers prevent food from traveling backward and propel the balled-up food through the GI tract.

**Serosa:** It is also known as adventitia. This consists of several epithelial layers and forms an external protective coat.

**Intestinal drug transport mechanisms**
Drug transport across the intestinal epithelium is mediated by active or passive transport processes (Fig. 1). Mechanism of transport depends mainly on the physicochemical properties of drug molecule. Active transport involves the movement of drug molecules against concentration gradient (i.e. from low to high concentration) by transmembrane proteins with expenditure of ATP molecules. Passive transport involves the diffusion of drug
molecules in the direction of concentration gradient. The rate of drug transfer is governed by Fick’s law of diffusion (Eq. 1).

\[
\frac{dQ}{dt} = \frac{DKA(C_1 - C_2)}{h}
\]

(Eq. 1)

- \( \frac{dQ}{dt} \) = rate of diffusion
- \( D \) = diffusion coefficient
- \( K \) = oil/water partition coefficient of drug
- \( A \) = surface area of the membrane across which drug transfer occurs
- \( h \) = thickness of the membrane through which diffusion occurs
- \( (C_1 - C_2) \) = difference in drug concentrations in area 1 and 2 respectively

Passive diffusion of peptides and proteins can be described by a combination of two processes:

**Paracellular transport:** This process involves the transport of molecules via water filled pores/channels between cells. Approximately 0.01–0.1% of the total intestinal surface area consists of water filled pores. Taking into consideration that the intestinal epithelium has a surface area of \( \sim 2 \times 10^6 \) cm\(^2\), paracellular route corresponds to \( \sim 200 \) to 2000 cm\(^2\). This surface area is sufficient for the absorption of small quantities (pM–nM range) of a protein adequate to exert their biological activity. This route is preferred by low molecular weight hydrophilic compounds such as small peptide fragments generated from the breakdown of proteins. Peptide and protein molecules are hydrophilic in nature with logP value < 0. These molecules enter cells mostly via paracellular route. However, the presence of tight junctions or zonula occludens between the epithelial cell layer of GIT severely limit penetration ability of polar macromolecules. The diffusion of polypeptides via paracellular route depends on their physicochemical properties, molecular dimension and overall ionic charge. The bioavailability of drugs decrease rapidly with increase in molecular weight beyond 700 Da. Unfortunately, most of the therapeutic proteins have molecular weight much greater than 700 Da and hence exhibit low bioavailability. The tight epithelial junctions of colon are impermeable to molecules with radii larger than 8–9 Å. However, in case of polypeptides with high conformational flexibility it is possible that even larger molecules can diffuse through the tight junctions the effect of secondary structure on the aqueous diffusion of a model peptide poly(L-lysine) through a microporous membrane. This study concluded that the change in secondary structure of poly(L-lysine) from the random coil to the \( \alpha \)-helix did not alter apparent permeability (\( P_{\text{app}} \)) and intrinsic diffusion coefficient (\( D_{\text{aq}} \)). However, the
**β**–sheet conformer significantly lowered $P_{\text{app}}$ and $D_{\text{aq}}$ values. This result was attributed to higher solution viscosity and extended **β**–sheet structure of poly(L-lysine). In another study, examined the effect of secondary structure and charge of a model polypeptide, poly(D-glutamic acid) on its permeability through negatively charged pores of synthetic porous membranes and Caco-2 cell monolayers. Poly(D-glutamic acid) exists as a highly negatively charged random coil conformer at neutral pH and below pH 5.0 it changes to α-helix conformer. Transport studies across Caco-2 cell monolayers revealed higher permeability of poly(D-glutamic acid) at pH of 7.4 (Fig. 2), while a completely opposite trend was observed in the moderately hindered diffusion case (Fig. 3). This observation may be due to the effect of electric field that plays a significant role in the permeation of solutes which are small relative to the pores.

![Fig. 2](image1)

Percentages of FITC-labeled poly(D-glutamic acid) transported across a Caco-2 cell monolayer at 37°C. Asterisks indicate a significant difference ($P < 0.05$) compared to pH 4.5. The TEER values before and after the transport experiments.

![Fig. 3](image2)

Percentages of poly(D-glutamic acid) transported across a track-etched polycarbonate membrane with an average pore diameter of 0.015 µm at 37°C. Asterisks indicate a significant difference ($P < 0.05$) compared to pH 7.4.

a. However, for large molecules sieving through the pores is dependent mainly on the molecular size which dominates the influence of electric field. This study concluded that charge and secondary structure of polypeptides play a significant role in determining the rate
of aqueous diffusion in a hindered diffusion model. Dodoo et al. studied the permeability of 14 synthetic model peptides labeled with an amino acid fluorophore on rat alveolar cell monolayers cultured on permeable supports. The results indicated that the peptides entered cells primarily via paracellular route and $P_{app}$ values were inversely proportional to the molecular size. Scientists have investigated the role of paracellular route in the absorption of peptides such as potent analogs of vasopressin octreotide, thyrotropin-releasing hormone (TRH), salmon calcitonin and peptidomimetic renin inhibitors. Novel strategies such as modification of drug molecule and modulation of tight junctions associated with the paracellular pathway were investigated to increase the penetration of macromolecules.

b. **Transcellular transport**: This process involves the diffusion of drug molecules through the apical and basolateral membranes. This route is ideal for lipophilic drugs which express relatively high affinity for the lipid bilayer of cell membrane. Many theoretical models based on molecular size, charge, hydrogen bonding, confirmation and lipophilicity have been developed to study transcellular transport of drugs molecules. Since cell membrane consists of lipid bilayer, it is widely accepted that lipophilicity plays an important role in determining the transport mechanism. However, early *in vivo* studies concluded that the intestinal absorption diminishes when lipophilicity is very high (usually log P > 5) the effect of lipophilicity, chain length and number of polar groups on the transport of model peptides in Caco-2 cell monolayers. Interestingly it was observed that the permeability of peptide depends on the number of polar groups that require desolvation before diffusion of peptide into the cell membrane rather than lipophilicity as observed in small organic molecules the relationship between structure and permeability of neutral and zwitterionic peptides prepared from D-phenylalanine and glycine across Caco-2 cell monolayers. The lipophilicity (log P) of peptides varied from −2.2 to +2.8. The results indicated no apparent correlation between the apparent lipophilicity and observed flux. Moreover, a strong correlation was noted for the flux of neutral series and the total number of possible hydrogen bonds of the peptide with water molecules. These results clearly indicate that the passive transcellular absorption of a peptide depends on the energy required to break water-peptide hydrogen bonds so the molecules can enter the cell membrane.
Intestinal drug transport mechanisms.

**Carrier mediated transport:** This mechanism involves the movement of small molecules, or macromolecules via membrane proteins (transporters). This is also known as facilitated diffusion or active transport process. It has been well established that intestinal absorption of di- and tri-peptides occurs via carrier mediated peptide transport systems. These oligopeptide transporters also help in the absorption of peptidomimetics such as amino-β-lactam antibiotics, renin-inhibitors and angiotensin converting enzyme inhibitors. Detailed understanding about the structural features of the peptide is required to target these transporters for protein delivery.

**Challenges 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. 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 peptide 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 $P < 0$. 

![Fig. 1.](image-url)
(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) 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 and minimize exposure of drug to proteolytic enzymes. Thick enteric coating formulation has been used to target both the ileum and colon due to delay in the release of drug for a sufficient period of time. However there is an 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.

(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.

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 demonstrated through his in vitro studies that the 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 the epithelial part of the 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.

Formulation approaches for oral delivery of proteins and peptides

Direct structural modification

One class of structural modifications under study is cyclization. The benefits of cyclization to oral peptide/protein therapy are evidenced by cyclosporine (CSA). CSA is a fungal-derived, non-ribosomal 11-amino acid peptide with a cyclic backbone and a single D-amino acid.[1]

While most naturally occurring proteins and peptides are composed of L-amino acids, D-amino acids are found in some naturally occurring non-ribosomally synthesized peptides. CSA is used most frequently as an immune system modulator for the prevention of solid organ rejection. This cyclic peptide is resistant to proteolytic degradation and also has higher than expected absorption after oral administration.[1] The superior oral bioavailability is thought to be due to a number of properties including decreased flexibility and hydrogen bonding characteristics. The cyclic nature of CSA incorporates seven N-methyl groups that reduce the number of hydrogen bond donors and the remaining four hydrogens bond intramolecularly. This reduction in intermolecular bonding reduces hydrophilicity. CSA has lipophilic side chain amino acids that further raise its lipophilicity and allows it to cross the gut wall.[1] Other peptides such as somatostatin and encephalin have demonstrated similar characteristics and improved oral absorption after cyclization. Generically, cyclization is usually carried out between side chains or ends of the peptide sequences through disulfide bonds, lanthionine, dicarba, hydrazine, or lactam bridges. While cyclization is an option for
some peptides, its widespread use is limited when larger peptides and proteins are needed for therapy.

PEGylation is a modification option for some peptides not amenable to cyclization. PEG is an amphipathic molecule that dissolves in organic solvents as well as in water. Both PEG and its metabolites are nontoxic and US FDA approved. PEG has been reported to be toxic at high parenteral doses, much higher than the amount of PEG a patient would be exposed to with current PEGylated therapies. If PEG toxicity is seen it usually presents in the kidney, as unmodified PEG is mainly cleared through the kidneys. Interesting, even when pathological changes were seen, no functional deficits resulted. Case studies exist that demonstrate high doses of PEG can induce acute tubular necrosis, and the use of PEG in colonoscopy bowel preparation is associated with an increased risk of acute renal failure in patients aged over 50. There is also evidence that repeat administration of PEGylated particles can lead to increased clearance rate, likely related to anti-PEG IgG and IgM antibodies. The structure of the PEG molecules, properties of the molecule being PEGylated and method of PEGylation all play a role in determining immunogenicity.

**Direct PEGylation** confers benefits in both protein absorption and systemic stability (described later in this paper). As an example, insulin PEGylated with a 750 Da version of PEG was formulated into a mucoadhesive tablet. After oral administration, insulin activity was demonstrated by the observed drop in blood glucose levels of approximately 50% 3 h after administration. Additionally, some activity of the orally administered insulin was seen up to 30 h after administration. PEGylation of another peptide, salmon calcitonin (sCT), resulted in resistance to intestinal enzymes, a nearly six fold increase in intestinal absorption and slowed systemic clearance compared with the unmodified version of sCT.

Vitamin B12 has been used to increase the oral absorption of a number of therapeutic proteins including G-CSF, erythropoietin, insulin and lutenizing hormone releasing hormone.\[^{43}\] By fusing therapeutic proteins to vitamin B12, it is possible to take advantage of the binding of vitamin B12 to IF, followed by the receptor-mediated absorption of the vitamin B12–IF conjugate. However, this system is limited by the quantity of B12 that can be absorbed, GI degradation, decreased activity of the protein therapeutic due to steric hindrances and loss of IF affinity for conjugated vitamin B12. For more information on the use of B12 to improve the oral delivery of protein and peptides.
Protein lipidization is another method that increases the bioavailability of orally administered proteins. Fatty acid conjugates of polypeptides demonstrate improved transport across biological membranes, higher stability and longer plasma half-lives. sCT was lipidized using reversible aqueous lipidization and thus it is categorized as a prodrug in this case. Compared with free sCT, the reversible aqueous lipidization sCT reported increased absorption and a 19-times higher AUC value. Caprates, medium-chain fatty acids, promote paracellular diffusion of Class III (highly soluble, low permeability) molecules such as peptides. In addition, triglycerides can be used to evade first-pass metabolism. While irreversible methods of lipidization allow for increased membrane permeability, the activity of such modified proteins may be diminished due to steric issues with the fatty acid chain.

Recently, stapled peptides have garnered interest due to their enhanced biochemical properties in the context of drug delivery. More specifically, these are α-helical peptides that contain a synthetic, hydrocarbon backbone linking various residues. This backbone, known as the staple, locks the conformation of the peptide, increasing its helicity and stability in solution have demonstrated the ability of a hydrocarbon-stapled BH3 helix to increase apoptosis in vivo. The enhanced stability of these peptides, along with increased cellular penetration capabilities, makes these molecules ideal candidates for future study in peptide delivery.

A final method of peptide modification to increase oral bioavailability is the substitution of natural L-amino acids with D-amino acids. One study demonstrated that a variety of peptides cleaved by chymotrypsin, elastase, papain (a cysteine protease found in papaya), pepsin, trypsin and carboxypeptidases are cleaved minimally or not at all by these enzymes when certain residues were replaced with D-amino acids. D-amino acid substitutions in MUC2, a mucin glycoprotein. The authors noted that the substituted peptide demonstrated high resistance to proteolytic degradation in vitro in both human serum and lysosomal preparations. Their work illustrated that simultaneously modifying both N- and C-terminal regions with D-amino acids conferred the greatest stability increases.

The above mentioned direct modifications of peptides and proteins are key strategies that have been implemented to increase stability and oral bioavailability. Many other direct modifications have been carried out, including certain prodrug methods, an overview of which is given in Table 3. [8,31,32,41,42,44,45,52,56]
Table 3: Direct modifications of peptides and proteins. Overview of direct modifications made to peptides and the resulting change in bioavailability.

<table>
<thead>
<tr>
<th>Method of modification</th>
<th>Subtype of modification</th>
<th>Successfully modified compound</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclization</td>
<td>CTA, nonenolactone, pseudopeptide</td>
<td></td>
<td>Reduced enzymatic activity, increased solubility</td>
</tr>
<tr>
<td>PEG</td>
<td>Direct, permanent modification</td>
<td>Insulin, salmon calcitonin</td>
<td>Resistance to enzymatic degradation</td>
</tr>
<tr>
<td>Prodrug</td>
<td>PEG-β-1b</td>
<td></td>
<td>Decreased aggregation, increased stability</td>
</tr>
</tbody>
</table>

Enzyme 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%). 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. First approach is the use of enzyme inhibitors such as aprotinin and soybean trypsin inhibitors, 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). 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. In another case study, polymer inhibitor conjugates such as carboxymethyl cellulose-Elastinal (CMC-Ela) have shown 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.

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.
**Absorption enhancers**

The optimal absorption enhancer should be reversible, nontoxic at the effective concentration and provide a rapid permeation enhancing effect on the intestinal cell membrane. One such compound class of absorption enhancers is chitosans. Chitosans are nontoxic, biocompatible, FDA-approved polymer derivatives of chitin that enhance the absorption of hydrophilic macromolecule drugs. In addition, due to their high MW, they are minimally absorbed from the gut, limiting the possibility of systemic side effects. It is thought that varying degrees of deacetylation of chitin confer different amounts of absorption enhancement, with >80% deacetylation affording the greatest promoter effect in cell culture. Chitosans have been used to enhance the absorption of molecules such as atenolol, insulin and 8-R-vasopressin. Further, chitosans appear to be quite safe at their effective concentration. Chitosans work by increasing paracellular permeability. By binding tightly to the epithelium via positive charges, chitosans cause redistribution of cyto-skeletal F-actin and the zonula occludens 1. Chitosans are limited by their ability to diffuse across the mucous layer, as evidenced by their decreased activity on mucus-producing cells. *In vivo* studies with chitosans demonstrated a threefold increase in octreotide absorption when the two were coadministered into the duodenum. Another study with trimethyl chitosan chloride, a chitosan derivative, had many favorable characteristics. Trimethyl chitosan chloride was able to reversibly interact with TJs, leading to widening of the paracellular route, and at the same time did not damage cell membranes or alter the viability of intestinal epithelial cells. *In vivo* studies in rats demonstrated that it was able to increase the oral bioavailability of a peptide when the two were coadministered. Overall, chitosans and their derivatives are a promising class of absorption enhancers.

Another class of absorption enhancers demonstrating potential includes the medium-chain fatty acids. C8, C10 and C12 fatty acids (caprylate, caprate and laurate, respectively) can enhance paracellular permeability of hydrophilic compounds. First, caprate is thought to work by inducing dilation of TJ. Interestingly, the lowest concentration that enhanced absorption was near the critical micelle concentration of each fatty acid. The order of increased absorption *in vivo* is caprate>laurate>caprylate. Sodium caprate (C10) is the most studied of the medium-chain fatty acids. It is thought to increase absorption of hydrophobic molecules via the paracellular and transcellular route. Unfortunately, a study reported that it can only significantly increase absorption for molecules up to 1200 g/mol, or 1.2 kDa (such as octreo-tide). At the effective dose of 13 mM, sodium caprate is nontoxic to epithelial cells.
Lectins are another type of absorption enhancer that have many of the characteristics of the ideal absorption enhancer. Lectins are proteins that specifically recognize and bind to sugar complexes attached to proteins and lipids. Lectins are also naturally resistant to proteolytic breakdown, making inactivity before reaching their site of action unlikely. They can be used to target luminal surfaces of the small intestine and trigger vesicular transport into or across epithelial cells. Lectins are also mucoadhesive, which further leads to increased absorption.

Toxins can also be used for absorption enhancement, so long as they do not cause permanent cellular damage. Zonula occludens toxin (ZOT), is one such compound. ZOT, a 45 kDa toxin made by *Vibrio cholerae*, has been demonstrated to increase the permeability of small intestine mucosa by reversibly affecting the structure of TJs. ZOT binds to ZOT receptors on the luminal surface of the intestine and causes cytoskeletal rearrangement related to changes in protein kinase C and binding to β-tubulin. TJs can be perturbed enough to allow the transport of agents across the intestinal mucosa, although the increased bioavailability of insulin was only 20%.[86] In a study with Caco-2 cells, incubation with 4 μg/ml ZOT for 30 min increased the permeability to insulin by 6.3-fold. Mediation of TJs may not be the only method by which ZOT works; a study demonstrated that a fragment of ZOT was able to increase the bioavailability of hydrophobic drugs by interacting with PGP. Additional work has been done to determine the smallest portion of ZOT that maintains activity.

Recently, coadministration of cell-penetrating peptides (CPPs; described later in more detail) with therapeutic peptides has been attempted in order to increase absorption of the therapeutic. In one study, insulin coadministered with CPPs consisting of six to ten repeats of arginine led to increased GI uptake of insulin. Interestingly, the study investigated both D- and L-arginine-based CPPs, and the D-based CPPs allowed for greater increases in insulin absorption, assumed to be due resistance of D-amino acids to proteases. It is important to note that the CPP was not fused to insulin; rather, they were co administered. A follow-up study demonstrated that electrostatic interactions between insulin and the CPP were responsible for the enhanced absorption of insulin. Another study revealed that the CPP penetratin was best able to increase ileal insulin absorption. Penetratin consists of basic amino acids (lys, arg) along with some hydrophobic regions. Use of CPPs as absorption enhancers represents a relatively new area of research that has the potential to add weapons to the absorptive enhancement arsenal.
Other classes of absorption enhancers have lost favor in recent years due to irreversible epithelial damage. Surfactants such as sodium dodecyl sulfate were shown to cause increased permeability of the GI tract to hydrophilic compounds, but also cause altered cell morphology and cell membrane damage. Sodium dodecyl sulfate shortened microvilli of cells and produced actin disbandment, structural separation of the TJs and damage to the apical cell membrane with even limited exposure. Certain in vivo rat studies support the increase in absorption and revealed the damage caused to be reversible. Bile salts such as sodium cholate and deoxycho-late were originally seen as safe and effective at increasing drug absorption; however, it is now understood that these particles are damaging after long-term use.

Mucoadhesive systems

These systems comprise of synthetic or natural polymers that can bind (adhere) to biological substrates such as mucosal membranes. The phenomenon of bioadhesion allows a greater amount of drug to be available at the target site resulting in desired therapeutic effect. The ability of the mucoadhesive polymers to adhere to mucin layer on the mucosal epithelium can improve oral bioavailability of protein and peptide therapeutics. Drug delivery systems comprising bioadhesive polymers are known to reduce the rate of clearance of drug molecules from the absorption site, thus prolonging the time available for absorption. Bioadhesive drug delivery systems also offer a controlled release of drugs and thus can reduce the frequency of drug administration. Increased oral bioavailability via delayed gastrointestinal transit induced by bioadhesive polymers was shown for the first time by Longer et al. A mucoadhesive polymer should possess ideal characteristics:

a. It should be hydrophilic in nature and be able to form strong adhesive bonds with mucosal membranes because of the presence of large amounts of water in the mucus layer.

b. Polymers with a high molecular weight are desirable because they provide more bonding sites.

c. It should possess optimum surface tension which can enable spreading of polymers onto mucosal/ epithelial cell layer.

d. It should contain adequate hydrogen bond – forming groups such as -OH and -COOH groups that provide strong adhesive bonds between the entangled polymer chains.

e. It should be non-irritant, non-toxic and non-allergenic in nature.

f. It should be chemically inert and may not react with the oral epithelium or the protein/peptide drugs.
g. The cost of the polymer should not be high, so that the final product remains competitive in the market place.

Mucoadhesive polymers were also found to inhibit proteolytic enzymes and/or modulate the permeability of tight epithelial tissue barriers. Bioadhesive polymers are generally classified into synthetic or semi-natural. Synthetic bioadhesive polymers are either polyacrylic acid or cellulose derivatives. Polyacrylic acid-based polymers include carbopol, polycarbophil, polyacrylic acid, polyacrylate, poly(methylvinylether-co-methacrylic acid), poly(2-hydroxyethyl methacrylate), poly(methacrylate), poly(alkylcyanoacrylate), poly(isohexylcyanoacrylate) and poly(isobutylycyanoacrylate). Examples of cellulose derivatives are carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, sodium carboxymethyl cellulose, methyl cellulose and methylhydroxyethyl cellulose. Chitosan and various gums such as guar, xanthan, crylamide-acrylate polymer (PHPA), poly (vinylpyrrolidone) and poly (vinyl alcohol) constitute semi-natural bioadhesive polymers. A wide range of bioadhesive formulations have been investigated for the oral cavity. For instance, luminal degradation of insulin, calcitonin and insulin-like growth factor-I (IGF-I) by trypsin and chymotrypsin was inhibited by employing carbopol polymers. Site specific targeting was achieved with lectins, that possess high affinity for carbohydrate binding with $K_d$ values of $10^4$–$10^6$, high diffusion coefficients and high resistance to proteolytic breakdown. Lectins prefer binding to receptors on the cell surface rather than mucosal gel layer. A significant enhancement in intestinal absorption of 9-desglycinamide, 8-arginine vasopressin (DGAVP) was observed in rats using the weakly cross-linked poly(acrylate) derivative (polycarbophil) dispersed in physiological saline. Surface conjugation of the bioadhesive agent, tomato lectin demonstrated higher intestinal uptake of orally administered inert nanoparticles in rats. Peptide and protein drugs formulated with chitosan–EDTA conjugates inhibited peptide and protein drugs from enzymatic degradation across the GI tract and greatly enhanced their oral bioavailability. Binding patterns associated with wheat germ agglutinin (WGA) and peanut agglutinin (PNA) to glycoproteins in human and rodent colon were examined in gastrointestinal diseases. The authors also investigated the feasibility of utilizing N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-lectin-drug conjugates to deliver therapeutic agents. This report suggested that HPMA copolymer-lectin-drug conjugates provide site-specific treatment of conditions such as colitis and Barrett’s esophagus.
Thiolated polymers (thiomers) have been widely employed as mucoadhesive polymers for enhancing oral delivery of hydrophilic macromolecular drugs. Unlike other mucoadhesive polymers, thiomers form covalent bonds with cysteine-rich subdomains of mucus glycoproteins via thiol/disulfide exchange reactions. These polymers form stronger covalent interactions than other non-covalent bonds such as hydrogen bonds, Vander Waal’s forces and ionic interactions of polymer with anionic substructures of the mucus membranes. Furthermore, thiolated polymers act as enzyme inhibitors, permeation enhancers and efflux pump inhibitors. These polymers are also capable of protecting the incorporated peptides and protein drugs against enzymatic degradation in the intestine. However, stability of thiomers in solutions and gels is a major concern which may reduce the efficacy of thiomers. These polymers are susceptible to early oxidation (pH ≥ 5) unless protected under inert conditions. A recent study aimed at utilizing pre-activated thiol groups to facilitate better stability, prolong retention time of dosage forms, offer mucoadhesion in order to enhance uptake and oral bioavailability. Poly(acrylic acid)-cysteine-2-mercapto nicotinic acid (PAAcys-2MNA) conjugates were synthesized by the oxidative disulfide coupling of PAA-cys (100-, 250- and 450 kDa) with 2-mercapto nicotinic acid (2MNA). In vitro mucoadhesion studies revealed that immobilization of thiol groups on PAA (100, 250 and 450 kDa) exhibited 1.7-, 2.5- and 452-fold improvement in mucoadhesive properties, respectively. Tablets based on PAA-cys-2MNA (100, 250 and 450 kDa) conjugates displayed 5.0-, 5.4- and 960-fold improvement in the mucoadhesion time relative to corresponding unmodified PAAs (Fig. 6). Results from in vitro permeation studies displayed the permeation enhancement ability for preactivated thiomers and was ranked as follows: PAA(450)-Cys-2MNA (h) > PAA(250)-Cys-2MNA (h) > PAA(100)-Cys-2MNA (h) on both Caco-2 cells and rat intestinal mucosa. Also, the apparent permeability of sodium fluorescein was observed to be 5.08-fold higher in Caco-2 cells for PAA(450)-Cys-2MNA (h) and 2.46-fold higher on intestinal mucosa for PAA(450)-Cys-2MNA (m), respectively, relative to sodium fluorescein in buffer only. Such enhancement in permeability as well as better stability render preactivated thiomers as promising macromolecular permeation enhancers and mucoadhesive polymers and may be suitable for non-invasive drug administration.
Comparison of the mucoadhesive properties of unmodified, thiolated and preactivated polyacrylates with an average molecular mass of A: 100 kDa; B: 250 kDa and C: 450 kDa as determined by the rotating cylinder method. Reproduced with permission from reference.

**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 physicochemical stability. Stability problem of emulsions may be overcome by dry emulsion formulations, which are prepared by spray drying, lyophilisation or evaporation. Liposomes have also been exploited to improve the bioavailability of proteins from the intestinal tract. 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. Langer and his colleagues developed polymerized liposomes with covalent double bonds to improve the stability of biomolecules against the harsh environments.

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. 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. Polymeric nanoparticles can be used to easily entrap and encapsulate therapeutic proteins and peptides and lead to the targeted area. It can be smoothly functionalized for off opsonisation and therefore has shown reduced toxicity towards the non-target areas (peripheral tissues) *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 the 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 the alimentary canal and lead to increased bioavailability of therapeutic protein. 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 microscopy has been investigated for prolonged penetration using fluorescein isothiocyanate dextran for in vitro and in vivo conditions. It was concluded that the drug loaded mucoadhesive nanoparticles showed prolonged penetration (5–5.56-fold) as compared to free FITC dextran through confocal microscopy.

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Nanoparticles

Nanoparticles are colloidal carriers with size ranging between 1 to 1000 nm, nevertheless,
particles with size greater than 200 nm. Nanoparticles can be broadly classified into two
types, nanospheres and nanocapsules. Nanospheres are matrix systems in which drug is
uniformly physically dispersed, whereas, nanocapsules are vesicles in which drug is
encapsulated by a polymeric membrane. The physicochemical and drug release properties of
nanoparticles vary with the preparation method. Compared to other colloidal carriers such as
liposomes and micelles, most of the nanoparticles are stable in the harsh GI environment.
They can be tailor made to target certain tissue and achieve controlled drug release
by altering the polymer features and surface chemistry. Nanoparticles are taken up by cells
via endocytosis process, which includes three subtypes phagocytosis, pinocytosis and
receptor-mediated endocytosis. Phagocytosis involves the assimilation of materials up to 10...
µm in diameter especially by macrophages, neutrophils, and dendritic cells. Pinocytosis is a cellular uptake mechanism, generally involves absorption of sub-micron material and substances in solution and its conducted by all cell types. Figure 7 explains the process of endocytosis and fate of nanoparticle after internalization into cell cytoplasm. First, the nanoparticles associate with the cell membrane and subsequently are endocytosed. Then nanoparticles escape from endosomes and degrade in the lysosome. Finally, therapeutic agent diffuses out from lysosome into cytoplasm and transport of therapeutic agent to target organelle takes place which is then followed by exocytosis of nanoparticles. Captivating the benefit of varying pH in the GI tract, pH sensitive nanoparticles can be tailor-made to deliver peptides and proteins to different parts of the intestine. Such nanoparticles are essentially prepared with either polyanionic or polycationic polymer and their mixtures. Mechanism of drug release from nanoparticles is mainly based on the drug dissolution property, swelling pattern of polymer or both of these at a particular pH. Nanoparticles can enhance drug stability, augment mucoadhesion, extend the residence time in GI tract, enhance intestinal permeability and improve the saturation solubility and dissolution rate. Most pH sensitive carriers have been widely used as enteric coating materials for a prolonged period of time and their safety has been approved. More recently, diethylene triamine pentaacetic acid (DTPA) is a complexing agent, known to disrupt intestinal tight junctions and prevent intestinal proteases by chelating divalent metal ions. Su et.al has made an attempt to incorporate DTPA in functionalized nanoparticles (NPs) for oral delivery of insulin. DTPA was conjugated to poly(γ-glutamic acid) (γPGA) to maintain the complexing agent concentrated on the intestinal mucosal surface, where enzyme inhibition and paracellular permeation enhancement are vital. NPs were prepared by mixing anionic γPGA-DTPA conjugate and cationic chitosan (CS). The γPGA-DTPA conjugate inhibited the activity of intestinal proteases significantly and made a transient and reversible enrichment of paracellular permeability. The NPs were responsive to pH alterations, CS/γPGA-DTPA NPs swelled with increasing pH and disintegrated above pH 7.0. Furthermore, the biodistribution of orally delivered insulin by CS/γPGA-DTPA NPs in rats was observed by confocal microscopy and scintigraphy. Experimental results showed higher absorption of insulin from CS/γPGA-DTPA NPs and absorbed insulin was evidently noticed in the kidney and bladder. CS/γPGA-DTPA NPs have produced a prolonged reduction in blood glucose levels; the oral intake of enteric-coated capsule containing CS/γPGA-DTPA NPs had shown maximum insulin levels at 4 hr after treatment. The relative oral bioavailability of insulin was approximately 20%.
Results from this study clearly indicated the potential role of NPs in delivering insulin by oral route.

![Diagram](image)

**Fig. 7.**

Steps detailing the cytosolic delivery of therapeutic agents via nanoparticles carriers. (1) Association of nanoparticles with cell membrane, (2) internalization of nanoparticles by endocytosis, (3) escape of nanoparticles from endosomes, (4) degradation.

Targeted drug delivery is a novel approach for augmenting the oral absorption and hypoglycemic activity of insulin by means of encapsulation in folate-(FA) coupled polyethylene glycol (PEG)ylated polylactide-co-glycolide (PLGA) nanoparticles (NPs; FA-PEG-PLGA NPs). FA-PEG-PLGA NPs (50 U/kg) demonstrated a two-fold surge in the oral bioavailability (twice hypoglycemia) without hypoglycemic shock when compared to subcutaneously administered regular insulin solution. Insulin NPs sustained the blood glucose levels for 24 hr, while, subcutaneous insulin exhibited a transient effect (<8 hr) with a severe hypoglycemic shock. This nano formulation of insulin is suitable for once-daily administration and would be adequate to regulate blood glucose levels for at least 24 hr. In a different study, goblet cell-targeting nanoparticles were designed to enhance insulin oral absorption. The insulin loaded NPs were made using trimethyl chitosan chloride (TMC) surface decorated with a CSKSSDYQC (CSK) cell targeting peptide. Rather than unmodified nanoparticles, the CSK peptide on the surface facilitated the uptake process of nanoparticles in villi. Increase in drug permeation across the epithelium and higher internalization of drug was facilitated by clathrin and caveolae mediated endocytosis of goblet cell-like HT29-MTX cells. Orally administrated CSK peptide modified nanoparticles had shown a better hypoglycemic effect with a higher relative bioavailability of 1.5-fold compared to unmodified NPs. Over all, oral delivery of insulin by CSK peptide modified TMC nanoparticles was effective in targeting goblet cells. Insulin loaded PLGA/HP55 nanoparticles were developed.
to improve the hypoglycemic effect of orally administered insulin. *In vivo* efficacy of nanoparticles was tested in diabetic rats. Upon oral administration (50 IU/kg) to diabetic rats, nanoparticles were able to decrease the blood glucose level rapidly with a maximal effect between 1 and 8 hr. The relative bioavailability of nanoparticles when compared to subcutaneous injection (5 IU/kg) in diabetic rats was 11.3% ± 1.05%. This study revealed that PLGA/HP55 nanoparticles might be used for oral delivery of insulin. Preactivated thiomers are biocompatible and improve mucoadhesion to a great extent. Thiomer nanoparticles are prepared by simple ionic gelation method. *In vivo* studies indicated enhanced bioavailability of protein-based drugs due to thiomer nanoparticulate formulations relative to formulations of non-thiolated polymers. Numerous thiomers have been developed and studied in terms of nanoparticulate carrier systems. Considering the low bioavailability of protein and peptide based drugs when administered orally, very encouraging results have been reached with thiomer based nanoparticles. Table 2 shows the outcomes and best features of thiomers for insulin delivery.

Table 2: Salient features of thiomer nanoparticles in insulin delivery.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Microemulsion</td>
<td>In vitro degradation studies, nanoparticles protected 44.7% of insulin in organic solvent compared to insulin solution</td>
</tr>
<tr>
<td>Thiomers</td>
<td>N.C. in vivo oral administration in rats showed improved bioavailability of nanoparticles</td>
</tr>
<tr>
<td>Thiomers</td>
<td>Oral and nasal application in rats induced glucosuria depression of insulin levels compared to those of insulin nanoparticles</td>
</tr>
</tbody>
</table>

Solid lipid nanoparticle (SLN) is another class of nanoparticles, also widely used in oral protein delivery. SLN does not involve the use of toxic organic solvent and hence provide improved protein stability during formulation. SLN has demonstrated improved oral bioavailability of several therapeutic proteins such as insulin, calcitonin and cyclosporine A. After oral administration of insulin loaded SLN to diabetic rats. Observed a substantial hypoglycemic effect during 24 hr. Relative bioavailability of insulin increased from 1.6% in oral solution to 5% when administered as loaded SLN. Ability of surface modified lipid nanostructures for oral delivery of salmon calcitonin (sCT) in rats. Following oral administration of sCT-loaded CS-coated nanoparticles, a significant and prolonged reduction in the serum calcium levels were obtained as compared to those obtained for sCT solution. Ability of nanoparticles to improve oral bioavailability of macromolecules by protection from harsh GI environment makes them a promising tool for oral protein delivery. In spite of
encouraging results, requirement of high dose and lack of control over delivery hindered development of marketed nanoparticulate formulations.

**Conclusion and future prospects**

Oral delivery of proteins and peptides is the most efficient way to replace the invasive route as well as a very interesting and promising area for research. The strategy for development of oral 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 the oral route such as Oral Recosulin, Octreolin® and Sandimmune® etc., in which a few are in clinical stage of development. As discussed in the review, nanotechnology offers various efficient carriers for the delivery of proteins such as solid lipid nanoparticles, nanostructured lipid carrier, liposomes, niosomes 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.

**REFERENCES**

