Enhancing the Oral Bioavailability of Peptide Drugs by using Chemical Modification and Other Approaches

Naibo Yin1, Margaret A Brimble1, Paul WR Harris2 and Jingyuan Wen1*

1School of Pharmacy, University of Auckland, Auckland, New Zealand
2School of Chemical Science, University of Auckland, Auckland, New Zealand

Abstract
In regards to patient compliance for drug delivery, oral drug delivery is generally the preferred route of administration. However, parental injection of peptide drugs has always been the primary method of peptide drug administration. This is a result of the poor oral bioavailability of peptide drugs, which are typically under 1%. The degradation of peptides in the gastrointestinal (GI) tract by peptidase enzymes and harsh pH, combined with the poor intestinal mucosal penetration properties of the non-drug-like peptide drugs have been identified as the major barriers towards improving the oral bioavailability. Nevertheless, oral delivery of peptide drug presents a significant challenge due to the enzymatic degradation by enzymes in the GI tract and the poor penetration of the peptides across gastro-intestinal epithelium membranes, particularly for adults. Therefore, a novel peptide drug analogue or pro-drug that both protect peptide drugs from degradation by the enzymes in the GI tract that also improves its penetration across the intestinal epithelium membrane would greatly advance the development of peptide drugs as effective candidates for the treatment of various diseases. So far several approaches are being investigated to improve the oral bioavailability of peptide drugs by different researchers. Indications suggest that chemical modification such as incorporation of unnatural amino acids, unnatural peptide bonds, cyclisation and pro-drug approaches as well as nanoparticles and microemulsions offer great potential for improvement and likelihood of enabling peptide drug to be administered orally. This review will focus on the chemical modification methods and other approaches (such as using variable nanoparticular delivery systems), that could be used to overcome the barriers involved in low oral bioavailability of peptide drugs.

Keywords: Gastrointestinal; Lymphatic system; Peptide drugs

Introduction
Peptides are composed of short chains of amino acid monomers linked together via peptide bonds and occur naturally in the human body. Peptides are very specific in activity when compared to small molecules when used as a drug candidate. Generally having fewer side effects, peptides have become popular candidates for drug design. In 2007, there are about 60 approved peptide drugs that are in clinical use and have generated approximately $13 billion USD as of 2010 [1]. Another 140 peptide candidates are in clinical trials, as well as another 500 to 600 in pre-clinical development [1]. The difficulty associated with marketing peptide drugs, however, is the low oral bioavailability as a result of physical and biochemical barriers of the gastro intestinal tract forcing invasive parental delivery methods to be the only practical method of delivery. Such parental injection methods include: intravenous, intramuscular and subcutaneous injections. Unfortunately, parental delivery methods make administration of peptide drugs difficult and painful, which leads to lower patient compliance and ultimately, reduced popularity of using peptide drugs on a frequent basis. Oral administration, on the other hand, would offer easy, convenient administration that can be sold over the counter.

Oral administration of peptide drugs is severely hindered by the physical, biological and chemical barriers of the gastrointestinal (GI) tract. Such chemical, biological and physical barriers present in the GI tract serve to primarily protect the body from pathogens, antigens or any other harmful substances while allowing both digestion and absorption of ingested nutrients or fluids for essential body functions. The chemical barrier for peptide drug delivery is attributed to the proteases and the low pH environments of the stomach that are both essential for the digestion of proteins required for the successful absorption of amino acids [2]. These same proteases that facilitate the hydrolytic degradation of protein in food, however, are also able to facilitate the hydrolytic degradation of peptide-based drugs due to their similarities in chemical structure and functional groups. Major gastrointestinal proteases that are involved in the process of hydrolysis of peptide or protein into amino acids include: pepsin, chymotrypsin, peptatin, trypsin, carboxypeptidase and aminopeptidases [2]. The absorption of proteins and peptides across the intestinal membrane as depicted in fig. 1 is also limited by the physical barriers presented by the unstirred water/mucous layer (UWL), the epithelial membrane of enterocytes (transcellular route) and the tight junctions between the apical ends of the epithelial cells (paracellular route). Furthermore, drug can be uptaken into the blood by receptor mediated endocytosis, or uptaken into the lymphatic system via m cells located at the Peyer's patches. The intestinal epithelial cells are specialised in the absorption of amino acids or dipeptides as opposed to the uptake of larger peptide and proteins. Furthermore, efflux systems located on the surface of intestinal epithelial cells as well as potential metabolism of any peptide drugs inside the intestinal epithelial and liver cells serve to further decrease the oral bioavailability of peptide drugs before it enters the systemic circulation. Micro-organisms located in the intestinal lumen are able to breakdown peptides by the release of peptide metabolising enzymes. This presents a biological barrier for peptide drug absorption [2,3]. As a result of the above factors influencing the absorption and stability of peptide drugs in the GI tract, peptide drugs generally show less than 1% oral bioavailability and therefore considered unacceptable for clinical usage as oral dosage forms [4,5]. Many different methods...
including chemical modification of peptides have been established as attempts to improve oral bioavailability of peptide drugs.

**Chemical Modifications**

**Peptide analogues**

Peptide analogues or peptidomimetics are peptide sequences utilizing unnatural amino acids or unnatural peptide bond linkages between amino acids. Such modifications create a resulting peptide sequence that is less susceptible to enzymatic degradation as naturally occurring proteases are designed to catalyse reactions involving natural peptides and natural peptide bonds. One difficulty in this approach is the activity of the drug must be retained. Unnatural amino acids and unnatural sterics of a peptidomimetic are required to be able to interact with the original intended receptor or targets. N-alkylation and α-alkylation of amino acids can provide steric hindrance against enzymatic degradation. Modification of peptide bonds can create bonds between amino acids that are resistant to peptideases that cleave peptides at peptide bonds to liberate amino acids. Examples of biologically active and enzymatically stable peptide bond substitutes previously used include: reduced amide bond, alkene, hydroxalkene, hydroxyethylamino, dihydroxyethylene and thioamides [6]. Reversal of stereochemistry from natural D-amino acids to L-amino acids has shown to increase resistance to proteases while retaining activity. Increase in lipophilicity or decrease in hydrogen bonding potential by chemical modification of a peptide can improve the cell penetrating ability of a peptide. It has been shown that a chain of methylphenylalanine had improved caco-2 cell culture penetration compared to the same peptide a peptide. It has been shown that a chain of methylphenylalanine had improved caco-2 cell culture penetration compared to the same peptide chain of phenylalanine [7]. Glucagon-like peptide-1 was found to be enzymatically cleaved at α2 by Joseph, J.W et al (2000). Replacement of αα with D-ala2 (chemical structures shown in Figure 2) showed an increase in drug stability, half-life and activity [8]. Mimicking the shape of secondary structures of peptides while changing the functional group of a peptide chain can also be achieved through the use of peptidomimetics. This can help increase enzymatic stability without the loss of activity.

**Peptide pro-drug conjugates**

Pro-drugs are conjugates of drugs that can be easily metabolized using enzymes in the human body or under physiological conditions to release the natural drug and non-toxic by-products. Pro-drugs for oral peptide delivery are designed to remain in the inactive pro-drug form while in the GI tract to be protected from by degradation in GI conditions. Pro-drugs can also improve the physical properties of a drug to increase uptake through the intestinal cell membrane. After bypassing these barriers, the drug is released from the pro-drug by metabolism. Therefore, readily cleavable linkers have been developed to maximise the drug recovery rate within the body. Lipophilic moieties such as long fatty acid chains have been common conjugates used for increasing the lipophilicity of hydrophilic peptides to enhance uptake. The conjugation of palmitic acid to Leucine2- enkephalin via an ester bond combined with the use of nanoparticle GCPQ formulation methods has shown an increase in activity and duration of effect compared to the unconjugated peptide in the same nanoparticle formulation [9]. Another example of increasing the lipophilicity of a peptide drug by conjugation of a fatty acid moiety is the attachment of 1,3-dipalmitoylgllycerol to insulin by an ester bond [10]. This study showed an increase in intestinal penetration of the conjugate compared to the native as well as an increase in stability to enzymatic degradation.

Cova lent attachment of a dimethylmalic anhydride analogue, 3,4-bis (decylthiomethyl)-2,5-furandione to leu-enkephalin showed increased resistance to aminopeptidase, which is the primary enzyme responsible for cleavage of natural leu-enkephalin. The area under curve (AUC) of the lipid conjugated group was found to be 21 folds greater than that of the unconjugated leu-enkephalin group and this was attributed to the increase in membrane penetration of the more lipophilic pro-drug [11]. Conjugation of a glutathione-methionine analogue in another study, to an L-dopa analogue displayed stability towards degradation in the stomach, improved absorption through the intestinal cell membrane as well as spontaneous cleavage and release of L-dopa in plasma conditions in a study by Pinnen et al. (2012) [12]. The conjugation of low molecular weight chitosan to the anti-diabetes drug exendin-4 showed high stability against enzymatic degradation due to the resulting charged nanoparticle-like structure formed. Exendin-4 was conjugated to the low molecular weight chitosan backbone via an easily cleavable disulphide bond. Oral administration of the conjugate was shown to be absorbed into the blood and produced an anti-diabetes effect in type 2 diabetes rat models [13]. The attachment of short carbohydrate units to peptides can also improve intestinal mucosa penetration. Conjugation of lactose, galactose and glucose to the N-terminus of the glutamic acid analogue of gonadotrophin release hormone and the N-terminus of the glutamine analogue of the same peptide showed an increase in caco-2 cell penetration with the highest increase being lactose conjugated to the glutamic acid analogue showing a 7.2 fold increase in penetration [14]. Glucagon-like peptide-1 was diconjugated to biotin via lys4 and lys14 and the conjugate showed a significant increase of the drug AUC in rat models.

**Peptide Cyclic Pro-Drugs**

Another pro-drug strategy is the cyclisation of peptides, which can enhance the cell membrane penetrating ability of a linear peptide. The constrained stereochemistry of the cyclic pro-drugs is thought to be harder for proteases to recognise when compared to the linear, freely rotating peptides. As with all pro-drug strategies, recovery of the original drug is important. To improve the regeneration rate for the
linear peptide cyclisation, readily cleavable linkers have been used in different studies. Coumarin-based linker (structure shown in Figure 3) for cyclic peptides has been shown to improve oral bioavailability for analogues of the tripeptide fibrinogen antagonist Arg-Gly-Asp. The coumarin-based cyclic prodrug has shown increase in lipophilicity as well as mucosal membrane penetration. The peptidomimetic prodrug was observed to be bioconverted into the peptidomimetic form and displayed antithrombotic activity in dog models [15]. Oral bioavailability was improved to 5-10% for the biotin conjugates [16]. It was found that cyclisation of a model hexapeptide (structure shown in Figure 4) caused conformational constraint, which leads to resistance to degradation by enzymes as well as enhanced penetration through a caco-2 cell culture [6,17]. A recent NMR study conducted by Nielsen, D.S et al. (2014) successfully improved the oral absorption and bioavailability in rats of a heptapeptide by introduction of functional groups that help rigidify the overall cyclic peptide [18]. Hill T.A. et al. (2014) showed cyclohexaleucine composed of natural amino acids only was able to show a respectable 17% oral bioavailability [19].

**PEGylation**

PEGylation of peptides involves the covalent attachment of polyethylene glycol (PEG), a non-toxic and non-immunogenic polymer, to a peptide. PEGylation is a commonly used strategy to enhance both pharmacokinetic properties as well as the pharmacodynamics properties of peptide-base drugs. PEG is an FDA approved compound and is non-toxic to use. PEGylation of compounds have also display decreased immune responses that can shorted drug half-life in the body. Earlier attempts at PEGylation used smaller PEG chains of PEG 5k (molecular weight 5kDa). More recent attempts at PEGylation of peptides conjugates uses longer PEG chains as more recent studies show greater activity for the longer PEG conjugates. PEGylation of peptides also can reduce immune responses associated with peptide drugs [20]. PEGylation of bovine lactoferrin (bLf) with PEG 20k and PEG 40k showed an increase in proteolysis resistance as well as a significant increase in intestinal uptake in mature rats [21]. In a continuation of this study, it was shown that both the PEG 20k and the PEG 40k conjugates showed an increase in biological activity compared to the unmodified bLf [22].

**Enzymatic inhibitors**

Enzymatic inhibitors, as the name suggests, are capable of inactivating certain enzymes. Co-administration of enzyme inhibitors specific to the inactivation of GI peptidases that catalyse the metabolism of the administrated peptide drug with the administration of peptide drugs can serve to decrease the degradation of peptides in the GI tract and hence increase the oral bioavailability of peptide drugs. Enzyme inhibitors for peptide drug delivery can be classified into: polypeptide protease inhibitors, peptides, amino acids, and inhibitors that are not based on amino acids [23].

Maiani et al. (2014) was able to demonstrate in a study, the decrease in insulin degradation with the co-administration of protease inhibitors to improve the oral bioavailability of insulin. A significant decrease of blood glucose levels in both lean and diabetes induced obesity rat models as well as a significant increase in plasma insulin levels 20 min and 135 min post-administration of oral insulin with the peptidase inhibitor have been shown in this study [24]. Bacitracin is another enzyme inhibitor that has been used to inhibit the degradation of various therapeutic peptides including insulin, methephamid and buserelin [25-27]. Aminoboronic acid derivatives are amino acid based enzyme inhibitors, which have shown to successfully enhance peptide drug delivery in earlier studies but have decreased in usage due to the pursuit of better alternatives [25]. Polypeptide protease inhibitors, on the other hand, have been used to a high extent as auxiliary agents to overcome the enzymatic barrier of orally administered therapeutic proteins due to their low toxicity and strong inhibitory activity [28]. A Peptidic enzyme inhibitor, aprotinin, is a small protein bovine pancreatic trypsin inhibitor commonly used to improve insulin bioavailability. Aprotinin was used by Kraling M et al. to achieve 6.2% oral bioavailability of insulin, an increase from 5.0%, which was observed using the same formulation approach without aprotinin [29]. Inhibition of enzymes however is known to cause side-effects such as systemic toxicity, disturbed digestion and hyperplasia of the pancreas [30].

**Absorption enhancers**

Another method used to improve peptide oral bioavailability is the co-administration of absorption enhancers. Absorption enhancers are a wide range of chemical compounds through a wide range of mechanisms. Absorption enhancers that have been reported in the literature with some success includes: ethylenediaminetetraacetic acid (EDTA), citric acid, salicylate, N-acyl derivatives of collagen [31-33] cyclodextrins [34], sodium caprylate [35], sodium lauryl sulphate [36] and sodium taurocholate [37]. Absorption enhances act via different mechanisms to increase the penetration of peptides through intestinal cell membranes. Mechanisms of action for absorption enhancers includes: opening tight junctions, changing the membrane fluidity and changing the mucous viscosity [38,39].

Chitosan acts as an absorption enhancer that is able to increase lactoferrin absorption and permeability through the intestinal membrane by opening the intercellular junctions [40]. One patent describes the use of an EDTA-chitosan conjugate for the enhancement of oral protein delivery [23]. In this patent, the covalent attachment of EDTA to chitosan was achieved by the formation of amide bonds between carboxylic acid groups of the polymer. Another example of an absorption enhancer are cell penetrating peptides (CPPs). Co-administration of cell penetrating peptides (CPP) with the peptide drug helps in the intracellular delivery of the macromolecules. Examples of CPPs used include: HIV-1 Tat, penetratin and oligoarginine [41].
study conducted by Liang et al. (2005) on the enhancement of the oral bioavailability of insulin showed the use higher oral bioavailability of fluorescent isothiocyanate labelled insulin covalently conjugated to HIV-1 tat fusion protein compared to the native peptide. Intestinal absorption across the intestinal epithelium showed the conjugate of insulin and TAT was 5-8 times higher than that of free insulin [42]. Fatty acids and glycerides are another class of absorption enhancers that can act as detergents or surfactants, which temporarily disrupt the phospholipid membrane, improving substance penetration through the affected membrane [43]. Studies on sodium dodecyl sulfate, sodium caprate, and long-chain acylaromatics shows increased permeability through the paracellular pathways [44]. Tomita et al. and Lindemark et al. proposed that sodium caprate is able to activate phosphopase C, causing upregulation of Ca++, which is able to open tight junctions and hence improve absorption [45,46]. The drawback of using absorption enhances, however, is the potential toxicity involved. Several reports have been made indicating that absorption enhancers can cause damage, or enter the systemic circulation due to their low molecular mass leading to systemic toxicity [47,48].

**Microemulsions**

Microemulsions are defined as isotropic, thermodynamically stable transparent systems composing of oil, water, surfactant and sometimes, co-surfactant forming particles with droplet size of < 200nm [49]. Microemulsions are typically classified into three classes or a combination of the three classes: oil-in-water (o/w), water-in-oil (w/o) and bicontinuous. The ratio of oil phase, aqueous phase, surfactant and in some cases the co-solvent in an emulsion determines the resulting type of emulsion formed. The type of microemulsion formed is also dependant on the type of surfactant used. Surfactants with a hydrophilic lipophilic balance (HLB) value > 12 primarily favours the formation of o/w emulsions whereas surfactants with a HLB value < 12 favours the formation of w/o emulsion [50]. The main advantages of microemulsions over colloidal systems such as suspensions and emulsions include: low viscosity, higher stability, improved solubility, ease of manufacturing, ease of upscale and improved bioavailability [50].

It has been reported by Wen et al. (2013) that microemulsions were successfully applied to enhance the oral bioavailability of the tripeptide glutathione [51]. Other peptide drugs such as calcine and cyclosporin have also been reported to have improved oral bioavailability when administered in microemulsion form [52-54]. Naicker et al. observed enhanced drug bioavailability for microemulsions as well as a reduction in adverse effects associated with the administration of cyclosporin A. The bioavailability another peptide, SKF-106760, was also observed to be enhanced for microemulsion formulations when compared to the unformulated aqueous solutions [54]. In recent years, self-microemulsifying drug delivery systems (SMEDDDS), which spontaneously emulsify when exposed to the fluids of the GI tract to form microemulsions, have been developed to enhance the oral bioavailability of protein drugs [55,56]. The study by Celebi et al. showed a 30% decrease in rat blood glucose levels after administrating the lecithin-based microemulsion insulin formulation (Cilek, Celebi et al.). The use of large amounts of surfactant has been a concern regarding microemulsion formulations, as surfactants can cause toxicity.

**Nanoparticulate Delivery Systems**

**Liposomes**

Liposomes are aqueous filled structures surrounded with one or more double layers of phospholipids or other amphiphilic lipids. Liposomes are generally spherical in shape with a size ranging from 20 nm to 10 μm and are classified into six different categories: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), multilamellar vesicles (MLVs), multivesicular vesicles (MVs), oligolamellar vesicles (OLVs) and giant unilamellar vesicles (GUVs). Liposomes are useful as drug delivery methods due to the ability to carry both lipophilic drugs as well as hydrophilic drugs. Lipophilic drugs are included in the phospholipid bilayer for delivery whereas hydrophilic drugs are trapped inside the interior aqueous cavity (Figure 5). It has been suggested that drug loaded liposomes can be taken up by endocytosis, transcellular pathways [57,58] as well as via Peyer’s patches [59].

A study by Takeuchi et al. showed enhanced calcitonin absorption with chitosan coated liposomes when compared with uncoated liposome formulations [60]. Pectin-coated liposomes have been shown by Sanko et al. to exhibit improved calcitonin absorption and activity [61]. It was concluded that the polymer cross-linking coatings increased uptake due to the increased retention time of liposomes in the intestine due to the polymer's mucoadhesive properties [61]. A study using insulin as a model polypeptide, Iwanaga et al. showed that a coating of polyethylene glycol or mucin sugar moiety was able to cause a gradual decrease in glucose levels following oral administration in rat models [62]. A Gly-Pro-Gly tri-peptide analogue for the treatment of post traumatic brain injury was shown by Bickerdale et al. to be more readily absorbed and have higher oral bioavailability relative to a saline formulation as seen by the faster appearance of the peptide in blood and greater total AUC respectively [63].

**Nanoparticles**

Alternative particulate carriers, including nano- and microparticles, are often chosen to overcome the problems concerning stability and entrapment efficiency of liposomes. Nanoparticles and microparticles are polymer or lipid fabricated particles having a size range between 1 - 1,000 nm and larger than 1 μm, respectively. Nanoparticles are produced by attachment or entrapment of drug molecules to the polymeric nanoparticle. To overcome one of the concerns of the usage of non-degradable polymers as nanoparticles, biodegradable polymeric nanoparticles coated have been developed. Hydrophilic polymers such as poly(ethylene glycol) (PEG) are especially useful as they are known to be long-circulating particles and have been used as potential drug delivery devices. Such polymers have demonstrated ability to deliver proteins and peptides [64]. Other commonly used nanoparticles used for drug delivery include: poly(DL-lactic-co-glycolic) acid (PLGA), gold, chitosan and gelatine [65]. The advantage of using nanoparticle formulations over other methods such as liposome formulations is the
Pectin polymers are pH sensitive polymers that are insoluble at lower pHs but dissolve in high and neutral pH environments. Coating of peptide drugs with pH sensitive polymers can protect the peptide drug from stomach environments. The pH range of the colon and small intestine, however, are very similar and this reduces the site specificity of this method [79]. Time-controlled release systems are another potential method for colonic drug delivery. Time-controlled release is achieved with enteric coated time-release tablets utilising a hydroxyl propyl cellulose layer [78]. This method however is highly dependent on GI transit time, which can vary from person to person and be affected by diet and GI related diseases [80].

**Conclusion**

The enzymes in the GI tract are specifically designed to break down proteins and peptides into their amino acid counterparts resulting in the low oral bioavailability for the peptide drug that is observed. Researchers have devised and improved upon various methods to improve the oral bioavailability of peptide drugs including the use of penetration enhancers, enzymatic inhibitors, formulation approaches such as liposomes, nanoparticulates, microemulsions, mucoadhesive polymers and colonic delivery. Chemical modification methods including peptide analogue design, peptide pro-drug design, cyclisation and PEGylation have also been focused areas of research for the improvement of peptide drug oral bioavailability. Modest success in improving peptide oral bioavailability have been displayed by such approaches, however, only a very limited number of peptide drugs are in current clinical use in oral formulation forms. Among these approaches, the chemical modification seems to elicit the pathway to administrate peptide drugs orally as this method can enhance both peptide drug enzymatic stability and permeability in vivo. Chemical modification, as well as the combination of chemical modification methods with other synergistic strategies shows the most potential in achieving successful oral delivery of peptide drugs as seen in many examples, most noticeably in the work conducted by Bickerdike et al.

### References


---

Mucoadhesive Delivery Systems

Most mucoadhesive delivery systems are formulated by using mucoadhesive polymers. Mucoadhesive polymers are multi-functional macromolecules, which in addition to their mucoadhesive properties increase the permeability of the drug candidates across epithelial membranes and simultaneously inhibit peptidolytic enzymes [73]. These polymers make close contact with the mucosal layer and therefore exert their effects within a limited area of the intestinal mucosa. Some of the mucoadhesive polymer/copolymers that have shown excellent bioadhesive properties include: sodium carboxymethyl cellulose, polyacrylic acid, traganth, polyvinyl methyl ether co-maleic anhydride, polyethylene oxide and methyl cellulose. Mucoadhesive delivery systems have increased residence time of the system at the absorptive mucosal membrane, leading to increased time available for absorption to occur and hence improved absorption of proteins and peptides [73]. Carboxyl and polyacarbophill show very good mucoadhesive properties at moderately acidic pH values. However, a major challenge posed for per-oral delivery is the presence of soluble mucus in the gastrointestinal luminal juices which deactivates the bioadhesive properties of these systems. Another limitation is the inability of the delivery systems to renew the mucoadhesive surface. Thus high mucus turnover rates impede prolonged adhesion to the mucosal surface.

Colonic Drug Delivery

Another strategy for oral peptide drug delivery is to target the colon. This is based on the premise that the overall peptidolytic or proteolytic activity of the colon is very low compared to that of the small intestine [74,75]. The residence time for a dosage form in this region of the gastrointestinal tract could also be relatively long (10-24 hours in rats), which may allow for the effective absorption of proteins and peptides [76,77]. Colonic drug delivery can be achieved by using pH-dependent release dosage forms, time-controlled release formulations or to exploit the unique enzymes produced by microflora located in the colon. Azo-aromatic pro-drug conjugates have been found to be resistant to digestion in the stomach and small intestine. Azo-aromatic pro-drugs can be cleaved in the colon, releasing the peptide for absorption [78]. Pectin polymers are pH sensitive polymers that are insoluble at lower pHs but dissolve in high and neutral pH environments. Coating of peptide drugs with pH sensitive polymers can protect the peptide drug from stomach environments. The pH range of the colon and small intestine, however, are very similar and this reduces the site specificity of this method [79]. Time-controlled release systems are another potential method for colonic drug delivery. Time-controlled release is achieved with enteric coated time-release tablets utilising a hydroxyl propyl cellulose layer [78]. This method however is highly dependent on GI transit time, which can vary from person to person and be affected by diet and GI related diseases [80].


