Intestinal Permeation Enhancers for Oral Peptide Delivery

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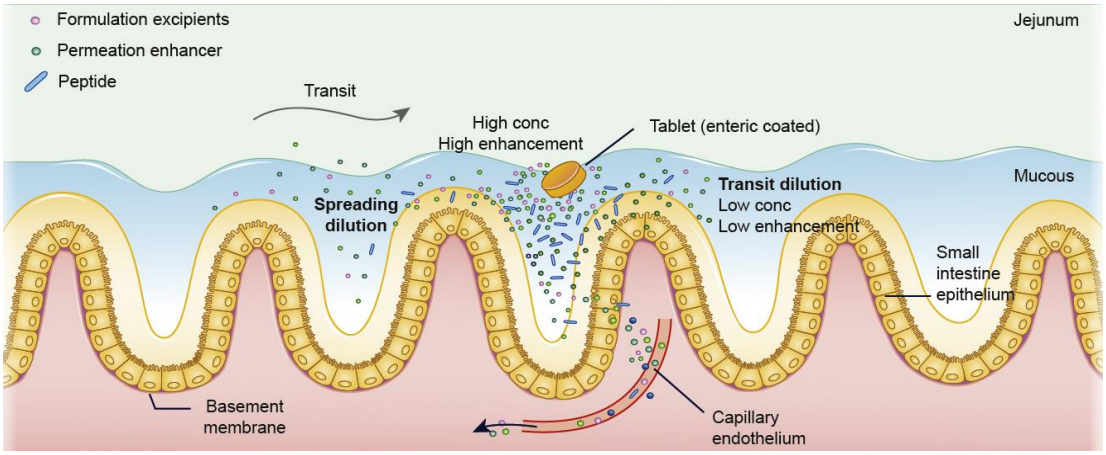
ABSTRACT

Intestinal permeation enhancers (PEs) are one of the most widely tested strategies to improve oral delivery of therapeutic peptides. This article assesses the intestinal permeation enhancement action of over 250 PEs that have been tested in intestinal delivery models. In depth analysis of pre-clinical data is presented for PEs as components of proprietary delivery systems that have progressed to clinical trials. Given the importance of co-presentation of sufficiently high concentrations of PE and peptide at the small intestinal epithelium, there is an emphasis on studies where PEs have been formulated with poorly permeable molecules in solid dosage forms and lipoidal dispersions.

KEYWORDS: Oral peptide delivery; intestinal permeation enhancers; paracellular transport; transcellular; solid dose formulation; surfactants; emulsions

GRAPHICAL ABSTRACT



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1. INTRODUCTION

Growth in global peptide markets has spurred development of technologies that enable oral delivery of poorly permeable drugs. Initial delivery strategies focused on inclusion of candidate excipients that protected the peptide from intestinal degradation and transiently altered intestinal permeability [1]. The majority of oral peptide delivery technologies that are currently in clinical trials use formulations with established intestinal PEs that have a history of safe use in man [2]. Recent clinical data suggests that inclusion of PEs in oral formulations can safely assist absorption of selected potent peptides with a large therapeutic index. For example, primary endpoints were met in a Phase III trial of octreotide formulated in an oily suspension with a medium chain fatty acid salt, sodium caprylate (C_8) [3]. In parallel, a new generation of PEs with more specific mechanisms of action are in preclinical research, and may confer improved safety and efficacy over those currently in development. This article summarises the progress of ~250 PEs that have been tested in preclinical intestinal delivery models (Table I, Table S1). An in-depth review of pre-clinical data is presented for PEs in proprietary delivery systems that have progressed to clinical development. The review by Aguirre *et al*. (this Issue [4]) evaluates the performance of technologies in clinical trials, of which most are enteric-coated solid dosage forms containing PEs. We focus here on how PEs alter intestinal permeability and on innovations that may further assist translation of safety and efficacy outcomes from pre-clinical models to man.

2. THERAPEUTIC PEPTIDES

A drug delivery system that facilitates oral peptide administration has long been desired. There are ~55 therapeutic peptides marketed as parenteral formulations (based on a 9 kDa cut-off in molecular weight (MW)) (Table II) and a further 140 in clinical development [5]. Compared to small molecules, peptides are attractive due to their specificity, potency, efficacy, and low toxicity. Clinical potential of unmodified injectable peptides can be hampered by a short plasma half-life ($t_{1/2}$) due to labile moieties and higher manufacturing costs relative to small molecules. A breakdown of marketed peptide products indicates that injection routes (61%) are the most common, followed by topical (11%), nasal (9%), oral (9%) and ophthalmic (4%), noting that bioavailability is typically low and variable from non-injectable routes [6].

Injection requirements are associated with lack of adherence to dosing regimens, hence the impetus towards long acting formulations that are administered less often. Thus, for glucagon-like-Peptide 1 (GLP-1) analogues, sub-cutaneous (s.c.) injection of exenatide has shifted from twice-a-day administration (Byetta®; Lilly, USA) to once weekly administration (e.g. Bydureon®, Lilly). This was achieved by development of a microsphere-based controlled release system [7], whereas competing approaches have attempted to improve stability and reduce recognition by the reticuloendothelial system by conjugating lipid moieties to amino acid residues or by fusing the analogue to albumen. Although needle fabrication technology has improved in the last 20 years, injections are still inconvenient in the longer term and can delay take-up and adherence to regimes necessitated by chronic diseases. In the case of type 2 diabetes (T2D), early initiation of insulin can slow the progressive destruction of pancreatic β-cells [8], but T2D patients frequently require dose adjustments related to peripheral hypoglycaemia [9]. Oral insulin may reduce such risks because it is absorbed via the portal vein and therefore imitates pancreatic secretion to the liver [10]. This can also reduce two other side effects attributed to s.c. insulin in the periphery: weight gain and lipodystrophy [11].

An oral peptide dosage form would likely reduce costs associated with sterile manufacture of injectables, cold chain, needle disposal, and staff/patient training, but these savings would be offset against the requirement for higher doses compared to injection. A commercial driver for oral peptides is life cycle extension and increased revenue from branded medicines based around new patents. Development of oral delivery systems for approved injectable peptides has the benefit of known pharmacology for the active pharmaceutical ingredient (API), good safety profiles (at least for the injected route) and established analytical detection methods. The most clinically-advanced oral peptide formulations are being developed for diabetes (insulin, GLP-1 analogues), osteoporosis (salmon calcitonin, sCT; teriparatide (PTH 1-34)), and acromegaly (octreotide). Anti-diabetic peptides account for ~40% of peptides in commercial oral peptide delivery programmes and Table S2 details selected patents filed on oral insulin over the last 30 years. Synthesis of injectable anti-diabetic peptides with long plasma $t_{1/2}$ values is also contributing to investment in oral peptide delivery systems (e.g. $t_{1/2}$ = 160 h for the GLP-1 analogue, semaglutide, Novo-Nordisk, Denmark [12]), as they may yield better oral pharmacokinetic (PK)

data than short-acting counterparts. Competition between GLP-1 analogues makes oral formulation a key battleground [5].

Development of non-injected dosage forms has had some commercial successes, including oral desmopressin (DDAVP®, Ferring, Switzerland), oral cyclosporin (Neoral®, Novartis, Switzerland) and nasal calcitonin (Miacalcin®, Novartis). The suitability of commercially available peptides for oral reformulation depends on their physicochemical properties (MW, solubility), chemical complexity, therapeutic considerations (route/frequency of administration, therapeutic index) and cost-effectiveness. Peptides typically exhibit high aqueous solubility and low permeability, properties that unofficially place them in the Biopharmaceutics Classification system (BCS) Class III. Nevertheless, some peptides with cationic and anionic functional groups exhibit complicated pH-dependent solubility, where solubility is high in acidic conditions at pH values below their isoelectric point (pI), and is relatively low at pH values at and above their pI. Many basic molecules rely on acid/base phenomena for dissolution within the stomach and subsequent absorption across the duodenum and jejunum, so peptides with low intrinsic solubility are problematic. For example, insulin dissolves in dilute acid but not at neutral pH, which could manifest as poor dissolution in the small intestine. Peptides that have a MW >6000 Da do not have any appreciable intestinal permeability when delivered orally, this makes insulin (5808 Da) especially challenging, with difficulty decreasing in the order of teriparatide (4118 Da) > exenatide (4187 Da) > sCT (3532 Da) > octreotide (1019 Da). In addition, there is a correlation between MW and susceptibility to proteolysis [13].

An ideal oral candidate peptide should therefore have a low MW, high potency, enzymatic/chemical stability (e.g. cyclised peptides, D-substituted amino acids), a high therapeutic index and be of relatively low cost to synthesise. Desmopressin (MW 1069 Da) contains stable amino acids; it has an oral bioavailability (F) of only 0.17%, so high potency is its key attribute [14]. Prandial insulin is more challenging because it requires three relatively high mealtime doses to reach the required plasma levels per day. The s.c. insulin dose required for management of Type 1 diabetes (T1D) of 0.5-0.8 IU/kg per dose (1.2-1.9 mg); if normalised for an oral system designed for an oral F of 10%-20%, a dose level of 6-20 mg would be required. A recent oral insulin clinical study included 8 mg (240 IU) insulin three times daily [15], whereas

exenatide is injected at a dose of 10 µg and has been tested orally at 15-fold higher doses using the same technology [16].

3. BARRIERS TO TRANSLATION OF PE-BASED ORAL PEPTIDE TECHNOLOGIES

Peptides have poor oral bioavailability due to peptidase sensitivity and low intestinal permeability. They may be sensitive to gastric pepsin and acid- dependent destabilisation of disulphide bridges, hydrogen bonding and electrostatic interactions; although stomach-related breakdown can be overcome by enteric coating (e.g. Eudragit®, Evonik, Germany; Kollicoat® (BASF, Germany) [17]. Enteric coating excipients exhibit pH-dependent dissolution due to deprotonation of weakly acidic functional groups at high pH values. Oral peptide formulations that are enterically coated must be administered pre-prandially to avoid premature release in the stomach when buffered by food. Gastric emptying time is therefore a consideration for peptides like insulin that require absorption to coincide with ingestion of a meal. In the fasted state, capsule dosage forms are consistently found in the small intestine 1 h post administration [18]. The lag time between dose and food intake is an important therapeutic consideration for peptides that require prandial administration (e.g. insulin), but less so for peptides like exenatide and octreotide. Requirement for pre-prandial administration also raises concerns around adherence, when the dosage form must be administered in complex regimes over an indefinite period. Application of Eudragit® coatings without inclusion of excipients that address peptide degradation and poor permeability ultimately will not increase oral F [19, 20].

Upon leaving the stomach the peptide is vulnerable to proteolytic degradation in the lumen, brush border membrane, and in the cytosol of small intestinal enterocytes. The pancreas can produce over 40 g of proteolytic enzymes [21] delivered in 2.5 L of pancreatic juice per day [22]. Large linear peptides including insulin, sCT, glucagon and secretin are sensitive to human intestinal fluid (HIF), while higher stability is noted for short and structurally-confined peptides with stable bonds (e.g. octreotide, cyclosporin and desmopressin) [13]. PEs can also have a dual benefit in inhibiting regional proteolysis, examples being sodium glycocholate [23] and ethylenediaminetetraacetic acid (EDTA) [24]). However, any PE that is a peptide may

itself be sensitive to proteolysis, examples being *zonula occludens* toxin (ZoT) and the C-terminal fragment of *Clostridium perfringens* enterotoxin (C-CPE).

Many PEs are surfactants, so it is possible to protect the peptide in lipoidal dispersions including microemulsions (e.g. Macrulin^{TM}, Provalis, UK) (Section 4.2.2.3). A leading PE-based technology appears to offer peptidase inhibition by non-covalent complexation of the peptide with a carrier (e.g. sodium salcaprozate, Eligen®, Emisphere, USA) (Case 14: Eligen®). Inclusion of peptidase inhibitors like aprotinin can improve oral peptide delivery, however established excipients with similar properties are less risky in terms of toxicology. Acidifying organic acids including citric acid (CA) and tartaric acid lower the optimal pH for proteolysis and can benefit oral peptide formulation, since if they reach a pH of 1-2 units below the isoelectric point, they can improve solubility (e.g. insulin). If however, the pH remains at the isoelectric point for the peptide, the solubility of the peptide will be low, and it may be sensitive to secreted bicarbonate. Acidifiers can also interfere in the dissolution and enhancement action of anionic PEs, some of which exhibit low dissolution and poor enhancement action at pH values below their pKa. For example, the pKa of another lead PE, sodium caprate (C_{10}) (the sodium salt of the medium chain fatty acid, capric acid) is 6.5; should an acidifier decrease pH to 5.5, over 90% of the molecule will exist as an insoluble oil. Further, the hydrophilic-lipophilic balance (HLB) of capric acid is 4.8, which is lower than C_{10} (HLB: 21.8) and ultimately well below the optimal HLB for permeation enhancement [2]. The reduction in luminal pH by co-encapsulated acidifiers can also decrease the dissolution of enteric-coated dosage forms, and efforts to overcome this include separate coating of granules prior to tableting to prevent such interactions. In addition, the cationic charge imparted on many therapeutic peptides (e.g. sCT, pI=10.1) in acidified conditions can increase entrapment in mucus by electrostatic complexation. Finally, some acidifiers chelate metals, which can reduce proteolysis due to removal of peptidase co-factors. Ca^{2+} is also an important component in epithelial tight junction (TJ) formation, and some studies suggested that CA can also increase intestinal permeability via chelation (Table I), although this hypothesis was challenged recently in an *in vitro* insulin permeability study in rat tissue mucosae where the data suggested that the main role of CA in oral peptide formulations is to reduce peptidase activity [25].

Protease inhibitors in oral peptide dosage forms include soybean trypsin inhibitor (SBTI)) [26], aprotinin [23], ovomucoids [27], EDTA [24], sodium glycocholate [28] and camostat mesilate [23]. Some improved absorption of peptides to an extent [23, 28]; a combination of an enteric coating with aprotinin significantly improved oral peptide bioavailability in rats [20]. A safety argument against the use of inhibitors is that impaired dietary protein digestion may occur. However, inhibitors may provide localised protection where the dosage form dissolves and not throughout the GI tract. Nevertheless, agents like aprotinin target ubiquitous biological functions, which raises concerns regarding suitability for oral peptides. SBTI was included in oral exenatide formulations at concentrations as high as 125 mg/dose in clinical trials [26]. Despite the GRAS status of soy protein isolate [29], purified SBTI can cause pancreatic hyperplasia and carcinoma in rats [30, 31] and systemic absorption is undesirable. To this end, retention in the intestine has focused on conjugation to non-absorbed polymers (e.g. chitosan-aprotinin [32], chitosan-EDTA [33]). It is noteworthy that pancreatic peptidases are responsible for only 20% of the degradation of ingested proteins, with the brush border enzymes accounting for the majority [34]. Therefore, inhibitors need also to access the brush border for optimum efficacy. One example was the protection at the brush border membrane achieved for metkephamid by inhibiting aminopeptidase N with puromycin, thereby improving oral F in rats from 0.5% to 3.5% [35]. Ovomucoids also inhibit intestinal serine proteases and are commonly isolated from egg white of avian species [27]. Despite successful peptidase inhibition, the apparent permeability coefficient (P_{app}) of insulin across rat jejunal mucosae was decreased by ovomucoids, so peptidase inhibition is not predictive of improved flux *per se* [36]. Combining peptidase inhibitors with PEs may therefore achieve improved permeability compared to either approach alone [37].

Mucous can decrease the rate and extent that peptides diffuse to the intestinal epithelium. The estimated (variable) mesh pore diameter of porcine jejunal mucous ranges between 200-2000 Å [38], much larger than the molecular radius of most candidate peptides for oral delivery (e.g. insulin <2 Å). Nevertheless, diffusivity (D) of peptides through mucous is affected by viscosity (ranging between 1000-10,000-fold greater than water at low shear [39]) and the MW of the peptide (approximating the dissolved peptide as a sphere) according to the Stoke-Einstein equation ($D = RT/6\eta\pi rN$). Poor diffusivity is directly related to residence time in the small intestinal

lumen, which depends on the peptide surface charge at a given luminal pH, the potential for non-covalent bonding, and susceptibility to proteolysis [40, 41]. Diffusivity measurements for cyclosporine and desmopressin were comparable at selected concentrations across porcine GI mucous, although they have comparable MW but different lipophilicity [42]. The capacity of mucous to reduce diffusivity may in part account for why mucolytics such N-acetylcysteine (NAC), either alone or in combination with non-ionic surfactant PEs (e.g. polyoxyethylene octyl phenyl ether (Triton® X-100, Dow Chemicals, USA) improve fluorescent dextran-4kDa (FD4) and sCT bioavailability in rat intestinal instillations [43, 44]. Despite NAC having established safety in man, with approvals in respiratory and abdominal conditions, there is little interest in combining PEs with NAC or other mucolytics. In part, this relates to high variability in mucous production, the requirement for high concentrations of mucolytics in the formulation, and because disulphide bond reduction can also degrade certain peptides [45]. The capacity of mucous to complex cationic peptides could be attenuated by electrostatic interaction between anionic mucins and cationic mucoadhesive oligomers/polymers (e.g. chitosans), which could reduce loss of peptides that are positively charged at the small intestinal pH. Malhaire *et al* (in this Issue [46]) address the impact of mucous on oral absorption of peptides in nano-carriers in more detail.

The primary barrier to oral peptide bioavailability however, is poor intestinal transepithelial flux, which is due to unfavourable physicochemical properties. Intestinal epithelial permeability of peptides is predicted to be low based on metrics outlined in Lipinksi "Rule of 5" [47], the BCS [48], the Biopharmaceutics Drug Disposition Classification System (BDDCS) [49], the Developability Classification System (DCS) [50] and the Nutraceutical Classification System (NCS) [51]. The "Rule of 5" predicts that a drug will exhibit poor oral bioavailability if it does not exhibit two or more of the following parameters <5 hydrogen bond donors, <10 hydrogen bond acceptors, <500 Da MW, Log P <5 [47], criteria that most peptides do not adhere to. In general, a Log $P_{OCTANOL:WATER}$ value for a peptide is likely to be below -1, unsuitable for passive transcellular diffusion [22, 52]. TJs at the paracellular route prevent movement of high MW solutes (>500 Da), and so this transport route is also not available for most peptides. Of note is that a peptide that does not comply with the "Rule of 5" does not necessarily preclude oral administration, as seen for

desmopressin (1-4 µg dose, 0.2 mg/tablet, 15 H-bond acceptors, 14 H-bond donors, MW 1069 Da, and a Log P: -4.2). Peptides with lower potency (e.g. octreotide: 50 µg dose, Log P: -1.4, H-bond donors: 13, H-bond acceptors: 12, MW: 1019), or greater complexity (e.g. exenatide: Log P: -2, H-bond donors: 58, H-bond acceptors: 67, MW 4187 Da) need formulation assistance to be developed for oral delivery. The amphipathic nature of peptides also gives rise to pH-dependent changes in partitioning behaviour as measured by the $LogD_{pH}$ metric. This has implications for the use of acidifiers that reduce proteolytic degradation [53], as peptides typically have high solubility and low permeability at pH values below their pI due to the protonation of basic amino acid side chains.

Beyond the intestinal epithelium, it is assumed that peptides freely diffuse through the basement membrane and lamina propria, and also passively permeate capillary endothelia endothelium owing to leaky TJs and fenestrations (20-100nm). For insulin and GLP-1, entry into the portal vein mimics physiological secretion, yet more refined studies are required to understand the extent to which intestinal brush border and hepatic metabolism influence oral peptide bioavailability. While the BCS classifies drugs as low permeability, distinction has been made between poor permeation and high metabolism (BDDCS) [49, 54]. This is an important distinction in oral peptide delivery as there may be requirement to combine intestinal PEs with delivery approaches that limit metabolic degradation, not just by peptidases. For example, cyclosporin (Sandimmune®, Novartis, Switzerland) can permeate the intestinal epithelium up to 86%, but the fraction absorbed (F_A) is 35% due to coordinated brush border cytochrome P450 metabolism and P-glycoprotein efflux, and a further 8% is lost through hepatic metabolism to yield an oral F of 27% [55]. Given that many conventional therapeutic peptides (e.g. exenatide, octreotide, liraglutide) have been designated BDDCS Class I (high solubility/extensive metabolism) through *in silico* modelling, it is more difficult to develop peptides within this category. The most promising strategies to limit metabolism include combining PEs with hydrophobisation, structural modification (amino acid substitution and N-terminal amidation) and nanoencapsulation. Other strategies to evade metabolism have been tested pre-clinically include inhibition of cytochrome P450 3A4 (e.g. cyclosporin [56]) or by targeting regions of the GI with lower metabolic activity [57].

Enteric coating also ensures that the peptide, PE, and other additives within the formulation are contemporaneously released in the small intestine. In rat studies, enteric coated tablets increased the efficacy of the PE, palmitoyl carnitine (PC), as measured by an increase in oral F of the transport marker, cefoxitin [58]. Innovative formulation strategies can optimise release and spreading in an epithelial region in order to (i) create a diffusion gradient that improves the rate and extent of peptide flux (ii) decrease luminal residence of peptide to limit exposure to pancreatic peptidases (iii) allow formulation additives to reach a threshold concentration required to improve flux (Fig. 1). Confining the PE and peptide to a small intestinal region will in most cases improve intestinal permeability. For example, the F_{ABS} of cefoxitin was 77% when co-instilled with PC in ligated rat jejunum, but was only 18% in the un-ligated tissue [58]. There are however some exceptions, such as with the use of paracellular PEs, which are required to spread over a wider area to ensure that sufficient number of TJs are opened [59].

Intestinal transit and the requirement for liberation from a dosage form make it difficult to achieve optimal presentation of the peptide and PE at the intestinal wall. Slow infusion/high fluid volume instillation into the small intestine mimics slow dissolution of solid dosage forms, and so it was not surprising that fast infusion of sCT (25 mg/5 mL/25 s) led to increased absorption in dogs compared to slow infusion (25 mg/20 mL/600 s) [58]. The duodenum and proximal jejunum have strong absorptive capacity, but targeting release in this region is hampered by greater brush border enzyme activity [60] and fast transit [18], which invariably disperses the peptide and additives in the dosage form over a larger area. Significant dilution and spreading of a radiopaque dye and CA occurred in the duodenum following initial disintegration from a capsule dosage form. In the lower GI (ileum) however, the dye was more likely to exhibit more optimal plug flow [53]. The average small intestinal transit time for a monolithic dosage form is just over 200 min, irrespective of its density and shape [61]. Given the approximate length of the small intestine (7m), transit can be estimated to ~3.5 cm/min, wherein lies significant difficulty for efficient use of PE. The peptide will quickly pass the optimum region of the small intestine where the peptide and PE are designed to be co-released. PEs that require several hours to increase intestinal permeability will therefore not have that opportunity in the small intestine owing to fast transit. For example C_{10} was a 5-fold more effective PE

at the lower flow rate of 0.1 mL/min compared with 0.2 mL/min in a rat intestinal single pass perfusion model [62]. The most promising PEs are therefore those that work quickly, allowing local co-presentation of the peptide and PE at the intestinal epithelium in high concentrations. Accelerated transit of a formulation (e.g. secretory diarrhoea, thyrotoxicosis) requiring intimate epithelial contact with the epithelium could therefore lead to a slower barrier modification that would result in erratic absorption.

Several strategies have been attempted to improve peptide and PE localisation at the intestinal wall. In contrast to the potential benefits of mucolytics to facilitate better diffusion to the small intestinal wall, others advocate use of mucoadhesive polymeric coatings to prevent spreading and improve co-presentation. The small intestine, however, has a high rate of mucous sloughing that has seen mucoadhesive polymer coating strategies become less popular [63, 64]. Thiolated polymers are mucoadhesives that form covalent disulphide bonds with mucin glycoproteins (reviewed in [65]). Mucoadhesion of polymers can be improved by functionalising with thiol (e.g. 4-fold for alginate with cysteine and 250-fold for chitosan with iminothiolane [66]). The potential of mucoadhesive polymers as additives in oral peptide delivery was highlighted in data showing relative bioavailability (sc) of insulin of 15% following oral administration in mini-tablets containing chitosan-6-mercaptonicotinic acid in rats [67]. Thiomatrix GmbH (Austria) has researched formats of thiolated chitosan and polyacrylic acid that combine mucoadhesion with permeation enhancement and enzyme inhibition. In addition, dosage forms containing superporous hydrogels (SPH^{TM}) swell and take up 100-200 times their original volume and can bring a co-delivered PE and peptide into direct contact with the epithelium. For example, oral F of octreotide in pigs was 16% with trimethylated chitosan [68], and enteric-coated SPH^{TM} improved intestinal residence time by 45-60 min in man [69].

Several investigations yielded insight into the importance of liberation and co-presentation of PEs and poorly permeable molecules in the small intestine. Co-delivery of C_{10} (100 mM) and FD4 in intestinal instillation in rats yielded an oral F of 33%, but staggering delivery of FD4 by 10 min after C_{10} exposure had F_{ABS} of only 9% [62]. Both *in vitro* [70, 71] and *in vivo* [72] testing proved that the epithelial

barrier can recover from PE-induced membrane perturbation, but it depends on the molecule, its concentration and the time of exposure [70, 71, 73]. Recovery of barrier integrity was slow in rats following intestinal exposure to both sodium dodecyl sulphate (SDS) and EDTA for 60 min, but rapid recovery was observed with C_{10} [74]. Similarly, gavage of phenol red and SDS together to rats increased absorption of phenol red, but staggering the delivery of phenol red by 15 min post-SDS exposure had only a modest effect on dye absorption [75]. In a kinetic analysis, co-delivery of C_{10} (100 mM) with cefoxitin for 15 min in rat perfusion was less effective in achieving bioavailability than co-administration of a lower concentration (50 mM) for a longer time period (30 min) [74]. In cases where dissolution of PE and payload are not closely matched, enhancement is weaker than when release is synchronised. For example, comparable dissolution of drug and PE was observed from matrix tablets containing sulpiride, C_{10} and HPMC, and this formulation had high F_{ABS} of 46% for sulpiride [76]. On the other hand, for multilayer tablets where dissolution of C_{10} (>95% at 30 min) was faster than sulpiride (<95% at 30 min), there was a consequential lowering of F_{ABS} (11%). A number of formulation strategies have been attempted to promote co-presentation including formulating peptide and PE with excipients that promote rapid disintegration and dissolution to facilitate optimal presentation at the intestinal epithelium [77]. Others attempt to reproduce pre-clinical effectiveness of fluid instillations by formulating fluidic solutions, lipoidal vehicles, or suspensions in liquid/semi-solid dosage forms that limit the role of dissolution in co-presentation strategies at the epithelium [78].

The majority of PEs in proprietary oral delivery systems are relatively mild surfactant-based detergents requiring high mM concentrations to alter permeability of mucosae *in vitro* and milligram to gram quantities to perform *in vivo*. Depending on the physicochemical properties of the PE and peptide, such high concentrations present difficulty for formulation and process optimisation. For example, salts of medium chain fatty acids have good flowability when granulated, but require inclusion of disintegrants to ensure rapid release from solid dosage forms. On the other hand, it is difficult to formulate tablets containing high quantities of non-ionic surfactants due to low melting points. For example, low temperature trituration and filtration of the PE, sucrose laurate (MP: 37-43°C) through a wire mesh (180 µm) yielded a sticky cohesive powder with poor flowability (Maher S, and Brayden DJ,

unpublished). At the high pressures required for tableting (1.5 kN, 3 kN) sucrose laurate underwent pressure-dependent solid-to-liquid phase transition which immediately reversed upon removal of the compression force, yielding waxy defective tablets with excessive hardness and poor disintegration. Such behaviour can be prevented by inclusion of other excipients, but this may lower the overall concentration of PE available for the formulation. While most peptides are more stable in the relatively quiescent solid form, there are stability considerations that can impact formulation. Inclusion of CA in oral peptide formulations can be problematic in cases where the peptide does not exhibit solid state stability at low pH values. For example, amorphous (lyophilised) insulin at low pH (3-5) decomposed to [desamidoA21] insulin or the covalent amide linked dimer, [Asp_{A21}-Phe_{B1}], which is accelerated by the presense of water [79]. Alternatively, acidifiers and alkalizing agents can attenuate chemical degradation [80]. This is not to say that aqueous solutions are preferable to solid peptide formulations, but one must take into consideration solid state stability of the final dosage form [81].

Low and erratic intestinal flux of peptides from oral formulations is likely to be influenced by intestinal fluid volume. Fasted state fluid volume ranges from 45 mL to 319 mL (83 mL median) that is distributed heterogeneously along the intestine, with an increase in fluid filled pockets (12 mL median volume) in the distal small intestine [18]. The most clinically advanced PEs obey concentration-dependence, so if the dosage form dissolves in a larger intestinal fluid volume, the concentration range required for permeation enhancement may not be reached. Hence high PE doses are required in oral dosage forms in order to overcome potential dilution effects [82]. On the other hand, if excessive concentrations of PE are required to compensate for dilution, there is the possibility of superficial mucosal damage in low volume compartments [83].

The average residence time in the colon (20-30 h) is far higher than that of the small intestine, and the colonic fluid volume is lower (mean fasted state volume: 13mL; mean fed state volume 11 mL [18]); these features could facilitate optimal co-presentation of peptide with PE. Efforts to target this region focus on regional differences in enzyme digestion, pH, time, and pressure [84]. Success in colonic targeting is largely restricted to delayed release formulations of small molecules to

locally treat inflammatory bowel disease (e.g. mesalazine, Pentasa®, Ferring, Switzerland) and also to gastro-resistant prodrug formulations that are activated by bacterial azoreductases (e.g. sulfasalazine, Salazopyrin®, Pfizer, USA). Specific advantages to colonic targeting for systemically-delivering therapeutic peptides are greater sensitivity of the colonic mucosa to PEs [85, 86] and lower levels of luminal and brush border peptidases [21]. However, delayed absorption and low and inconsistent luminal volumes [18] will lead to variable dissolution and reduce access to the colonic mucosal surface. In sum, oral peptide formulation targeting to the colon in association with PEs ultimately has a rather weak rationale for systemic delivery.

4. INTESTINAL PEs

Intestinal PEs are a candidate excipient class that transiently increase permeability of co-administered payloads across the small intestinal epithelium (Fig. 2). The use of PEs has long been met with suspicion, which is influenced by the diversity of substances that alter the intestinal barrier and induce toxicity. Table I and Table S1 together list over 250 substances that have demonstrated permeability enhancement action in pre-clinical studies. These include a range of natural-, semi-synthetic- and synthetic substances: solvents (e.g. ethanol [87]) to chelating agents (EDTA [88]), surfactants (sodium caprate (C_{10}) [89]), endogenous secretions (bile salts [90]), drugs (acetylsalicylic acid [91]), and high MW polymers (e.g. polysaccharides [92]) and bacterial toxins [93]). In 1961, sodium EDTA (50 mg/kg) enabled absorption of heparin following oral administration in gelatin capsules to dogs [94]; three years later it was tested by rectal and sublingual delivery routes in man [95]. Since then, C_{10} was approved in Scandinavia and Asia to improve rectal bioavailability of ampicillin from a suppository (Doktacillin™, Meda Pharmaceuticals, Sweden), although the efficicay of this approach was correlated to non-specific damage to the rectal mucosae rather than paracellular permeability modification [83].

Some PEs are marketed for delivery of poorly absorbed drugs by other administration sites including topical (ethyl oleate/glyceryl monooleate in Minitran® patch; Meda Pharmaceuticals) and buccal (bile salts in Oralyn®; Generex, Canada). Although there are currently no marketed oral peptide formulations containing PEs, several formulations are in clinical development and Chiasma Pharma (USA/Israel) recently filed an NDA for oral octreotide (Mycapssa™). The Eligen® carrier (Sodium N-[8-

(2-hydroxybenzoyl) Amino] caprylate (SNAC) was approved for oral delivery of vitamin B_{12} (Eligen® B12, Emisphere, USA) and is in Phase III for oral delivery of the GLP-1 analogue, semaglutide (Novo Nordisk, Denmark).

PEs act either paracellularly via the opening of TJs or transcellularly through an increase in plasma membrane permeability, or a combination of both (Fig. 2). The number of substances that have been reported to increase transcellular intestinal epithelial permeability outweighs those that increase paracellular permeability by over 10 to 1 (Table I, Table S1). This is not surprising as it is far easier to non-specifically perturb the intestinal mucosae than to selectively open junctional complexes, but it is important to note that <5% of transcellular PEs have progressed to clinical assessment for oral peptide delivery. This indicates high attrition of substances that do not address (i) significant but temporary enhancement, (ii) epithelial recovery and (iii) established safety profile in man. New generation TJ modulators specifically target the paracellular route, and promising results from pre-clinical studies could translate into technologies suitable for clinical trials.

4.1 PARACELLULAR PEs

A system of categorisation designates TJ openers as either 1st generation or 2nd generation. For the most part, 1st generation act through alteration of intracellular signalling mechanisms involved in modulation of TJs, while 2nd generation directly disrupt homophilic interactions at cell adhesion recognition (CAR) sequences between TJ or *adherens* junction (AJ) proteins in adjacent epithelial cells (Fig. 2). The elucidation of proteins that regulate TJ function, in particular actomyosin, occludin, claudin, and tricellulin, has provided molecular understandings of actions induced by both 1st and 2nd generation TJ openers. In the 1980-90s, 1st generation modulators included cytochalasins [96, 97], C_{10} [89], ZoT [98], nitric oxide donors [99], and EDTA [88]. These PEs aided the development of 2nd generation modulators, in particular, sequences targeting the extracellular loop of E-cadherin [100], occludin [101], and claudin (C-CPE [102]) (Table S1). Many patent filings disclose 2nd generation PEs that disrupt AJ and TJ complexes [103]. Selected 1st generation TJ modulators are more clinically advanced, and continuing efforts to discover novel PEs in this category are ongoing. Omitted from discussion in this Section are paracellular PEs that have multimodal action, including C_{10} and chitosan derivatives. In addition,

PEs that increase epithelial permeability in MDCK monolayers were omitted if they have not also been reported to increase permeability in GI-related models.

4.1.1 Paracellular PEs emerging from the study of toxins

A number of toxins have been shown to alter paracellular permeability ([104] and Table S1); knowledge that has assisted in our understanding of TJ structure/function. For example, cytochalasins are a group of small molecule fungal metabolites that cause actin-mediated disbandment of TJ [105]. Likewise, although a large number of patents have been filed for ZoT and its capacity to generate oral insulin delivery in rats [106], the more important output was the discovery of zonulin, an endogenous mediator of epithelial permeability involved in intrinsic control of barrier integrity [107]. An associated discovery was that of larazotide acetate (Alba Therapeutics, USA), an 8-mer peptide that promotes TJ assembly [108] and is currently in clinical development for management of coeliac disease [109, 110].

In their native form, toxins are not realistic candidate PEs due to safety considerations. The initial discovery of protein-based toxins like ZoT, melittin, and CPE was followed by structure activity studies to identify modified analogues that retained enhancement action but not the elements pertaining to toxicity. This approach was effective for CPE (C-CPE) and ZoT (AT1002 [111]), but not melittin [112]. Native ZoT (~45 kDa) increased small intestinal permeability via PKC-dependent cytoskeletal contraction [113], and SAR studies localised the enhancement action to the 12 kDa carboxyl terminal region (termed Delta G) and subsequently to its first six amino acids [114]. This short peptide sequence was designated AT1002 (H-FCIGRL-OH) (Alba Therapeutics (USA)) [111]. While the enhancement action of AT1002 was reversible, high mg concentrations were required to induce a 40-fold enhancement of Lucifer Yellow in Caco-2 monolayers [111] and in addition, the peptide did not increase duodenal absorption of cyclosporin A unless co-administered with a peptidase inhibitor (bestatin) and a surfactant (benzalkonium chloride) [115]. Melittin is a small cell penetrating- and antimicrobial peptide that also modulates the function of a number of mammalian receptors and signalling proteins (e.g. calmodulin [116]). While melittin was an efficacious PE in Caco-2 monolayers [117], there was a reduction in its efficacy in isolated GI tissue mucosae [118] and in rat models [119]. Substitution of selected amino acid sequences in melittin was effective in eliminating

the peptide's ability to form pores in mammalian membranes, but the capacity for permeation enhancement was lost [112]. Viral protein 8 (VP8) is a PE derived from rotavirus that improved enteral delivery of insulin in diabetics rats [120]. A number of patents have been filed for VP8 relating to oral insulin delivery [121]. VP8 altered the distribution pattern of TJ-associated claudin, ZO-1, and occludin, but did not decrease overall expression. Sequence analysis of VP8 showed that regions of this protein have sequence homology with extracellular loops of claudin and occludin. If prospective studies demonstrate that VP8 acts through direct disruption of homophilic interactions between claudins or occludin in adjacent enterocytes, analogues of VP8 could become promising 2nd generation PEs.

4.1.2 Paracellular PEs that bind claudins

CPE is one of at least 14 toxins produced by *C. perfringens* which triggers food borne illness through the formation of ion selective channels in enterocyte plasma membranes, resulting in fluid secretion and Ca^{2+}-dependent enterocyte apoptosis [122]. SAR analysis found that the N-terminal was responsible for pore formation, and the C-terminal sequence (C-CPE$_{184-319}$) was the first protein reported to modulate the function of claudin [102]. While C-CPE is a toxin, its capacity to bind claudins and the design of novel claudin modulating sequences warrants distinction from conventional toxins.

Claudins are a family of TJ proteins (~23 kDa) having at least 25 members; they are expressed at different sites within the body in distinct combinations typically containing 3-5 family members. Claudin proteins are positioned at the apical most point of TJ stuctures organized at the apical neck of polarized intestinal epithelial cells. All claudin family members contain four transmembrane domains that form two extracellular loops and can establish cis- and trans-interactions that are both homophilic and heterophilic in nature to establish the perm-selective paracellular barrier properties of a specific epithelium. These extracellular adhesion points are therefore the most targetable TJ component for modulation of the paracellular barrier. PEs that bind either the first (Claudin-1$_{53-80}$ peptide [123], C_1C_2 [124] and C-CPE [125]) or second (C-CPE [102]) extracellular CAR motifs interfere in TJ formation leading to an increase in paracellular permeability (Table S1).

Comparison of the enhancement action of C-CPE with C_{10} revealed comparable efficacy in rats, but at a 400-fold lower dose for C-CPE (0.1 mg/mL versus 40 mg/mL) [93]. However, these experiments were performed in closed intestinal rat loops, and C-CPE had a lag time of ~60 min before jejunal enhancement of FD4 flux could be detected, whereas C_{10} maximally enhanced FD4 flux in <60 min. Slow onset of enhancement action is not uncommon for peptides that directly modulate the function of claudin as both claudin-derived peptide [123] and the claudin-1 peptidomimetic (C1C2) [124] required long incubation times before inducing a significant alteration in epithelial permeability. Pre-treatment with C-CPE for 4 h prior to administration of PTH improved F_{ABS} of the peptide from 0.6% (co-administration) to 2.7% (pre-treatment) when delivered in a rat jejunal loop, which suggests slow recovery of barrier integrity with this PE [126]. However, formulators cannot use pre-treatment in development of an oral peptide dosage form. Removal of the first 10 amino acids from native C-$CPE_{184-319}$ yielded a truncated structural variant (C-$CPE_{194-319}$) that had a fast onset of action in rat jejunal loops. However, the concentration required to elicit such a response was high at 4 mg/mL (F_{ABS}: 1.3%) and the potency relative to C_{10} was reduced to only 10-fold [126]. Recombinant engineering of C-CPE through random mutation at positions 304 (S-to-A), 305 (S-to-P), 307 (S-to-R), 309 (N-to-H), and 313 (S-to-H) yielded a variant termed m19, which exhibited a 2.1-fold increase in enhancement action compared to native C-CPE, as measured in rat jejunal loops [127].

4.1.3 Paracellular PEs that target E-cadherin and Ca^{2+}

An increase in intracellular Ca^{2+} can increase epithelial permeability through the activation of calmodulin, which activates myosin light chain kinase (MLCK) to phosphorylate actomyosin II, culminating in disbandment of TJs [128]; a mode of action proposed for C_{10} [129, 130]. In contrast, bilateral or basolateral depletion of extracellular Ca^{2+} from cultured intestinal epithelial monolayers increased paracellular permeability by disrupting Ca^{2+} dependent E-cadherin at AJs [131]. Depletion can be achieved by incubating cells or tissues in low Ca^{2+} buffers or using chelating agents (ethyleneglycoltetraacetic acid (EGTA) [131] and EDTA [132]). In addition, barrier alteration is reversed by restoring Ca^{2+} levels [133]. While the AJ does not solely control the gate function of the paracellular route, it plays an important role in formation and modulation of TJs through indirect binding to TJ-associated proteins

(ZO-1) and cytoskeletal reorganisation [134]. For example, treatment with EGTA can activate Mg^{2+} myosin ATPase that is followed by cytoskeletal contraction and disbanding of the TJ [135].

Chelation of extracellular Ca^{2+} remains one of the most widely studied approaches to alter paracellular permeability that has been pursued with excipients including EDTA, EGTA, and CA; frequently as sodium salts (Table I). EDTA forms water soluble complexes with several metals of physiological importance. Although the Ca^{2+}-EDTA chelate is weak compared to lead, zinc, copper, and iron, this interaction is sufficient to alter intestinal paracellular permeability through sequestering the Ca^{2+} required by E-cadherin [133] and by activating PKC [136] (reviewed in [137]). It is not clear how PKC activation results in decreased TJ permeability, since activation by phorbol myristate acetate phosphorylates and inactivates MLCK, leading to a decrease in MLC phosphatase (MLCP) and an increase in TJ barrier integrity [138]. EDTA also improved intestinal permeability in a number of pre-clinical delivery models (Table I).

The ability of EDTA to chelate and deplete extracellular Ca^{2+} can also protect the peptide, as Ca^{2+} is a co-factor for many proteases. The permitted use concentration of EDTA typically ranges between 0.01-0.1% w/v, and the FDA Inactive Ingredients List permits levels of up to 5mg in oral tablets. In theory, if a tablet containing 5mg of EDTA was to dissolve to completion in 5-50 mL of intestinal fluid, the expected regional concentration would range from 0.1 to 0.01% w/v. The permeation enhancement concentration of EDTA tested in animal models ranges between 0.03-0.6% w/v (Table I). In one study, its concentration was 50 mg/mL (5% w/v), which far exceeds the regulatory limits [94]. *In vitro*, the enhancement action of EDTA can often be erratic when solely added to the mucosal surface as opposed to bilateral addition. Apical addition of EDTA (2.5 mM) in Caco-2 monolayers had only a 2-fold increase on the permeability of FD4, whereas basolateral EDTA application produced a 10-fold increase and a bilateral application of the chelator resulted in a 322-fold increase in the permeation of this solute [132].

Oramed Pharma (Israel) has developed Peptide Oral Delivery (POD^{TM}), which improved oral delivery of antidiabetic peptides in dog and pig studies, a progam that

is now in Phase 2 trials. Formulations of exenatide used in a canine duodenal cannulation model contained 150 mg of sodium EDTA [26], a quantity that could result in an estimated luminal concentration of ~0.3-3% (w/v); which is higher than the maximum recommended levels in the FDA Inactive Ingredients List. Oramed have also combined EDTA with insulin (8 mg, SBTI [125 mg], aprotinin [150,000 IU] and omega-3 fish oil [1 mL]) [139]. A formulation containing insulin (8 mg), Carrier (≤150 mg), Adjuvant $_{A}$ (≤ 125 mg), and Adjuvant $_{B}$ (≤ 24 mg) improved glycaemic control in type 1 diabetics [140]. The mode of enhancement action of EDTA at high concentrations has not been comprehensively studied. Pre-clinical testing showed that the effects of high concentrations of EDTA (50 mg/mL;~5% w/v) can be blocked by Ca^{2+} salts, which suggests that enhancement mechanism remains coupled to a paracellular mode of action. Given the role of Ca^{2+} in cell metabolism, attempts have been made by others to avoid the use of chelators by selectively targeting E-cadherin using peptides that scramble intercellular contact interactions [141].

4.1.4 Paracellular PEs that target occludin

Elucidation of the occludin structure, along with reports showing the effectiveness of C-CPE in modulating claudin expression, led to the rational design of a prototype 2nd generation TJ modulator that targets occludin. Peptide sequences that correspond to the first extracellular loop motif of occludin ($OP_{90\text{-}113}$, $OP_{90\text{-}135}$ and $OP_{90\text{-}103}$) can scramble homophilic interactions between adjacent epithelial cells and improve intestinal permeability [101]. However, even the shortest occludin peptide ($OP_{90\text{-}103}$) was only active when added bilaterally (68-fold benefit) or basolaterally (11-fold benefit) to epithelial monolayers on Transwells®. Efficacy (enzymatic/physical stability, targeting) of $OP_{90\text{-}103}$ was improved by synthesis of an all D-form, inclusion of peptidase inhibitors, and conjugation to a hydrocarbon moiety (C_{14}-$OP_{90\text{-}103}$) but, to our knowledge, there has been no further progression of this PE or others that target occludin. A number of other occludin binding peptides have shown enhancement action in epithelial models derived from kidney but there are no equivalent reports in GI delivery models [142].

4.1.5 Paracellular PEs and cytoskeletal reorganisation

Cytoskeletal control of TJ permeability is a widely studied target to improve oral peptide delivery. The phosphorylation of MLC by MLCK results in a conformational shift in the structure of myosin II in enterocytes resulting in cytoskeletal contraction and increased TJ permeability; possibly through ZO proteins and/or cingulin [143]. This is a transient physiological process under non-pathological conditions, but activated MLCK can be associated with chronic inflammatory conditions of certain pathologies [144]. A number of PEs increase MLCK activity including EGTA [135], cytochalasin B [145], cytochalasin D [146] and C_{10} [146]. The cellular control of cytoskeletal organisation is dynamic, with contraction occurring when MLC is phosphorylated and relaxation occurring when MLC is de-phosphorylated via an endogenous phosphatase. Given such dynamic control of the phosphorylation state of MLC, selected inhibition of MLCP prevents dephosphorylation, thereby indirectly increasing the level of MLC-P without the necessity for MLCK activation. Two rationally-designed candidates, permeant inhibitor of phosphatase (PIP) peptide 640 (RRDYKVEVRRKKR-NH_2) and PIP peptide 250 (RRFKVKTKKRK-NH_2), target the interaction between sub-units of MLCP; specifically interaction between protein phosphatase 1 (PP1) and MYPT1 (myosin phosphatase target subunit) (PIP640) and the interaction between PP1 and CPI-17 (C-kinase-activated protein phosphatase-1 (PP1) inhibitor–17kDa) [147]. Both PIP 640 and PIP 250 were all D-forms and cationic amino acids were introduced to mimic the behaviour of cell penetrating peptides (CPPs). Both peptides increased FD4 permeability across Caco-2 monolayers and also insulin delivery in rat intestinal loop instillations (F_{REL} 4% (PIP 640) and 3% (PIP 250)) (Table S1). PIPs caused a gradual decrease in transepithelial electrical resistance (TEER) in Caco-2, but the high concentrations of PIP 250 used in instillations were effective with a shorter onset time.

4.2 TRANCELLULAR PEs

Permeability enhancement is achieved for the most part by PEs acting on the plasma membrane including fatty acids, acylcarnitines, acylated amino acids, alkyl polyethoxylates, glyceryl polyethoxylates, channel forming peptides, bile salts, glycerides, sucrose esters, polysorbates, enamines, and salicylates (Table I, Table S1). The majority of these are surfactants, and while several thousand surfactants are used in a diverse range of pharmaceutical, cosmetic and industrial applications, only a few meet the criteria for success (Section 4). Transcellular PEs can be broken down into

(i) surfactants that alter the integrity of the enterocyte plasma membrane to enable better transepithelial flux of the co-administered molecule (this Section), (ii) complexing agents that bind to the molecules and transiently increase their passive transcellular diffusion (Section 4.3), and (iii) non-surfactant PEs with poorly defined modes of action (Section 4.4).

4.2.1 Soluble surfactant PEs

Surface active agents share the capacity to preferentially adsorb at the boundary between distinct phases of matter to lower interfacial tension, which is a direct consequence of structural amphiphilicity. They are broadly categorised as soluble (this section) or insoluble (Section 4.2.2) amphiphiles, which depends on the relative contribution of the hydrophobic moiety (hydrocarbon chain length, degree of branching, aromaticity) and the hydrophilic moiety (anionic, non-ionic, amphoteric or cationic). While both soluble and insoluble amphiphiles are surface-active and are surfactants *per se*, the term is more commonly applied to soluble amphiphiles.

The most common surfactant-based PEs are soluble in an aqueous continuous phase, and the most effective enhancers are those that exist in high concentrations in the monomeric form, but not so high as to impede their ability to insert into enterocyte plasma membranes. Soluble surfactants can be sub-divided based on their physical structure at high concentration, with sub-group 1 exhibiting lyotropic mesomorphism, a capacity to form supramolecular cubic, hexagonal or lamellar liquid crystal structures. Most PEs lie within the soluble sub-group 1, including fatty acid salts, non-ionic surfactants (polysorbate 20) and acylcarnitines. Surfactants in soluble sub-group 2 do not exhibit lyotropic mesomorphism [148], although this group also contains PEs that have progressed to clinical evaluation (e.g. bile salts). The hydrophobic moieties of sub-group 1 are usually aliphatic, while sub-group 2 are aromatic. This distinction is important because the behaviour of bile salts differs from aliphatic PEs, making it difficult to correlate physicochemical properties and permeation enhancement. A wide range of metrics relate surfactant structure and physical behaviour to transcellular permeation enhancement, including the critical micelle concentration (CMC), HLB, as well as the magnitude of change to surface tension ($\Delta\gamma$), and aggregation number (n).

4.2.1.1 CMC in permeation enhancement

In dilute form, the monomeric surfactant exists as a molecular dispersion (<1nm) and above the CMC they self-associate into micelles, which are thermodynamically stable, isotropic dispersions that lie within colloidal dimensions (1-500nm). Although there is no linear relationship between CMC and permeation enhancement, it represents the concentration of surfactant that exists in solution (monomeric form), which is ultimately responsible for detergent action and transcellular permeation enhancement. CMC is an indication of the solubility of the monomeric form, as any further addition leads to formation of micelles. Therefore, the CMC value indicates the amount of free surfactant available to diffuse from the bulk vehicle to the plasma membrane to initiate enhancement. It is a common misconception that detergent effects only begin above the CMC; however, it is unclear if micelles can alter membrane permeability directly. For example, C_{10} exhibits detergent-based perturbation of Caco-2 monolayers at 10 mM [149], but its CMC in physiological buffers is ~26 mM [150]. While the intestinal epithelial enhancement capacity of MCFAs clearly increases above their CMC, this is because micelles are a reservoir for efficient replenishment of free monomer lost from solution upon interaction with enterocytes, in addition to solubilising fragments of cell membrane following detergent action.

In general, the higher the CMC the greater the detergent effect, which is why anionic surfactants are more effective PEs compared to non-ionic surfactants, although these actions also correlate with a higher level of membrane damage. The typical CMC range for non-ionic and amphoteric surfactants is 0.1-1 mM, which means that the free surfactant monomer concentration is far lower than for anionic surfactants [151-153]. In addition to a lower free monomeric concentration, non-ionic surfactants commonly form larger micelles, which make them effective drug solubilisers. Non-ionic surfactants can however, still exhibit strong enhancement. Despite their higher CMC values, the efficiency by which surfactants penetrate the plasma membrane often decreases at much higher CMC values [153]. From a molecular perspective, surfactants that have very high aqueous solubility have a significant imbalance in their HLB. For example, ionic surfactants (e.g. carboxylates) with very high CMC values normally have very short hydrocarbon tails that are less efficient at perturbing phospholipid bilayers (e.g. hydrocarbon chains $<C_6$). Likewise, non-ionic surfactants that have very high HLB values have either very short alkyl chains ($<C_4$) or very long

ethoxylate moieties (E_{20}+), both of which influence their interaction with the plasma membrane.

The relationship between CMC and enhancement action is confounded when using the surfactant at concentrations below their CMC value. Consider the CMC values in water of an homologous series of fatty acid carboxylates, C_6 (CMC = 1000 mM [154]), C_8 (CMC = 351 mM [155]), C_{10} (CMC = 95.5 mM [155]), C_{12} (CMC = 23 mM [155]), C_{14} (CMC = 6.9mM [155]), C_{16} (CMC = 3.9mM [156]), C_{18} (CMC = 1.8mM [156]). When comparisons are made between C_8-C_{12} at relatively low concentrations (e.g. 10 mM, the common concentration used in Caco-2) the detergent effect is strongest for C_{12} because its greater hydrophobicity will permit more efficient insertion into the plasma membrane. While C_{14} to C_{18} are even better able to insert into the plasma membrane, the free monomer is only present at 6.9-1.8 mM concentrations, indicating that there is less free monomer available for longer chain fatty acids. If the concentration of C_{10} is increased to 100 mM (the level of C_{10} used in animal models), the monomer concentration of C_{10} (95 mM) that is available to alter permeability is far in excess of that of C_{12} (23 mM), indicating that C_{10} will demonstrate more efficacious enhancement compared to C_{12}. This point is important in candidate development, because a PE that is the most effective in Caco-2 monolayers and isolated intestinal mucosae (where effective concentrations lie below the CMC), might not be the most effective in animal models. Furthermore, C_6 and C_8 have significantly higher CMC values than MCFA owing to very high aqueous solubility, but their short alkyl chain length does not favour membrane penetration. It is not always the case that surfactants with low CMC values are more efficient at inserting into the plasma membrane. Due to unusual stereochemistry a lower molar ratio of cholate (0.5) was required to completely solubilise PC bilayers compared with sodium deoxycholate (0.7) despite a higher CMC (16 mM versus 6 mM) [157]. Nevertheless, sub-CMC concentrations of deoxycholate had significantly greater enhancement action compared with cholate in Caco-2 monolayers [158].

Any molecule or physical change within the dispersion that lowers the free monomer concentration can alter the rate and extent of enhancement. Sequestration of free surfactant into mixed surfactant micelles with endogenous or dietary surfactants (e.g. bile salts, phospholipids, mono- and di-acyl glycerol), emulsification of dietary lipids

and/or adsorption to solid particulates all have the potential to alter enhancement action. Although the formation of mixed micelles does not reduce the CMC of a surfactant PE, the sequestration of free monomers into mixed micelles with endogenous surfactants effectively removes the monomeric surfactant and limits enhancement action. Enhancement action of alkyl maltopyranosides (C_{10}-C_{14}) across Caco-2 monolayers were therefore attenuated in the presence of bile salts and lecithin due to removal of monomers into mixed micelles [159]. PEs cannot avoid endogenous bile salts within the upper GI, where levels are 2-6 mM in the fasted state and 7-16mM in the fed state [160], although one would expect such loss of efficacy to be reduced at *in vivo* PE dose levels above their CMC, as free monomer concentration would be efficiently restored and can offset initial removal into mixed micelles by the presence of bile salts. Low concentrations of surfactant-based PEs often cause a rapid decrease in TEER in Caco-2 monolayers, but then no further decrease is detected over time [150, 161]. Initially, the predominant monomeric form is likely to have stripped phospholipids from the plasma membrane to reduce TEER and increase paracellular permeability, but then the free monomer concentration is reduced due to incorporation into the membrane and into mixed micelles. It is clear therefore that the free monomer concentration of surfactant significantly varies depending on the environment.

The capacity of ionic surfactants to form micelles can be impacted by increasing electrolyte concentration or by altering pH to reduce surfactant ionisation. Given that micelle formation is an index between water/hydrocarbon repulsion and ionic repulsion between head groups, an increase in ionic strength of the counter-ion can neutralise repulsion between head groups and ultimately reduce the CMC (e.g. bile salts [157]). On the other hand, decreasing the pH of C_{10} below its pKa leads to the formation of insoluble capric acid, which does not form micelles, and effectively converts C_{10} into an insoluble less efficient PE surfactant that interacts poorly with the intestinal epithelium.

4.2.1.2 HLB in permeation enhancement

High HLB values (10-20+) are assigned to surfactants with high aqueous solubility and low values (1-10) to those with low solubility. In general, the most effective PEs in advanced pre-clinical or clinical testing are low MW, anionic/non-ionic surfactants that have HLB values of ~15-25 (e.g. C_{10}, lauroyl carnitine). It is however, not

possible to predict enhancement potential for a candidate PE from HLB alone, but prediction accuracy can be improved when values are aligned with other common metrics including CMC, change in surface tension ($\Delta\gamma$), and aggregation number as well as structural information (MW, alkyl chain length (C_x), ethoxylate chain length (E_x) and pKa). Detergent effects are evident at HLB values of 13-18, yet many anionic detergents and PEs have HLB values above this range, such as the most effective carboxylates (C_{10}, HLB 21) and sulphates (SDS, HLB 40). A high HLB value therefore does not necessarily correlate with a high CMC, nor will it predict a strong detergent efficiency or capacity for permeation enhancement. While both the CMC and HLB give an indication of surfactant dispersibility, the key difference is the inability of HLB to distinguish between solubility in the monomer and micellar forms. For example, Poloxamer 188 (P188) ((polyoxyethylene-polyoxypropylene-polyoxyethylene ($E_{80}P_{30}E_{80}$)) has a HLB value of 29, which might suggest a high CMC (high free monomer solubility), yet monomer solubility for P188 is low (CMC 0.8 mM), indicating good solubilisation but poor detergent and permeation enhancement effects.

4.2.1.3 Surfactant structure in permeation enhancement

Surfactant structure plays a significant role in enhancement action. Ionisable functional groups in charged surfactants prevent close packing of hydrophilic head groups and the efficiency with which new surfactant monomers are inserted into micelles. This ultimately leads to higher CMC values, improved detergency, and enhancement action compared with non-ionic surfactants. The CMC for a comparable series of dodecyl hydrocarbon chains varies by three orders of magnitude depending on the hydrophilic head group: dodecyltrimethylammonium chloride (CMC 20 mM), SDS (CMC 8 mM), disodium 1,2-dodecylsulphate (CMC 40 mM) and $C_{12}E_6$ (CMC 0.09 mM). Divalent counter ions can also reduce repulsion between ionised hydrophilic moieties leading to significant lowering of the CMC. In some cases, divalent ions are known to complex and neutralise surfactant charge leading to precipitation of fatty acid (for example as Ca^{2+} salts) and decreased enhancement action [162]. Therefore dietary Ca^{2+} could have an impact on enhancement action; for example, milk altered the interaction of both fatty acids and bile salts with colonic epithelial monolayers [163].

Cationic and anionic surfactants are more commonly used to improve penetration through skin because the stratum corneum is more resilient that the intestinal epithelium. When these surfactants are used to improve flux across the skin they are referred to as penetration enhancers. The behaviour of zwitterionic surfactants is similar to mild non-ionic surfactants and some examples show promise as PE candidates in intestinal tissue (e.g. dimethyl palmitoyl ammonio propanesulfonate (PPS) [164]). Ethoxylates are commonly used non-ionic surfactants and ethoxylate chain lengths between E_{10} and E_{20} are more effective PEs than E_{20}-E_{40}, furthermore, alkyl chain lengths between C_{12} and C_{16} had the strongest enhancement of poorly permeable marker molecules [165]. In rat stomach, $C_{12:0}$ and $C_{18:1}$ from an alkyl series of C_{12}-C_{18} exhibited the strongest enhancement action with ethoxylate chains between E_{10} and E_{20} [165]. The hydrophobic moiety of the surfactant also influences enhancement action. Traube's rule states that for a homologous surfactant series, the concentration required for an equal lowering of surface tension in dilute solution decreases 3-fold for each additional methyl functional group. Such a rule indicates that the surface saturation for surfactants will occur at lower concentrations, which is why CMC decreases so significantly with increasing alkyl chain length, and goes some way to explaining why there is such a significant increase in intestinal epithelial permeation enhancement in MCFA moving from C_8 to C_{12}. When the CMC and efficiency of membrane insertion are both considered, the most effective alkyl chain length for MCFA and alkyl sulphates is between C_{10} and C_{12} [150, 166]. Fatty acids of uneven chain length are less widely studied than conventional fatty acids, but they also demonstrate efficacy (e.g.C_9, C_{11}, $C_{11:1}$ [70]). Enhancement action of $C_{11:1}$ (FD4, F_{ABS} 21.8%) was comparable with C_{10} (FD4, F_{ABS} 22.4%) in rat jejunal intestinal instillations. However, performance $C_{11:1}$ (FD4, F_{ABS} 40.2%) in solid dosage forms was improved in the same model compared to C_{10} (FD4, F_{ABS} 26.3%). The difference in CMC between C_{10} (23 mM) and saturated $C_{11:0}$ (5 mM) would suggest that a higher level of C_{10} will be available to enhance permeability in instillations at a concentration of 100mM, yet unsaturation of the $C_{11:1}$ (17 mM) at carbon 10 significantly raises the CMC. Here, the increase in CMC for a longer alkyl chain with improved membrane perturbation appears to strengthen the permeation enhancement potential of $C_{11:1}$ compared with both $C_{10:0}$ and saturated $C_{11:0}$ [70].

4.2.1.4 Mechanism of action of soluble surfactants

The capacity of surfactants to alter intestinal permeability relates to detergent-like action, where the monomeric form adsorbs and penetrates the mesophasic plasma membrane leading to removal of membrane constituents. The concentration of surfactant that can be accommodated in the plasma membrane influences permeability, and so enhancement is a function of both the physicochemical properties of the PE and the composition of the enterocyte plasma membrane. For example, the phospholipid profile of enterocytes is dependent on the species, the levels of maturation along the villi, the region of the intestine, and even within specific micro-domains of individual enterocytes [167]. Diet can also alter composition of the plasma membrane, which can influence fluidity and permeability [168]. The plasma membrane itself can therefore contribute to intestinal region-specific enhancement and variable efficacy of surfactant-based PEs. For example, it is more difficult for the surfactant Triton® X-100 to penetrate membrane domains rich in sphingomyelin (SM) compared with phosphatidylcholine (PC) [169]. Although both phospholipids are insoluble swellable amphiphiles, SM forms more tightly packed bilayers due to higher hydrocarbon saturation [170] and strong association with cholesterol [171] (reviewed in [172]).

Surfactant-based PEs increase transcellular flux by altering the integrity of the plasma membrane of intestinal epithelial cells. There is initial adsorption of the surfactant, followed by penetration of the monomeric form into the outer layer of the phospholipid bilayer, and then a flip-flop into the inner leaflet [173]. Studies in model membranes indicate that the bilayers can accommodate some surfactant without loss of integrity [152]. At a certain concentration, the penetration of surfactant may fluidise the plasma membrane. For example, insertion of octylglucoside into unilamellar phosphatidyl choline (PC) vesicles led to an increase in diphenylhexatriene fluorescence anisotropy [174]. An increase in membrane fluidity is facilitated by an initial swelling of the bilayer in to a more expanded state (e.g. swelling upon insertion of octylglucoside into unilamellar PC vesicles [152]). In this state, the surfactant may lead to an increase in membrane permeability without causing lysis and micellar solubilisation. For example, increased permeability of phosphatidylcholine (PC) bilayers to a marker solute (Mn^{2+}) was observed following treatment with sodium deoxycholate [175]. The insertion of a non-native surfactant into bilayers alters the natural packing integrity of cholesterol, phospholipids and

proteins, which alters membrane fluidity. C_{10} appears to disrupt intermolecular forces and packing organisation of brush border membrane vesicles (BBMV), as indicated by a decrease in fluorescence polarisation of two fluorophores that specifically label the interior and exterior of the bilayer [176, 177]. Similar behaviour was observed with other fatty acids and bile salts [158].

A threshold of surfactant is eventually reached where no further expansion of the bilayer can occur without destabilisation. In membrane models, the continuous addition of surfactant results in linear increase in surface pressure to a plateau, above which surface pressure decreases and membrane integrity is reduced (e.g. Brij® 70 [175]). Because mixed micelles are not detected immediately following a drop in surface pressure [165], it is possible that phospholipids (PC and SM) and proteins are dislodged and exuded into the luminal fluid, an event that could be associated with increased permeability without initial lysis [152]. The driving factors for removal of membrane constituents are the high surface pressure, the dynamic adsorption/desorption of soluble surfactant, and their ability to bind proteins and lipids. Above this concentration, the destabilising effect of surfactant on the membrane causes buckling, lysis and fragmentation in to laminar membrane segments that are exuded into the luminal media. These fragments form laminar cell membrane fragment-surfactant mixed micelles, as illustrated in the Small and Mixed-Disc models [148]. Further addition of surfactant decreases the ratio of phospholipid, protein and cholesterol in the mixed micelles and reduces their size [178].

4.2.1.5 Lead soluble surfactants as intestinal PEs

Soluble surfactants represent the largest category of PEs, with some being used in oral peptide clinical trials (Aguirre *et al* in this Issue [4]). In many cases the extent of permeation enhancement is still relatively low because either the CMC is too low to permit efficient alteration in membrane integrity or because their alkyl chain length does not permit insertion into the plasma membrane. At the other extreme, strong detergency may go beyond that of endogenous detergents and might not be safe. The leading candidates are discussed in following sections.

Case 1: C_{10}

The capacity of C_{10} to clinically improve intestinal drug absorption was demonstrated in rectal suppositories of ampicillin, for which it gained approval in Sweden (1992) and Japan (1985). Doktacillin™ suppositories were marketed by Astra Pharma (Sweden) and contained ampicillin (250 mg), C_{10} (25 mg) and hard fat (950 mg, Pharmasol™ B-105, NOF Corp. Japan). The inclusion of C_{10} in this formulation improved rectal F of ampicillin in man from 13% to 23% [83]. Doktacillin™ is now part of the portfolio of Meda (Sweden), but it is no longer marketed. In the early 1990's studies demonstrating enhancement action and mode of action where carried out in Caco-2 monolayers [162], rat intestinal mucosae mounted in Ussing chambers [179], and rat *in situ* intestinal closed loops [20] (Table I).

In the last 30 years, C_{10} became a candidate PE in a number of proprietary formulations of biomolecules such as proprietary oligonucleotides [180], desmopressin [181], acyline [182], low MW heparin (LMWH) [72] as well as poorly permeable small molecules (e.g. alendronate [181], pemetrexed [181], and zoledronic acid [89])). C_{10} is the key MCFA in the Gastrointestinal Permeation Enhancement Technology (GIPET™, Merrion Pharmaceuticals, Ireland [183]) and enteric-coated oral solid dose formulations containing C_{10} have been in clinical trials for oral delivery of insulin and GLP-1 analogues [181]. C_{10} is also listed in patent filings by Biocon (India) as an additive to improve oral delivery of a PEGylated alkylated insulin (reviewed in [2]). C_{10} is efficacious in both small and large intestine, although it was more effective in the colon for FD4 and insulin in rat intestinal instillation [20, 70, 118, 184, 185]. Disparity is also noted between the effective enhancement concentrations of C_{10} *in vitro* and *in vivo*, which relates to differences in GI transit, release kinetics, dilution, interaction with bile salts and dietary lipids.

Typical effective concentrations of C_{10} vary between those for Caco-2 monolayers (10 mM; 2.5 mg/mL buffer [119]), isolated tissue mucosae (10 mM; 2 mg/mL buffer [119]), loop gut instillations (50-100 mM; 10-20 mg/mL saline [118]), and mini-tablet insertion to rat loops (500 mM; 100 mg/mL saline [70]). Efficacious concentrations in larger species include those for intra-duodenal intubation in dogs (185-500 mM; 550-1650 mg/15mL [180]), intra-duodenal administration to pigs (500-1000 mM; 2.5 g [186]) as well as oral tablet delivery in dogs (150-990 mg) and man (660 mg) [187]. In general, the formulations tested in pre-clinical models have to be refined for

clinical application for practical reasons. When mini-tablets (50 mg) containing FD4 (10 mg), C_{10} (30 mg) and microcrystalline cellulose (Avicel® PH102, FMC Biopolymer, UK) were inserted into the rat small or large intestine with a small volume of PBS, the F_{ABS} of FD4 was improved from 7% to 26% in jejunum, and from 2% to 30% in colon. In rats, the oral F of a short cyclic peptide that functions as a GP IIB/IIIA receptor antagonist, DMP 728, was increased from 2.4 to 6% when delivered in size 9 microcapsules containing ratios of C_{10} (40%), PEG (13.4%), PEG1450 (13.4%), water (26.8%) and the DMP 728 salt (6.3%) [188]. Formulation of DMP 728 in hard gelatin capsules containing 150 mg of C_{10} improved absorption in dogs by approximately 3 fold with an increase in oral F from 5% to 19%, again with considerable inter-subject variability. Relating pre-clinical data in rodents to man is difficult because normalised doses of active and excipient used in rats are far higher than the doses that can be administered in clinical trials. For example, administration of 10 mg of FD4 to rodents (40 mg/kg) would normalise to a dose of nearly 2.8 g in man.

The ability of C_{10} to improve oral absorption of oligonucleotides was tested in pigs and dogs, and in clinical testing by Ionis Pharma (USA, formerly Isis Pharmaceuticals). Oral delivery of enteric-coated tablets with an oligonucleotide (240 mg) and C_{10} (990 mg) yielded an oral F of 1.7% in beagles, although a therapeutic threshold was achieved [180]. Disintegration of this oligonucleotide formulation occurred in less than 60 min, where the rate of C_{10} release was linear from 5% at 15 min to 85% after 45 min, suggesting that ~25% of C_{10} was released in 30 min at an approximate rate of 9 mg/min. Although no data is known about release *in vivo* or on the local concentration that is present at the intestinal mucosa, it is probable that slow release along with relatively fast transit and variable fluid volume results in jejunal apical membrane exposure to low mM concentrations of C_{10} for a short duration, consistent with *in vitro* studies. As the concentration of C_{10} that is effective *in vitro* is less effective in intestinal instillations, slow release from the Ionis formulation may have been responsible for the poor data in dogs. Delivery of C_{10} (2.5 g; 500-1000 mM) with 250 mg oligonucleotides to pigs via jejunal intubation was equally disappointing (F for Ionis-2503: 1%, F_{ABS} for Ionis-1004838: 3%). In man however, delivery of Ionis-104838 (100 mg) in enteric-coated capsules loaded with immediate release mini-tablets containing C_{10} (660 mg) improved oral F to 10% compared to

s.c., with the authors concluding that delivery of 4 × 100mg capsules delivered weekly may lead to the required systemic absorption [187]. Nevertheless, both inter- (F_{REL}: 0.02-28.1%) and intra- (F_{REL}: 0.03-28.1%) patient variability was high, and Ionis discontinued oral development of oligonucleotides.

Case 2: acyl carnitines in CA-based formulations

Acyl carnitines are soluble surfactants that improve intestinal permeability in animal models (Table I, Table S1). The presence of a quaternary ammonium imparts a positive charge at physiological pH values and, as the pKa of a carboxylate is 3.8, acyl carnitines exist as zwitterions in the small intestine and their CMC values are lower than anionic surfactants (e.g. lauroyl carnitine chloride (LCC): 1.2 mM) [189]. Several carnitine esters, in particular palmitoyl carnitine chloride (PCC), improved rectal absorption of cefoxitin and a somatostatin analogue in dogs [190]. PCC also improves absorption when formulated in enteric coated tablets and delivered to dogs (200 mg cefoxitin, 600 mg PCC) [58]. Formulation of DMP728 (18-20 mg) with LCC (100 mg, enteric coated) or PCC (100mg, non-enteric coated) in gelatin capsules improved oral bioavailability in dogs almost 2-fold to 21% and 17%, respectively [188]. Both LCC and PCC are the main PEs in Peptelligence™ [191], the oral peptide delivery system of Enteris BioPharm (USA). Peptelligence™ is an enteric coated solid dosage form containing the API, acyl carnitine, and CA. The CA-containing particles are coated to prevent interaction with the enteric coating. In 2009, Peptelligence™ was licensed to Tarsa Therapeutics (USA) for clinical development of an oral formulation of recombinant sCT, which successfully achieved its primary endpoint in a phase III trial of Oral Calcitonin in Postmenopausal Osteoporosis (ORACAL) [192] (discussed by Aguirre *et al* in this Issue [4]). Importantly, the dose of sCT (0.2 mg, 1200 IU) in Peptelligence™ tablets containing 500 mg CA was 6-times higher than the nasal preparation (200 IU Miacalcin®, Novartis, Switzerland) and 12 times higher than the s.c. dose (100 IU Miacalcin®, Novartis).

The iteration of Peptelligence™ used in the ORACAL trial did not contain an acyl carnitine [192], despite their inclusion in clinical trials by Enteris with other peptides [191, 193]. In preclinical testing, oral sCT absorption was increased in rat by 10-fold when LCC was included, increasing F_{ABS} to 8% [194]. The Peptelligence™ formulation also contains a disintegrant to accelerate dissolution and hence facilitate

more rapid absorption following gastric emptying. However, the residence time of enteric coated monolithic formulations within the stomach is erratic (4-120 min). This is because ingestion of dosage forms is not synchronised with muscular contractions (migrating myoelectric complex) that bring about gastric emptying. Within the study protocol for oral PTH 1-34, patients were directed to self-administer the Enteris formulation 2 h after their evening meal; which depending on the nature of the diet could impact on gastric emptying time [195]. In optimisation of Peptelligence™ formulations containing CA and LCC, the polymer used for enteric coating of capsules influenced both T_{MAX} and oral F of an LHRH analogue in dogs in the rank order of Eudragit® L30 D55 (10% w/w, duodenal release T_{MAX}: 111 min, F: 3%) < Eudragit® L30 D55 (15% w/w, duodenal release T_{MAX}: 116 min, F: 4.6%) < Eudragit® L30 D55, and FS 30 D (12% w/w, duodenal/colonic release Tmax: 152 min, F: 7.2%) [191]. These data suggest that delayed release can impact oral peptide delivery.

The dose of acyl carnitine used in the clinical studies has not been reported, although an iteration tested in a proof of concept study contained CA (473 mg), taurodeoxycholic acid (75 mg), LCC (75mg) and sCT (0.82 mg) in Eudragit® L30-D55-coated capsules. The mean C_{MAX} of calcitonin for 5 patients was reported to be 5-10 times above the targeted therapeutic plasma level [194]. It is not clear if the interaction between ingredients has an impact on enhancement, especially the dissociated form of CA and the quaternary ammonium ion of LCC, but testing in rodent instillations [194, 196] and oral delivery in dogs [197] clearly indicate the benefit of combining CA and LCC. For example, duodenal delivery of sCT (0.1 mg) with just CA (77 mg) had a F_{ABS} of 0.7% compared with a F_{ABS} of 5.4% in a formulation containing sCT (0.1 mg), CA (48 mg), and LCC (5 mg) [194]. Likewise, LCC (55 mg) in the absence of CA had minimal effect on absorption of sCT from enteric coated capsules, yet the combination of LCC (56 mg) with CA (565 mg) improved oral absorption [198]. Effort has been afforded to assess the effect of regional pH on absorption of sCT, as well as several other biopharmaceutical considerations [53, 82, 197, 199]. These studies show the relationship between intestinal pH and absorption of sCT, and while the magnitude of CA's effect on absorption of sCT is less than the combination of CA with LCC, the current NDA

reflected a desire to omit PEs for regulatory reasons, whereas CA is an accepted excipient [192, 200]

The action of CA has been attributed to stabilisation of sCT by reduction of intestinal pH [53, 198]. Following gastric emptying enteric coated capsules containing CA (656 mg) and radiopaque dye took 40 min to disintegrate, after which the pH remained below pH 5 for 90 min [53]. A correlation was found between reduction in luminal pH and improved absorption of sCT in beagle dogs [198]. However, there is a lack of clarity on the effect of CA on barrier integrity. While relatively low concentrations (4% w/v) of CA had no impact on TEER in isolated rat jejunal mucosae mounted in Ussing chambers [53], such a concentration is lower than that present in rat intestinal instillations (192 mg/mL; 19 % w/v) and potentially lower than the regional concentration following release from dosage forms. The luminal pH measurements from pH 1 to pH 1.5 [198] tend to correlate with more concentrated solutions of CA (10-40 %w/v). CA (0.57 % w/v) and the basic citrate form increased the permeability of mannitol, but not FD4 in isolated rat colonic mucosae [25]. CA had stronger enhancement action than salicylic acid, taurocholic acid, and polysorbate 80. In the same study, an enteric-coated capsule formulation containing sCT (0.5 mg) and CA (10mg) induced a F_{ABS} of 1.8% in rats [201]. The existence of CA in the non-dissociated form at low pH impedes its ability to chelate extracellular Ca^{2+} and hence alter paracellular permeability. At the same time, increased intestinal permeability in isolated rat colon by CA and tartaric acid has led to the theory that absorption of acidic forms of organic acids results in intracellular acidosis (leading to an increase in intracellular Ca^{2+} and subsequent contraction of actomyosin) or depletion of ATP (resulting in Ca^{2+}-ATPase inhibition, phospholipase activation and cytoskeletal destabilisation) [202]

Case 3: ethoxylates

The enhancement action of a diverse group of non-ionic surfactants has been reported in pre-clinical delivery models. These comprise medium and long chain sucrose esters, ethoxylated sorbitan esters, medium chain ethoxylates (C_XE_Y where C_X is the hydrocarbon number and E_Y is the ethylene oxide number) and ethoxylated glycerides (Table I, Table S1). The naming convention for the hydrophilic moiety of non-ionic surfactants makes it difficult to identify suitable candidates. For instance,

polyethylene glycol (PEG) has the International Non-proprietary Name (INN) macrogol, but it is frequently referred to as polyoxyethylene (POE), polyethylene oxide (PEO) and ethoxylate (E_Y), depending on the regulatory jurisdiction, supplier, and end user. Proprietary names, identification codes and even rebranding can also impact tracking of non-ionic surfactants, for example polyethoxylated-30 castor oil is marketed under the trade name Cremophor® EL (new trade name Kolliphor® EL).

Fatty acid- and fatty alcohol-ethoxylates enhanced oral and rectal bioavailability of heparin and small molecules in animal models, in particular those within the series C_{10}-C_{16} and E_8 to E_{25} [203, 204], although strong enhancement is sometimes reported outside this range (e.g. $C_{16}E_2$ [203]). A wide range of surfactants in this series are classified as excipients in several regulatory jurisdictions, primarily in topical delivery as emulsifiers, wetting agents and solubilisers. As might be expected, surfactants in this series that are permitted for use in enteral delivery have longer hydrocarbon chain length and ethoxylate chain length that lie outside of the optimal region for detergent- and enhancement actions (e.g. $C_{18}E_{40}$ (Myrj® 52; Croda, UK) [205]). The most effective PEs in this category are however, potent detergents (examples including $C_{12}E_9$ and $C_{12}E_{23}$ [206]) that were originally categorised as strong enhancers with slow recovery (Class III, see section 6).

Fatty ethoxylates typically form viscous liquids, unctuous semi-solids, and/or malleable soft solids that are difficult to incorporate into oral solid dosage forms compared to salts of fatty acids. For example, $C_{12}E_8$ (50mg) increased the P_{app} of [^{14}C]-mannitol by 6-fold across isolated rat colonic mucosae compared with a 9-fold increase for C_{10}, but $C_{12}E_8$ is a liquid at room temperature. Formulation development strategies can however, occasionally permit transformation of liquids into solids for solid dose formulation (e.g. through the adsorption of liquid on to granulated magnesium aliminometasilicate [207]). The requirement for a relatively high quantity of adsorbent, and other excipients, reduces the quantity of the non-ionic surfactant per unit dose, which could compromise the overall enhancement action of the dosage form. On the other hand, non-ionic surfactants can be incorporated more easily into microemulsions and oily suspensions and this is an important research area.

$C_{12}E_9$ is one of the most efficacious PEs in the C_XE_Y ethoxylate series with comparable enhancement action to EDTA in rectal administration [208] and to C_{10} in jejunal and colonic instillation in rats [209] (Table I). The suspension of insulin in a mixture of corn oil and $C_{12}E_9$ (BL-9-EX, Nikko, Japan) improved rectal absorption of insulin from suppositories [210]. In a similar study, rectal suppositories (2 g) of insulin dispersed in a hard fat base (Witepsol® W35, Cremer Oleo, Germany) with $C_{12}E_9$ (1%, 20 mg) reduced plasma glucose level in streptozotocin-induced diabetic dogs, but when higher concentrations of $C_{12}E_9$ were used, enhancement action was reduced, which led the authors to suggest that micellar solubilisation of insulin reduced the free concentration of insulin [211]. Formulation of non-ionic surfactants in gel-based preparations for rectal administration gives further insight into how non-ionic surfactants might be presented to the intestinal epithelium. Inclusion of $C_{12}E_9$ improved absorption of eel calcitonin following rectal delivery in a polyacrylic acid-based hydrocolloid gel (Carbopol® 941, Lubrizol, Japan) [212].

Alkyl phenol ethoxylates are a series of detergents sold under the trade names, Triton® (Dow, USA), Ipegal® (Rhodia, France) and Nonident® (Shell Co, Netherlands). Several of these surfactants have been tested in oral delivery models (Table S1). In particular, octyl phenol ethoxylate (average $E_{9.5}$) (Triton® X-100) is a detergent that, unlike sodium lauryl sulphate, does not denature membrane proteins [213]. A combination of Triton® X-100 (5% w/v) and the mucolytic, N-acetylcysteine (1% w/v) improved F_{ABS} of sCT from 0.1% to 1.6% in rodent gut loop instillations [44]. Comparison of several octyl phenol ethoxylates within the range of $E_{7.5}$-to-E_{100}, showed a centred symmetrical pattern of enhancement versus HLB that peaked at an ethoxylate chain length of 15 (HLB: 15) [43]. Likewise, in the case of nonyl phenol ethoxylates, significant enhancement of phenol red absorption was observed in the rat gut perfusion model for E_9 (HLB: 13), $E_{10.5}$ (HLB: 14) and E_{20} (HLB: 16), but not E_{30} (HLB: 17), E_{50} (HLB: 18) or E_{100} (HLB: 19) [214]. Furthermore, there was a good correlation between membrane perturbation and enhancement.

Case 4: fatty acid- and ethoxylated sugar esters

Fatty acid esters of monosaccharides (Sorbitan, glucose) and disaccharides (sucrose, maltose), and their ethoxylated variants give rise to a diverse range of surfactants with

application in oral delivery. Ethoxylated sorbitan esters (polysorbates) are common excipients used in oral delivery of poorly soluble drugs (e.g. polysorbate 20 and polysorbate 80 [215]). Several polysorbates exhibit modest to poor intestinal permeation enhancement capacity that is lower than that of anionic surfactants (Table S1) [216]. While polysorbates have relatively high HLB values, the quantity of free surfactant available to elicit membrane detergency is low, which is reflected in modest enhancement (e.g. polysorbate 20, CMC: 0.006%; polysorbate 85, CMC: 0.002% [217]). polysorbate 80 (PEG-20 sorbitan monooleate) did not improve rectal absorption of insulin (up to 5%) from an acrylic gel delivered to rodents, unlike $C_{12}E_9$ [212]. However, when dispersed in oily vehicles (e.g. glyceride base (Miglyol®; Sasol, South Africa)), enhancement effects of polysorbates was improved compared to that seen in aqueous dispersions [218]. For example, polysorbate 20 (PEG-20 sorbitan monolaurate) improved rectal absorption of marker sulfanilic acid by 4-fold but when dispersed in Miglyol® it was improved by 33-fold. Inclusion of polysorbate 60 in suppositories [containing a 1:1 mixture of hard fat (Witepsol® H15) and PEG (400:1000:6000 in a part ratio of 1:8:1)] improved rectal absorption of insulin in rabbits [219].

Sucrose esters are a group of non-ionic surfactants formed by esterification of sucrose with up to eight fatty acids, giving rise to a variety of functionally and structurally diverse non-ionic surfactants. This group of surfactants are permitted in oral formulations in major regulatory jurisdictions with functions including solubility enhancement, lubrication and emulsification [220]. Excipient grades of sucrose esters are not purified forms of monoesters rather they are supplied as heterogeneous mixtures containing percentages of sucrose mono-, di- and poly- esters. For example, sucrose laurate (D-1216, Mitsubishi Science, Japan; Table I) contains 80% sucrose monolaurate (HLB of 15) but also contains 20% of the di-, tri- and poly-ester. Given that sucrose di-laurate, with a HLB value of 5 is practically insoluble in water, the dispersions formed by commercial sucrose esters are not simple micellar systems; rather they are complex dispersions that range from mixed micellar systems to microemulsion pre-concentrates composed of monoester (surfactant) and diester/polyester (co-surfactant/oil). This is similar to other oil-in-water (o/w) microemulsion pre-concentrates including Labrasol® (Gattefosse, France), which are

composed of 90% surfactant (PEG-8 C8/C_{10} glycerides) and 10% co-surfactant/oil (medium chain glycerides).

The effect of five food-grade sucrose esters (0.25-1% w/v) was compared in a rat intestinal loop study, with sucrose stearate (75 % monoester) demonstrating higher efficacy than sucrose laurate (80% monoester) using a marker molecule, carboxyfluorescein (CF) [221]. The $AUC_{0\text{-}240min}$ of (CF) was improved by 5-fold with sucrose laurate (1% w/w) and to 6-fold with sucrose stearate (0.5% w/w). Rectal insulin absorption was improved with sucrose caprate and sucrose laurate, but not with sucrose palmitate or oleate (all at 80% monoester) [185]. Interestingly, both sucrose caprate and sucrose laurate induced comparable CF intestinal permeation enhancement to MCFAs. Sucrose laurate also demonstrated comparable enhancement action to C_{10} and Triton® X-100 in a rat duodenal instillation of FD4 [222]. Importantly, enhancement of sucrose laurate was MW-dependent, with the greatest effect seen on absorption of FD4 (14-fold), and FD10 (8-fold), but no effect on FD70 absorption was observed [222].

These data and the regulatory status present a strong case for development of sucrose esters for oral peptide delivery. However, in a comparison of enhancement action between C_{10} and the pure form of sucrose monolaurate, a far higher quantity of the latter was required to deliver comparable enhancement to C_{10} for the P_{app} of ^{14}C mannitol across isolated rat intestinal mucosae (Maher and Brayden, unpublished). Given that 500 mg doses of C_{10} have been tested in oral dosage forms designed for man, the level of sucrose laurate that would be required to improve oral delivery in dosage forms *in vivo* requires further evaluation. Furthermore, sucrose esters are malleable/waxy solids that are difficult to manipulate in the solid form especially at high temperature. Of concern were the low quality tablets that formed when high quantities of sucrose laurate (42 mg and 8 mg fluorescein) were made into standard mini-tablets. These tablets exhibited sticking, tackiness, and poor disintegration (>60 min) [223]. While sub-optimal formulation and process properties may be addressed, the relatively low level of sucrose laurate capable of being encapsulated within the formulation is problematic.

Case 5: alkyl maltosides and glucosides

Alkyl maltosides and glucosides have a similar structure and function to sucrose esters, the distinction being that their lipophilic moiety is typically linked to the carbohydrate via glycosidic or thioglycosidic bonds. In general, the most effective disaccharide maltosides have better enhancement capacity than the most effective monosaccharide glucosides (Table I, Table S1). The order of enhancement for boosting CF absorption in rat rectal instillations was dodecyl maltoside (DDM) (10 mM, F_{ABS}: 73%), decyl maltoside (20 mM, F_{ABS}: 62%), decyl glucoside (20 mM, F_{ABS}: 61%), octyl glucoside (30 mM, F_{ABS}: 48%), dodecyl glucoside (20 mM, F_{ABS}: 26%), and it is noteworthy that the highest DDM concentration was more effective than C_{10} (50 mM, F_{ABS}: 35%) and C_{12} (50 mM, F_{ABS}: 68%) [224]. Direct comparisons do not, however, consistently indicate that maltosides are more effect than glucosides, as variation in both CMC values and efficiency of membrane perturbation impacts permeation enhancement. For example, the CMC of hexyl glucoside is 250 mM, but despite such a high free monomer concentration, its lower capacity as a detergent reduces its efficacy for membrane perturbation and PE (F_{REL} insulin: 8%, buccal insulin absorption in rats). Glucosides with more hydrophobic side chains have lower CMC values but generate higher levels of membrane perturbation; giving rise to better permeation enhancement (e.g. dodecylmaltoside: CMC 0.6 mM, F_{REL} insulin: 30%) [225]. A greater improvement in epithelial permeability was observed with decyl glucoside (F_{REL}: 18%, CMC: 3mM) compared to decyl maltoside (F_{REL}: 6%, CMC: 1.6 mM), the data suggesting that the balance between free surfactant and membrane perturbation facilitated better enhancement for the glucoside [225]. On the other hand, the more hydrophobic dodecyl glucoside adversely changes the balance between CMC (lowered CMC to 0.13 mM) and membrane perturbation, which reduced enhancement action (F_{REL}: 7%). However, the balance between CMC (0.6 mM) and membrane perturbation is clearly better with dodecyl maltoside, which induced good intestinal permeation enhancement (F_{REL}: 30%) [225].

The most prominent members of this group of PEs are DDM and tetradecyl maltoside (TDM), which form part of the Intravail® platform (Aegis Therapeutics, USA) that is currently focused on nasal peptide delivery [226] but is also being examined in preclinical testing for oral delivery of octreotide and buccal delivery of desmopressin. Both DDM and TDM have been tested in oral delivery models (Table I). DDM (0.1%

w/v, 26-fold) had a greater effect on intestinal FD4 permeability compared to TDM (0.1% w/v, 6-fold) in isolated rat colonic mucosae [227]. In a follow on study, TDM improved colonic absorption of sCT from rat intestinal instillations [228]. However, like many surfactants, the actions of TDM were region-specific, in the rank order of colon>ileum>jejunum. This effect was also observed with decyl maltoside in rat intestinal instillation with CF [224]. Colon specific delivery of CF (1 mg) with DDM (1 mg) improved oral F_{ABS} from 17% in oral solution to 68% in enteric coated colon specific chitosan capsules in rats [86]. However, convincing colonic delivery data for systemic absorption of peptides has not yet been presented for these PEs [229]. There are also reports of enhancement efficacy in the upper GI; DDM increased insulin absorption following duodenal instillation in rats by 13% [230]. Oral formulation of the thyrotropin-releasing hormone (TRH) analogue, azetirelin, in enteric coated capsules containing lactose (120 mg), DDM (15 mg) and CA (100 mg) improved oral F_{ABS} from 15% to 44% in beagle dogs [231]. It is noteworthy that CA was first loaded into gelatin capsules that were subsequently inserted into the enteric coated dosage form, and that over 90% dissolution occurred in <30 min. Furthermore, the F_{REL} of octreotide was 4% when delivered as an oral solution to rats with 0.5% DDM, EDTA (0.1%) and sodium acetate (pH 4.5). At higher concentrations, DDM was less effective e.g. 1.5% (F_{REL}: 0.7%) and 3% (F_{REL}: 0.5%) [232, 233]. In the same study, absorption was biphasic with T_{MAX} at both 10 min and 30 min, which has not been observed in other pre-clinical delivery models. Intravail® (0.3% DDM in PBS) also improved oral absorption (F_{REL} s.c.) of the 7-mer peptide, [D-Leu-4]OB3 from 1% to 4% in mice [234]. Both studies are impressive, especially as both [D-Leu-4]OB3 and octreotide were administered via oral gavage.

Case 6: bile salts

Bile salts are another of the most widely tested intestinal PEs in oral peptide delivery (Table I, Table S1). In initial PE classifications, common bile salts were defined as Class II (moderate efficacy, fast recovery) [206]. Many studies have focused on their role in intestinal physiology and pathophysiological conditions (e.g. [235]). The most widely tested bile salts in oral peptide delivery are sodium deoxycholate, cholate, taurodeoxycholate, and taurocholate, all of which exhibit detergent characteristics, but have varying extents of free surfactant monomer and capacity to interact with plasma membranes [236]. Each of these bile salts can reduce the insulin degrading activity of

intestinal brush border homogenates [237]. More specifically, sodium taurodeoxycholate (2 mM) inhibited enzymatic activity of endopeptidases-2 (47%) and DPPIV (32%) [237]. Unlike fatty acids, no clear order of intestinal permeation enhancement can be delineated between bile salts analogues (Table I), but improved intestinal permeation has been observed for a range of poorly permeable payloads: calcitonin [82], heparin [238], HRP [239], insulin [240], acylated calcitonin [241], a proprietary cyclopeptide [242], rhEGF [243], interferon [244], ebiratide [245], EPO [246].

A number of proprietary oral peptide delivery systems list specific bile salts in patents (e.g. Generex [247], Biocon [248], Oramed [139], Aegis [249], Unigene [194], and Nod Pharma [250]). Sandoz Pharma (Basel, Switzerland) evaluated an oral formulation of octreotide (4 mg) containing either ursodeoxycholate (100 mg) or chenodeoxycholate (100 mg) administered to healthy volunteers in hard gelatin capsules [90]. While only modest absorption was noted for the dosage form containing ursodeoxycholate (F_{ABS}: 0.26%), the formulation containing chenodeoxycholate improved F_{ABS} to 1.26 % and AUC ($ng \cdot h \cdot mL^{-1}$) to 56% of the i.v. dose (100 µg). On the other hand, delivery of insulin (150 IU) and sodium cholate (50 mg) in an enema had only modest effect on rectal absorption of insulin in healthy volunteers [251].

Biocon have a portfolio of patents on a proprietary PEGylated and alkylated form of insulin, IN-105. Although this analogue reduced post-prandial glucose excursion in phase II trials [252], it failed to adequately reduce glycated haemoglobin (HBA1c) in phase III. Additional patents refer to application of PEs in delivery of the conjugate. Several bile salts significantly enhanced glycaemic control of Biocon anti-diabetic peptides in rat, including sodium cholate, glycodeoxycholate, ursodeoxycholate, taurocholate, taurodeoxycholate [248]. An oral liquid formulation of IN-105, in which patients were dosed with IN-105 (0.06 - 0.25 mg/kg), contained sodium cholate (30 mg/mL), capric acid (5 mg/mL), lauric acid (5 mg/mL) and CA (62.5 mg/mL). Effort to improve absorption of IN-105 from a solid dosage form has focused more on other PEs including C_{10}, SLS and cyclodextrins [253]. In one example, IN105 was formulated with granulated C_{10} and a super-disintegrant (crospovidone (Kollidon®

CL, BASF)), a formulation shown to improve oral IN-105 delivery in beagle dogs [253].

Bile salts are also amenable to oral solid dose formulation as their free flowing powder solid form has similar physical characteristics to that of MCFA salts. This is emphasised by inclusion of sodium taurodeoxycholate in Peptelligence™ formulations developed by Enteris Pharma [194], where sodium taurodeoxycholate had comparable effect on oral bioavailability of sCT (F_{ABS}: 1.3-1.4%) compared with lauroyl carnitine when tested in enteric coated (hypromellose phthalate) gelatin capsules. In another iteration of this dosage form, F_{ABS} of sCT (10mg) was 1.5% following oral administration to dogs with CA (600 mg) and taurodeoxycholate (80 mg) [194]. In an alternative presentation, oral insulin absorption was improved upon delivery in enteric coated particulates, which contained insulin (625 IU), soybean trypsin inhibitor (200 mg) and sodium cholate (615 mg).

Case 7: alkyl sulphates

Comparison of a range of alkyl sulphates of C_6-to-C_{14} shows that maximal membrane perturbation (as quantified by protein release) is observed in the order decyl>lauryl>myristyl>octyl>hexyl [254] (Table I, Table S1). SDS is the most prominent alkyl sulphate tested in oral peptide delivery and one of the most widely used anionic surfactants in formulation science (Table I). While the terms SDS and sodium lauryl sulphate (SLS) are interchangeable names for the C_{12} hydrocarbon tail, monographs state that SLS must contain ≥85% SDS (with other alkyl sulphates making up the remaining 15%); indicating a subtle difference between SDS and SLS. The interaction of SDS with biological membranes differs in comparison to other surfactant-based PEs, as it is strongly denaturing [153]. Nevertheless, SDS is an approved excipient and a quantity of up to 95 mg (SLS-USP) is listed in the FDA Inactive Ingredients List. SDS was originally categorised as a Class III agent (strong enhancement and slow recovery) and relatively low concentrations are required to enhance permeability in Caco-2 monolayers (e.g. 0.4 mM SDS [255] versus 13 mM C_{10} [162]). Although rats showed recovery of barrier integrity following oral gavage of SDS, the relatively long time for recovery from even small quantities (20 mg/mL) suggest that application of this excipient in routine oral peptide delivery could lead to progressive deterioration. Nevertheless, Biocon also evaluated SDS (as SLS) as a PE

to improve oral IN-105 in beagle dogs [253]. Each tablet contained IN-105 (6 mg), SLS (50 mg), crospovidone (15 mg, Kollidon® CL, BASF) as well as mannitol (78 Pearlitol® SD 200, Roquette Pharma, France), silica (0.75 mg, Aerosil® 200, Evonik Corp) and magnesium stearate (0.75 mg).

4.2.2 Insoluble surfactants

Insoluble surfactants reduce surface tension by forming monolayer films at the surface of water, although they are practically insoluble and do not form stable micelles. This group can be sub-divided into non-swelling amphiphiles that do not exist in the aqueous phase (e.g. di- and tri-glycerides, cholesterol, long fatty acids) and swelling amphiphiles which can exist as lamellar liquid crystals (e.g. phospholipids, monoglycerides) [148]. The dispersion behaviour of insoluble surfactants can be explained according to HLB values. Surfactants of HLB 1-4 do not disperse and readily separate, HLB 3-6 disperse with difficulty, HLB 6-10 form course emulsions, HLB 10-13 form cloudy to clear dispersions and HLB 13-20+ form micellar solutions [256].

This PE category includes medium chain mono- and di-glycerides (e.g. monocaprin [257], MGK^{TM} [258] (Table S1)). While insoluble surfactants have significantly lower intestinal permeation enhancement capacity compared with the soluble surfactants, this group is important because (i) membrane phospholipids and cholesterol are insoluble surfactants and their interaction with soluble surfactants forms the basis for enhancement action of the soluble class, (ii) some soluble anionic surfactants can undergo a shift to an insoluble form depending on their pKa and the pH of the vehicle, and (iii) because they contribute enhancement action in lipid -based delivery systems in clinical development in oral peptide delivery. Examples include those present in oily suspensions (e.g. Transient Permeation Enhancement, TPE^{TM}, Chiasma, Israel), reverse micelles (Soligenix Inc., USA) and microemulsions (Macrulin^{TM}, Provalis, UK). In most cases, insoluble surfactants are lipophilic oils, malleable semi-solids or waxes that function as both lipids and hydrophobic surfactants within the Lipid Formulation Classification System (LFCS) [259]. For example, dutasteride is formulated as a solution in mono- and di-glycerides of C_8 and C_{10} in soft gelatin capsules (Avodart®, GlaxoSmithKline, UK)). The physicochemical attributes of insoluble surfactants are problematic for formulation of hydrophilic peptides because

the latter do not dissolve in oils, therefore formulated peptides require course dispersion in oil (in suspensions, reverse micelles, or w/o (micro) emulsions), which could present physical stability issues. If not properly stabilised, suspended drug forms are susceptible to aggregation and caking during storage, which can impede dissolution.

The physical behaviour of fluidic, insoluble surfactants in the presence of low concentrations of water presents the possibility for the formation of reverse micelles, where an aqueous micellar core is formed around an oil continuous phase. In simulations, hydrogen bonding between glycerol backbones of glyceryl monolaurate and glyceryl dilaurate formed reverse micelles [260]. If the size of the aqueous reverse micelle core can be optimised for solubilisation of a hydrophilic peptide, this type of micelle can protect it because it would partition within the nano-sized aqueous core and would be inaccessible to peptidases. A problem with application of reverse micelles and w/o dispersions is deterioration when diluted in aqueous environments. The addition of a large volume of water to a balanced system containing droplets of water-in-oil can result in phase inversion, cracking, or diffusion of the peptide to the diluent aqueous phase [261]. An oily dispersion of anhydrous reverse micelles of insulin was formed by freeze-drying an aqueous dispersion of insulin-ursodeoxycholate-phospholipid, followed by dispersion in an oil phase (polysorbate 20/caprylocaproyl glycerides) [262]. Dilution of this dispersion in water led to the formation of a course emulsion, but it destabilised the reverse micelle leading to release of 60% insulin into the aqueous environment. The authors concluded that the modest absorption of insulin in cannulated rabbit duodenum related to the enhancement of surfactant-based PEs rather than flux of the peptide in the reverse micelle form. In other examples where phase inversion was not observed, release of insulin from soybean phosphatidylcholine reverse micelles in medium chain triglycerides was low at 7-12% over 24 h, yet bioavailability was still observed from oral administration to diabetic rats [263]. Physical and chemical stability of reverse micelles has been demonstrated for 12 months [262]. Soligenix (USA) has developed a number of proprietary dispersions to assist oral delivery of peptides including liposomes (Orasome^{TM}, now discontinued) and reverse micelles (Lipid Polymer Micelle, (LPM^{TM})) [264]. The LPM^{TM} system is a reverse micelle comprising medium chain mono- and di-glycerides and glyceride ethoxylates, where patent disclosures

include reverse micelles prepared from medium chain polyoxylglycerides (both PEG-6 (Softigen® 767, Cremer Germany) and PEG-8 (Labrasol®, Gattefosse). Included are stabilisers (e.g. gelatin, poly-lactide and or poly-glycolide) that slow the rate of phase inversion upon dispersion in luminal fluid. In rat duodenal instillations, LPM™ increased F_{REL} of leuprolide to 28% compared with 0.23% in solution form. When insoluble PE surfactants exist in semi-solid or solid forms, they can still be formulated in hard gelatin capsules. Overall, insoluble surfactants induce only modest intestinal permeation enhancement, slow release, and poor dispersion. This surfactant group are better suited for rectal formulations, as maintenance of intimate contact with the rectal mucosae is possible from suppositories [265].

Case 8: acyl glycerols

Medium and long chain acyl glycerols or glycerides are excipients used as vehicles, emulsifiers and solubilisers in the delivery of poorly soluble drugs (e.g. Capmul®; Abitec, USA and Imwitor®; Sasol, South Africa). Medium chain monoacyl glycerols such as monocaprin and monocaprylin (Capmul® 708G; Abitec, US) are generally the most effective intestinal PEs within this category [266], although efficacy as oral PEs is still moderate compared with soluble surfactants (Table I, Table S1). The non-ionic glycerol moiety is a weak hydrophilic moiety compared with anionic, cationic or medium-to-long ethoxylate groups. Thus, monoglycerides have poor aqueous solubility and low HLB values (e.g. glyceryl monoctanoate, (HLB 6.6), glyceryl monolaurate (HLB 5.2)). The rank order of enhancement of acyl glycerols in rectal delivery of a small molecule, cefmetazole, from an oily suspension was monocaprylin (F_{ABS}: 37%) > monocaprin (F_{ABS}: 18%) > monolaurin (F_{ABS}: 14%) [266]. In contrast, monocaprin was more effective than monocaprylin for rectal delivery of cefoxitin in rats [267]. Acyl glycerols are useful in rectal delivery because they can be formulated as suppositories that intimately contact the rectal mucosa, whereas when orally-delivered in gelatin capsules, they have poor dispersibility in small intestinal fluid. Non-dispersible lipoidal vehicles are an important class within the LFCS [268], and are used for delivery of molecules with low aqueous solubility. In addition, the NDA for oral octreotide in an oily suspension (Chiasma's Mycapssa™) contains glyceryl monocaprylate and glyceryl tricaprylate, illustrating the relevance of these agents for oral peptides. In many instances, acyl glycerols comprise the core oil phase and are also co-surfactants in complex dispersions

containing mixtures of soluble and insoluble surfactants. In such complex dispersions, there is only a moderate intestinal permeation enhancement action of acyl glycerols compared to those of soluble surfactants (C_8, or glyceride ethoxylates). The intestinal epithelial surface area exposed to acyl glycerols is increased by formulation in emulsified forms with soluble surfactants such as Cremophor®, demonstrating the positive interaction between the two types of surfactants [269].

Nikkol MGK^{TM} is a mixture of glycerides that has demonstrated intestinal epithelial permeation enhancement in rat [266] and dog models [270]. MGK^{TM} is composed of glyceryl monocaprylate (54%), glyceryl dicaprylate (36%), glyceryl tricaprylate (6%) and caprylic acid (3%) [266]. For example, rectal absorption of encephalin in rats was improved from F_{ABS} 4% to 20% in the presence of these glycerides [271]. Rectal delivery of insulin in an MGK^{TM} oily suspension had only a modest effect on rectal absorption in dogs (15% reduction in plasma glucose), but when insulin was first dissolved in water and emulsified in MGK^{TM} to form a w/o emulsion, plasma glucose level decreased to 40% for 1 h [272]. Monocaprylin, dicaprylin and tricaprylin improved rectal F_{ABS} of cefmetazole from 0% to 37%, 7% and 3.8%, respectively. Glyceryl monocaprylate (Capmul® 708G) also improved rectal absorption of gentamycin in rabbits, to a level that was half that of C_8 [273]. While monoglycerides are typically the most effective glyceride PEs, intestinal enhancement also occurs with medium chain triglycerides. The extent of enhancement by triglycerides is largely attributable to the free fatty acids and mono-/di-glycerides liberated by intestinal lipases. For example, trilaurin had no effect on absorption of cefoxitin in rat intestinal instillations *per se*, but addition of lipases enabled it [274].

4.2.2.1 Permeation enhancement from complex lipoidal dispersions

The combination of insoluble surfactant with soluble surfactant, water, co-solvent and/or oil gives rise to a wide range of pharmaceutical dispersions from mixed micelles to liposomes, solid lipid nanoparticles (SLNs) to course emulsions and microemulsions. These delivery systems are principally formulated in the o/w orientation, and are typically used for oral delivery of drugs with low aqueous solubility [275]. A growing number of delivery systems based on lipoidal dispersions improve oral peptide delivery. In many cases, the surfactants used in the stabilisation of lipoidal dispersions also function as transcellular PEs [161]. Some of the

surfactants used in the formation of lipoidal dispersions (e.g. w/o microemulsions or w/o/w multiple emulsions) are less efficient PEs, so improved absorption more likely relates to improved dispersion and subsequent interaction of lipids with the epithelium.

4.2.2.2 Permeation enhancement from oil-in-water systems

Course or simple emulsions are thermodynamically unstable, anisotropic dispersions with a droplet size of approximately 0.2-10 μm [276]. Fixed oils are typically emulsified with soluble surfactants to form o/w dispersions that can be delivered as liquids or in gelatin capsules. The first formulation of oral cyclosporin (CsA) was a pre-concentrate course o/w emulsion (Sandimmune®, Novartis) containing corn oil, linoleoyl polyoxyl-6 glycerides (Labrafil® M2125CS), ethanol (12.7%) and glycerol in soft gelatin capsules. Oral F of CsA in Sandimmune® was erratic with values ranging from 10%-89%. In contrast, microemulsions are optically isotropic, thermodynamically stable dispersions with a typical oil droplet size of <0.2 μm; their smaller droplet size relative to coarse emulsions provides a larger interface for digestion and ultimately permits more efficient uptake via endogenous lipid absorption pathways. The second generation CsA formulation (Neoral®, Novartis) is an o/w microemulsion pre-concentrate that contains corn oil mono- di and tri-glycerides, polyoxyl 40 hydrogenated castor oil **(**Cremophor® RH40), dl-α-tocopherol, ethanol, glycerol and propylene glycol. The oral F of CsA from Neoral ranges between 20-50% [277]. As most therapeutic peptides have high aqueous solubility, they do not partition in oil droplets, and hence o/w systems do not protect the peptide from peptidases, although the soluble surfactants may improve permeability. Several excipient suppliers provide pre-concentrated fluidic or semi-solid vehicles that have a high proportion of oil/co-surfactant and soluble surfactants, making it possible for hydrophilic peptides to be dispersed as either oily suspensions or molecular dispersions that form by solubilisation of the peptide in hydrophilic micellar structures.

Excipients used in formulation of emulsions and microemulsions are often complex mixtures of non-ionic surfactants, co-surfactants, co-solvents and/or oils that are supplied as pre-concentrated vehicles with differing degrees of dispersibility in aqueous environments. In some instances mixtures of co-surfactants alone (e.g.

glycerides, fatty acid esters of propylene glycol) provide an environment for dissolution of lipophilic drugs, but alone are poorly dispersed in water and have poor intestinal epithelial permeation enhancement. Such mixtures can be formulated with high HLB value-soluble surfactants to form vehicles that can be mixed under high shear to form simple emulsions or provided in premixed forms that spontaneously emulsify in an aqueous media. The Bancroft rule indicates the phase (oil or water) in which the surfactant is soluble, dictates whether oil (o/w) or water (w/o) droplets are formed, and so most oil in water emulsions form due to the presence of surfactant with solubility in the external aqueous phase (i.e. soluble surfactants). Although the soluble surfactant is in dynamic equilibrium with the emulsion interface and micellar structures [278], the free soluble surfactant monomer is still capable of eliciting transcellular enhancement action. For example, macrogolglycerides (50 mg/kg) improved absorption of heparin following jejunal delivery in rats *in vivo* [279]. These data are not surprising as the most effective non-ionic surfactants tested in oral peptide delivery have similar hydrocarbon (C_{8}-$_{12}$) and ethoxylate ($_{8\text{-}20}$) chain length [280]. Not all ethoxylated lipoidal vehicles alter intestinal permeability, for example, Kolliphor® EL (BASF, Germany)[281] and Cremophor® RH60 [282] are poor PEs. Complex mixtures of macrogolglycerides and free glycerides form vehicles that are capable of perturbing the intestinal epithelium, reducing TEER, and increasing transcellular flux *in vitro* [283]. These include Labrasol®, Gelucire® (Gattefosse, France), Kolliphor® (BASF, Germany), Capmul® (Abitec, USA), and Labrafil® (Gattefosse). However the concentration of surfactant in these dispersions that is available to elicit transcellular enhancement is difficult to ascertain compared with pure surfactant.

Case 10: Labrasol® and Gelucire® 44/14

Labrasol® is composed of 90% free PEG and medium chain polyoxylglycerides (termed macrogolglycerides in the EU) and 10% medium chain glycerides. The major PE component in Labrasol® is the mixture of the non-ionic surfactants, C_8 and C_{10} mono- and di- glyceride ethoxylates (E_8) (caprylocaproyl macrogol-8 glycerides). Labrasol® improved permeation of poorly permeable macromolecules (e.g. LMWH [161], erythropoietin [284], and FD4 [285]) in pre-clinical models (Table I), and this feature has been outlined further in patents (e.g. [286]). Labrasol® can also improve jejunal and colonic absorption of insulin, although F_{ABS} was low at 0.2%, and less

than the effects observed with other non-ionic surfactants [287]. Comparison of Labrasol® with C_{10} in rat intestinal loop instillations showed a 6-fold lower capacity of the former to boost absorption of LMWH [161]. Nevertheless, dispersions of Labrasol® have demonstrated strong enhancement action in particulate dispersions: adsorption of Labrasol® to carbon nanotubes formed liquid filled nanoparticles that when mixed with sodium starch glycolate (Explotab®, JRS Pharma, Germany), casein, and EPO (100 IU/kg) formed a solid dispersion that improved F_{ABS} of EPO from 0.6% to 11.5% in rats [288]. Labrasol® has also been transformed into a solid for delivery of macromolecules. For example, heparin (25mg) was dispersed in Labrasol® (0.5mL) followed by solidification using Fluorite RE (Eisai Co. Ltd., Japan), Neusilin® US2 (Fuji Chemical Ind. Co. Ltd., Japan) or Sylysia™ 320 (Fuji Silysia Co. Ltd., Japan), and loaded into enteric coated capsules for oral delivery in dogs [289]. Dissolution of heparin occurred in the order Sylysia™ 320 > Fluorite RE > Neusilin® US2, and enhancement of heparin bioavailability occurred in the order Fluorite RE (19%) > Sylysia (13%) > Neusilin US2 (5%). Solidified forms of Labrasol® can be prepared with relatively low quantities of carrier (Neusilin®), but the preparation of powders that exhibit the necessary characteristics for solid dose formulation (flowability, tableting, tablet disintegration, dissolution, hardness, uniformity) reduces the effective quantity of Labrasol® in the tablets (Maher S, and Brayden DJ, unpublished). Formulation of rhPTH 1-34 was achieved in a w/o microemulsion consisting of water (15%) and oil (85%), with the oil phase consisting of 6:2:1:1 of Labrasol®, medium chain triglycerides (Crodamol® GTCC, Croda, UK), macrogol-15 hydroxystearate (Kolliphor® HS15, BASF, Germany) and tocopherol acetate [290]. This dispersion delayed enzymatic degradation of rhPTH 1-34, increased permeability across Caco-2 monolayers, and improved bioavailability to 5% and 12% in rats by oral gavage and intestinal instillation, respectively. It is however noteworthy, that the daily dose of PTH in that study was in excess of the recommended daily injectable dose in man. Gelucire® 44/14 is a semi-solid excipient mixture containing 20% C_{12} glycerides and 80% C_{12} mono- and di- glyceride ethoxylates (E_{32}) (lauroyl macrogol-32 glycerides). Although Gelucire® 44/14 (HLB: 14) has a comparable HLB to Labrasol® (HLB: 12), and both belong to Class III of the LFCS, the ethoxylate chain for Gelucire® 44/14 is above the most effective length for permeation enhancement. In rodent studies there was less absorption of EPO [284] and LMWH [161] for Gelucire® 44/14-containing formulations compared to

Labrasol®. Gelucire® 44/14 is also a constituent in an experimental multiple emulsion (water-in-oil-in-water) that improved bioavailability of calcein from 1.8% to 8% [291].

Case 11: innovative lipid blends

Oral delivery of antidiabetic peptides in fluidic dispersions has been disclosed in patent filings (Table S2). In one embodiment, oral delivery of a high dose of insulin in a non-aqueous dispersion of propylene glycol, Capmul® MCM, Pluronic® F127, and PEG 3350) in enteric-coated capsules lowered blood sugar in dogs [292]. In another, self -emulsified lipoidal vehicles showed efficacious permeation enhancement of hydrophobic forms of insulin (30-60 nmol/kg) in rat and canine intestinal instillations [293]. The formulation was based on five key ingredients, propylene glycol, Tween® 20, Labrasol®, and diglycerol caprylate blended in different proportions. There was no obvious concentration dependency observed with the PEs (Labrasol® and Tween® 20), rather the most efficacious absorption enhancement was observed with dispersions forming the smallest particle size. In general, self-microemulsified delivery systems demonstrated increased intestinal permeation enhancement relative to self-emulsified dispersions. Delivery of hydrophobised forms of insulin were also improved in solutions of propylene glycol injected into canine small intestine [294]. Other iterations evaluated in oral delivery of modified insulins include combinations with propylene glycol, medium chain monoglycerides (Capmul® MCM C_8, MCM C8/C_{10}), propylene glycol monocaprylate (Capmul® PG8), and Labrasol® [294]. In these patent filings, enhancement was demonstrated from enteric-coated formulations containing physiological insulin doses (0.17 mg/kg), which suggests that lipoidal dispersions may have promise.

Sigmoid Pharma (Ireland) has performed preclinical testing for oral delivery of sCT by formulating it in a lipoidal dispersion composed of an oily mixture (medium and long chain triglycerides (Miglyol® 818, Sasol), diethylene glycol monoethyl ether (Transcutol® HP, Gattefosse) and PEG-35 castor oil (Kolliphor® EL, BASF)), which is mixed with an external aqueous phase containing gelatin and a PE (C_{10}, sodium taurodeoxycholate or coco-glucoside) to which sCT was added [295]. This dispersion was then extruded into cold oil to form semi-solid minispheres (1-2 mm). The physical properties of these lipoidal minispheres have not been reported, although the

presence of Kolliphor® EL and a gelatin emulsifier suggest the formation of an o/w emulsion. While this dispersion had only a modest effect on intestinal absorption of sCT in rat instillations, the application of particulate forms of semi-solid lipoidal dispersions formulated in an enteric-coated hard gelatin capsules offers potential.

4.2.2.3 Permeation enhancement from water-in-oil systems

A number of peptides have been dispersed within the aqueous core of w/o microemulsions including arginyl-glycyl-aspartic acid (RGD) peptide, vasopressin, calcitonin and insulin [296]. Provalis (UK) developed a w/o microemulsion (Macrulin^{TM}) to improve oral delivery of insulin. Macrulin^{TM} is composed of an external oil phase (Labrafil® M1944CS) with an aqueous disperse phase stabilised with lecithin and alcohol [297]. The optimised dispersion exhibited physical stability for at least six months at 4°C, 25°C and 40°C, although pseudo-ternary phase diagrams prepared during the preparation of the microemulsion clearly indicate the destabilising effect of water [297]. Intra-gastric delivery of 200 IU insulin in this microemulsion lowered blood sugar in diabetic rats to the same level as the s.c. dose (0.3 IU), but dose correction revealed a pharmacological activity (PA) of less than 0.2% [298]. Delivering Macrulin^{TM} to healthy patients via intra-duodenal intubation increased plasma insulin levels and lowered blood sugar [299]. Development of Macrulin^{TM} has since discontinued, but the potential for optimised enteric-coated formulations that regionally release fluidic w/o microemulsions in the small intestine warrants future study.

The aqueous environment of w/o dispersions can be tailored to suit the optimal solubility and stability for peptides leading to improved bioavailability [296]. For example, the PA% of insulin was 5% in dogs when dispersed in a lecithin-based w/o microemulsion and delivered in capsules coated for colonic release [300]. Significant improvement in oral insulin absorption in rats was reported from an experimental w/o microemulsion formulated with the cationic surfactant, didodecyldimethylammonium bromide (DMAB), propylene glycol (co-solvent) and tracetin (oil) [301]. In that study, pseudo-ternary phase diagrams suggest that the microemulsion retained its isotropic properties with significant aqueous dilution [302]. In general however, w/o microemulsions that are stabilised with soluble surfactants (such as DMAB), are sensitive to destabilisation and phase inversion following dilution. Oral delivery of

w/o systems to average sized rats, where the total fasted volume is less than 0.2 mL [303], could mask potential phase inversion in man, where the fasted fluid volume is significantly higher [18]. Analysis of phase inversion of a w/o microemulsion (Miglyol® 812, Capmul® MCM, Tween® 80, and water) that improved intestinal uptake of a marker peptide showed that the dispersion was susceptible to phase inversion when diluted 5-fold with water, and partial inversion following 2-fold dilution [304]. Not all w/o microemulsions invert at such low ratios of water, as inversion has been reported in the presence of 100-fold to 1000-fold excess water [261], indicating that careful selection of dispersion additives can influence stability. However, improved stability will compromise enhancement action as the presence of soluble PE surfactants is one of the driving forces for phase inversion. Therefore, w/o microemulsions require a delicate balance between transcellular permeation enhancement and physical stability. The most effective strategy to delivery w/o systems is therefore within enteric-coated systems where the maximal volume of fluid that is likely to be encountered in the proximal small intestine is relatively low. A panel of w/o microemulsions were evaluated in delivery of the RGD peptide following intra-duodenal delivery in rats [305]. There was no clear correlation between particle size and enhancement, but dispersions with higher concentrations of Capmul® MCM and Cremophor® EL had a greater effect on bioavailability. The most effective w/o microemulsion (RGD; $F_{ABS:}$ 29% versus saline F_{ABS}: 0.5%) consisted of saline dispersed in propylene glycol dicaprylocaprate (Captex® 200; Abitec, USA), Capmul® MCM, lecithin (Centrophase^{TM} 31, Central Soya Company Inc., USA) and Cremophor® EL. The inclusion of established PEs (e.g. C_8 and C_{10}) in w/o emulsions added a boost to bioavailability of calcein administered via intra-duodenal instillation in rats [306]. A microemulsion containing these PEs improved bioavailability by 27-fold from 1.3% in solution to 36% in the w/o dispersion.

4.2.2.4 Permeation enhancement from multiple emulsions

Water-in-oil-in-water (w/o/w) emulsions are thermodynamically unstable multiple emulsions that are difficult to stabilise, which is a factor that restricts their application in delivery of proteins (reviewed in [307]). Intestinal delivery of peptides in w/o/w emulsions was first tested over 45 years ago [308] and several prototypes have been shown to improve peptide permeability in animal models, including insulin [309] and sCT [310]. The excipients used in the stabilisation of multiple emulsions can also alter

intestinal permeability (e.g. SLS [309]). PEs can be added to either the oil phase (e.g. long chain fatty acids [311]) or either the external [312] or internal [313] aqueous phases. Furthermore, many of the insoluble surfactants used in the stabilisation of w/o emulsions have associated intestinal permeability enhancement action (e.g. fatty acids [314, 315]). A multiple emulsion containing either eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) in the oil phase increased absorption of insulin in rat colon and rectal instillations, although significant variability was reported [316]. Dilution of an insulin multiple emulsion with sodium taurocholate accelerated proteolytic degradation of insulin by pancreatin, suggesting that the dispersion could be destabilised in the small intestine [313]. The inclusion of peptidase inhibitors and PEs in such emulsions raises questions about their overall capacity to protect and improve intestinal permeation. This is not surprising, as lipases destabilise multiple emulsions leading to release of constituents in the internal water phase [317]. Other factors such as oil droplet diameter, composition of the oil phase, and constituents of the external phase also impact performance [318]. While physical stability of multiple emulsions have been reported for up to 2 years at 25°C, those loaded with insulin and stored at 15°C had stability for only 1-3 months [319], and the lack of stability in the presence of the peptides seems to be a widespread problem [320].

4.2.2.5 Particulates in PE-based lipoidal systems

The dispersion of nanoparticles or microparticles within lipid based drug delivery systems is a research area of growing interest. Nanoparticles for oral peptides were developed on the premise that they can protect the peptide from pre-systemic digestion and shuttle the cargo across the intestinal epithelium. However, the inability of most prototype particles to be sufficiently internalised by the epithelium has hampered development. A somewhat weaker rational is that dispersion of peptide-loaded particulates within fluidised lipoidal delivery vehicles could facilitate better presentation of the nanoparticle at the intestinal epithelium and improve absorption of the released peptide. Furthermore, encapsulation of the peptide within a solid matrix could improve stability of the peptide within the lipoidal vehicle. The term solid-in-oil-in-water (s/o/w) generally refers to microparticles or nanoparticles that are dispersed in an oily vehicle that is then emulsified in an external aqueous phase [321]. The term has also been applied to reverse micelles within o/w emulsions, despite micelles existing in a dynamic fluidic state of matter, as opposed to more rigid

colloids and course suspensions [322]. Insulin loaded reverse micelles were prepared in the lipophilic surfactant, sucrose erurate (HLB 2, ER-290, Mitsubishi Kagawa, Japan), and dispersed in soybean oil containing sodium cholate and sucrose laurate. When mixed with water, the micelles formed an o/w emulsion with kinetic stability for 30 days. Oral delivery of this dispersion to rats reduced blood sugar over 3-6 h [322]. Further iteration of this dispersion led to stabilisation of the o/w emulsion droplets into a more rigid multi-molecular film with hydroxypropylmethylcellulose phthalate (HPMCP), which also functions as an enteric coating for controlled release [323]. Polyelectrolyte complexes of insulin and chitosan formed 100 nm diameter nanoparticles when dispersed in an oily vehicle (oleic acid, glyceryl-6 dioleate (Plurol® Oleic, CG, Gattefosse, France) and Labrasol®). When administered orally to fasted streptozocin rats, the PA of insulin was 2.6% over 24 h when diet was unrestricted and 7% when diet was controlled [324].

Case 13: TPE^{TM}

The most clinically advanced oral peptide delivery is TPE^{TM}, developed by Chiasma (Israel) for the delivery of octreotide [325-329]. In 2015, an NDA for oral octreotide was submitted to the FDA under the brand name, Mycapssa^{TM}. Chiasma has also demonstrated enhancement with iterations of TPE^{TM} in rectal (GLP-1 [330]) and nasal (IFN [330]) delivery as well as oral delivery of several other peptides (including insulin, hGH, teriparatide, exenatide [329]). TPE^{TM} is an oily suspension of hydrophilic peptide that forms a coarse dispersion when mixed with other additives (soluble surfactant and suspending agent) and dispersed within an oil phase. TPE^{TM} consists of enteric-coated capsules/tablets (acryl-EZE®, Colorcon, UK) containing a lyophilised mixture of octreotide, C_8 or C_{12} and Povidone, dispersed in an oil phase composed of glyceryl monocaprylate, glyceryl tricaprylate, and polysorbate 80 [331]. The formulation improved absorption of octreotide in both rat and monkey [331] and in clinical trials [3]. Kinetics of the absorption process indicated that the actions of the oily suspension were partially reversed after 10 min and fully reversed between 30-60 min [331]. Reversibility is not unique to these oily suspensions, as similar barrier recovery was observed with other permeation enhancers [62]. Chiasma also reported an upper MW limit to enhancement by TPE^{TM} (10 kDa) and safety has been demonstrated by daily administration to primates over a 9 month period [331]. While the overall effectiveness of TPE^{TM} has been determined in phase III trials conducted

over 13 months in acromegaly patients, 58% of patients required up-titration to 40 mg, 60 mg or 80 mg (40 mg bid) to maintain response, the later representing an 800-fold increase in dose relative to the s.c., although a therapeutic threshold was achieved and there was low variability in plasma octreotide level [332]. Nevertheless, the very high dose required for such a low MW peptide challenges the potential wider application of this delivery system in its current format.

C_8 is the best known PE in TPE™ compositions but glyceryl monocaprylate also demonstrates enhancement action [331] (Table I). While C_8 is structurally similar to C_{10} and C_{12}, it is less effective in head-to-head testing in Caco-2 monolayers [150], rat intestinal loop models [20, 161], and rat rectal infusions [166, 333]). However, alone it improved rectal bioavailability of cefoxitin by over 3 fold from 5% to 17 % in healthy volunteers; highlighting its ability to improve intestinal permeation [334]. In rat intestinal instillations, the reported level of C_8 in TPE is 5.5 mg/mL (~33 mM), which lies within the range where enhancement action has been reported in other studies (33-50 mM, Table I). Delivery of FD4 in an admixed solution with C_8 (1.65 mg) improved absorption in rodent intestinal instillations, but an equivalent concentration of the surfactant within TPE™ further increased AUC by 5-fold [331]. The presentation of peptide and PE in an oily suspension therefore has an advantage over presentation in solution, hence the novelty of TPE™. This vehicle could also be responsible for why TPE™ was more effective in the small intestine compared with colonic delivery; which differs from several reports for MCFA (including C_8) when delivered in aqueous solution or mini-tablets [70].

4.3 PEPTIDE HYDROPHOBISATION

The hydrophobisation of a peptide aims to improve passive transcellular permeation, and can be achieved by either covalent (e.g. alkylation or bile acid conjugates [335]) or physical complexation (e.g. hydrophobic ion pairing (HIP) [336] or non-ionic interactions). A diverse group of complexing agents induce peptide hydrophobisation including polyelectrolytes (e.g. chitosan, oligo-L-arginine PEG_{2000}), monoprotic complexing agents (e.g. fatty acids, bile salts) and small molecule carriers (e.g. SNAC). HIP is the ionic complexation of an ionisable peptide group with a counter ion of comparable charge to conventional hydrophilic counter ions in salt formation, but lower capacity for solvation due to the presence of a hydrophobic moiety. The

neutralised complex lowers the aqueous solubility of the ionisable drug to an extent dictated by the nature of the hydrophobic moiety of the counter-ion. Ad-mixture typically leads to precipitation in water and an increase in lipophilicity, which should improve passive transcellular flux across epithelia [337]. Surfactants are effective complexing agents because they have strong ionisable functional groups and distinct hydrophobic regions.

Hydrophobisation is effective for low and high MW species, with improved fluxes across model membranes observed over two log orders of MW from oxytocin-derived tetra peptide (MW 448 Da) to bovine serum albumen (66 kDa) [338]. Similar to pharmaceutical salt formation, different pH and counter-ions are used for physical complexation of acidic and basic drugs. Detergents like SDS have long been known to undergo HIP with peptides and proteins when mixed in stoichiometric proportions to oppositely-charged amino acid side chains [339]. As most therapeutic peptides are amphoteric, complexation can be performed at pH values above their pI where the peptide displays an anionic charge and where a cationic complexing agent is used (e.g. Nα-deoxycholyl-L-lysine-methyl ester (DCK)), or below their pI where the peptide holds a cationic charge and therefore an anionic complexing agent is used (e.g. SDS). Insulin with a pI of 5.5 has six acidic and six basic functional groups, and when the pH is adjusted to 2.5, each of the acidic and basic functional groups are protonated to yield an overall +6 charge. In an acidic solution, complexation, for example with SDS (1:6 ratio of peptide:SDS) leads to a 3.4-fold increase in log P. In cases where the pH is above the pI, the majority of acidic and basic functional groups are deprotonated, resulting in overall anionic charge (-6). The pH at which complexes form can impact dissociation kinetics, because complexes formed at low pH dissociate at the pH in the small intestine, while those formed at high pH dissociate in the stomach. Although it is noteworthy that complex dissociation is not always evident at pH values that are predictive of dissociation [340]. It seems logical to complex the peptide in the anionic form at physiological pH in order to avoid pH-dependent complex dissociation prior to drug absorption. However, there are safety concerns regarding the internal use of cationic detergents as complexing agents. More importantly, while the proportion of anionic functional groups available for complexation can be high at physiological pH, the individual pKa values for basic amino acids means that not all cationic amino acid side chains are deprotonated at

physiological pH, and so positive charges in the molecules could impede passive transcellular diffusion.

In cases where surfactants are weak acids or weak bases, consideration must be given to their pKa relative to the isoelectric point of the peptide, and ultimately the pH of the environment, because the low pH environment required to protonate weakly basic amino acid side chains in the peptide will often result in protonation of the acidic counter ions in the complexing agent leading to precipitation of the complexing agent. As such, it is often necessary to consider strongly acidic or strongly basic surfactants where pH does not influence their ionisation in physiological conditions (e.g. benzalkonium chloride, SDS). Other factors that influence complexation efficiency include the type and number of ionisable functional groups in the peptide as well as the ionic strength of the environment [341]. Ionic complexation does not always lead to precipitation from an aqueous solution, as different carriers impart different levels of hydrophobicity. Testing a series acyl sulphonates of different chain lengths with insulin showed that the dodecyl and decyl chains led to complete peptide precipitation, octyl led to partial precipitation, while hexyl and butyl did not precipitate the peptide [338]. At the same time, the order of flux through a methylene chloride layer followed the order $C_{12}>C_{10}>C_8>C_6>C_4$ with flux ranging from 0.1×10^{13} $mol \cdot cm^{-2} \cdot s^{-1}$ to 3.2×10^{13} $mol \cdot cm^{-2} \cdot s^{-1}$. This is not surprising, as shorter chain surfactants have lower affinity for both self-association and association with proteins [342].

Several surfactants used in HIP are also established PEs that can alter barrier integrity e.g. SDS, sodium deoxycholate and fatty acids. The quantity of PE used in complexation is low relative to those used to alter the barrier, but this quantity is a function of potency of the peptide. For example, each 100 IU of insulin (3.5 mg) requires only 1 mg of SDS to achieve a saturated complex (1:6 molar ratio), which is likely to be below the quantity of SDS required to alter intestinal permeability. The required concentration of SDS can be still lower for more potent peptides like exenatide. However, in cases where the complexing agent is added in excess of stoichiometry proportions and above its CMC, the complex can be solubilised into micelles [340]. There has not yet been significant research evaluating strategies to formulate hydrophobised peptides. The loss of aqueous solubility is often

accompanied by improved dispersibility in solvents of lower polarity. For example, the insulin-SDS complex is soluble at a concentration of 3 mg/mL in octanol without loss of secondary structure. This compares with solubility in octanol at a concentration <0.03 mg/mL for native insulin [343]. Good solubility of insulin-SDS complexes has also been observed in propylene glycol (>0.9 mg/mL), PEG 400 (>0.14 mg/mL) and ethanol (>0.9 mg/mL) [343]. Furthermore, dispersion of hydrophobised insulin in lipid-based delivery systems has been proposed for insulin:distearyldimethylammonium bromide (DSAB) or insulin:PC complexes [344]. Therefore, hydrophobisation offers the prospect of formulating peptides in a wide range of established non-aqueous delivery vehicles already developed for poorly soluble drugs (e.g. CsA).

The data from HIP complexation has only been reported in pre-clinical models. Hydrophobisation of insulin using the semi-synthetic bile salt, DCK (Mediplex, South Korea [345]) in a 1:10 molar ratio increased the Log $P_{\text{METHYLENE CHLORIDE:WATER}}$ by 146-fold from 0.08 to 11.64 and increased transcellular flux across Caco-2 monolayers by 15-fold versus the native peptide [337]. Oral delivery of the insulin:DCK complex improved oral insulin absorption in rats by 6-fold [346]. In dogs, absorption of the insulin-DCK complex (42 IU/kg) was comparable to the i.v. route albeit at a higher dose [337]. DCK has also been shown to hydrophobise other drugs including ceftriaxone [347], heparin [348], and risedronate [349].

HIP complexation of acidified insulin with sodium deoxycholate increased Log P by two log orders from 0.004 mg/mL to 0.4 mg/mL, which was accompanied by a 23-fold increase in relative bioavailability in rats to 12% when formulated in poly(lactic-co-glycolic) acid (PLGA) nano-capsules [350]. Other examples of HIP complexation include heparin with deoxycholylethylamine [348] and insulin with PC [351]. HIP was also used as a strategy to prevent acylation of octreotide during release from microparticles composed of lactide and glycolide [352].

Case 13: Eligen®

Hydrophobisation can also be performed by exploiting weak dipole-dipole interactions. Eligen® (Emisphere, USA) is a family of several hundred proprietary carriers that physically interact with a wide range of drugs to improve passive

permeation across the intestinal epithelium. The most widely tested of these carriers are SNAC (sodium N-[8-(2-hydroxybenzoyl)amino]caprylate), 5-CNAC (N-(5-chlorosalicyloyl)-8-aminocaprylic acid), 4-CNAB (4-[(4-chloro-2-hydroxy-benzoyl)amino]butanoic acid), SNAD (N-(10-[2-hydroxybenzoyl]-amino)decanoic acid), 5-CNAB (monosodium N-(4-chlorosalicyloyl)-4-aminobutyrate) and 4-MOAC (N-[8-(2-hydroxy-4-methoxy)benzoyl]amino caprylic acid). Eligen® carriers have been evaluated for oral delivery of insulin, sCT, Peptide YY_{3-36}, PTH, hGH, and several GLP-1 analogues including semaglutide (Novo-Nordisk). There has been more clinical testing performed on Eligen® carriers than any other PE delivery system, yet there is considerable debate on how they alter intestinal permeability.

Initial research suggested that these acylated amino acids self-assemble to form microspheres [353] and that constituents thereof could improve oral delivery of sCT [353]. SAR testing indicated that lipophilicity [354] and hydrogen bonding [355] may play a role in intestinal permeation enhancement induced by Eligen®, although a consistent effect was not observed. A preliminary screen of 11 carriers showed that an optimal bell-shaped window of lipophilicity was required to improve oral heparin absorption [356]. Analysis of the interaction of Eligen® carriers with rhGH found a correlation between drug absorption and stabilisation of protein structure [356, 357]. When hGH was mixed with 4-[4-[(2-hydroxybenzoyl)amino]phenyl butyric acid (E414) [358] there was a peak shift in electrophoretic migration suggesting physical interaction. Effective carriers also bound to specific residues within the protein structure, but not with specific amino acids *per se* (e.g. to His^{21} in helix 1, and to Tyr^{164} Arg^{167} Lys^{168} Asp^{171} Thr^{175} in helix 3, but not to all His residues) [357]. While many Eligen® carriers have acidic functional groups, their interaction is not exclusively with amino acids that have basic side chains. In fact, the most effective ones interact with the anionic molecules, cromolyn [359], ampicillin, and heparin [360].

Emisphere reported the capacity of SNAC to improve permeation of heparin across Caco-2 monolayers and to induce inhibition of anti-Factor Xa in rat intestinal instillations [360] and in cynomolgous monkeys [361] (Table I). The SNAC-heparin dispersion was prepared to a final pH of 7.5-8.5 and contained SNAC (100 mg/mL) and heparin (33 mg/mL) dissolved in 25% v/v propylene glycol [361]. The effective

dose range of SNAC (88-300 mg/kg) was significantly higher than that used for conventional PEs, and this placed requirements on both dosage form capacity and the use of potent peptides and proteins. The interaction between SNAC and heparin was based on increased lipophilicity through hydrogen bonding and/or hydrophobic interactions [362]. In relation to carrier-peptide interactions, analysis by 4-4-bis-1-phenylamino-8-naphthalene sulphonate (bis-ANS) fluorescence showed that SNAC increased the lipophilic surface area of insulin through non-covalent bonding and/or conformational changes to the peptide, leading to exposure of hydrophobic peptide regions amenable to transcellular permeation [363].

While improved permeation of insulin can be uncoupled from an effect on barrier integrity, there are contradicting reports on the nature of the interaction between Eligen® carriers and the intestinal epithelium at concentrations required for oral delivery. For example, low concentrations of SNAC (17 mg/mL) in Caco-2 monolayers improved transepithelial permeation of insulin but not mannitol, suggesting that neither alteration to barrier integrity nor TJ opening was involved in the mechanism [363]. In another Caco-2 study however, SNAC caused complete loss of TEER and a 36-fold increase in mannitol permeability at the concentration (50 mg/mL) required to improve heparin permeation in an instillation model [360]. In isolated rat colonic mucosae, SNAC concentrations >50 mg/mL increased P_{APP} of mannitol and reduced TEER [364]. These two studies used such high concentrations that loss of TEER was inevitable and is likely associated with epithelial damage, so no conclusions on mechanism can be made. There are also reports that challenge the theory that SNAC acts through hydrophobisation. For example, SNAC did not cause an increase in the partition of cromolyn in either octanol (Log $D_{pH7.4}$) or chloroform, but increased epithelial membrane fluidity as measured by fluorescence anisotropy [365]. Although SNAC has demonstrated surface action (CMC: 56 mM in PBS (pH 7.4) [365]), the distribution of hydrophilic functional groups in the more hydrophobic salicylamide region of the carrier do not give rise to efficient detergent action, and this suggests a basis for the high concentrations required to induce transcellular permeability *in vivo*. It therefore remains unclear whether the high concentrations of SNAC required for oral peptide delivery simply relate to a weak detergent action or whether there is true carrier-based delivery. It bears noting that SNAC is a weak acid (pKa 5.08 [366]) and so could undergo HIP complexation with peptides at

physiological pH, although HIP cannot explain the hydrophobisation of anionic drugs like heparin.

Whatever the mode of action, SNAC was granted GRAS status in 2009 and Emisphere recently marketed Eligen-B_{12}^{TM}, an oral vitamin formulation containing SNAC [367]. This carrier had a no observed adverse effect level (NOAEL) of 1 g/kg/day in rats [368], well above the doses used in oral peptide formulations. SNAC was tested in a number of clinical studies especially with heparin in phase III (PROTECT), where an oral liquid dose of heparin-SNAC failed to meet its primary endpoint; moreover compliance in this trial was low due to the bitterness of SNAC in solution, which had previously been noted in proof of principle clinical testing [369]. The dose of SNAC that was required in preliminary clinical evaluation of oral heparin (90,000 IU) was 2.25 g delivered in a15 mL volume every eight hours pre-or post-prandially [369]. Higher doses of SNAC (10.5 g) delivered to patients by nasogastric intubation were accompanied by emesis in healthy volunteers. Heparin-SNAC was subsequently formulated into soft gelatin capsules and delivered orally to patients where it improved absorption of heparin relative to an unenhanced formulation, but it has sub-optimal pharmacodynamics relative to the s.c. form [370]. Ultimately, delivery of heparin in an optimal oral solid dosage form was not feasible in one unit dose due to the large dose requirements of carrier and payload (1.9-2.8 g), even before the addition of formulation or process excipients [371]. A conservative estimate of 4 × 750 mg tablets three times daily indicated that such an oral heparin dosage form might only be suitable for short term use. Given the challenges facing oral heparin, development of heparin-SNAC was discontinued. Lower quantities of SNAC and other Eligen® carriers have been tested in oral peptide delivery due in part to increased potency of such peptides relative to heparin. For example, 150 mg of SNAC was tested in oral preparation of PYY and GLP-1 [372]. In a 90 day trial, patients that received up to 40 mg of insulin daily in 4 divided doses saw a reduction in HbA1c of 0.74% when their initial baseline was between 7-8.9% [373]. SNAC has also been disclosed in patents filed by Oramed, where an oral insulin formulation containing insulin (6 mg) and a synergistic mixture of SNAC (250 mg) and SBTI (125 mg) significantly lowered blood sugar levels in preliminary clinical testing [374]. Administration of insulin (400IU) and SNAC (2.1g) in capsules (4 × size OOO gelatin) significantly increase plasma insulin level between 20-50 min and lowered

plasma glucose levels between 30-50 min [375]. More recent developments relate to the licensing of Eligen® to Novo Nordisk for oral delivery of insulin, GLP-1 (NN9924) and selected structural analogues (e.g. semaglutide [376, 377]). In a 600 patient phase II trial, daily oral delivery of semaglutide (40 mg) formulated with SNAC lowered HbA1c by 0.7-1.9% compared with 1.9% for the s.c. group [378]. The oral dose of semaglutide was 300-fold higher than the s.c. dose (1 mg), and Novo Nordisk have proceeded to phase III(a) (PIONEER) trials with oral doses of 3, 7, and 14 mg semaglutide. In animal testing, semaglutide (10 mg) was formulated in an oral tablet containing SNAC (150, 300, or 600 mg), povidone (2, 4, or 7 mg), sodium starch glycolate (Avicel® PH102; 36, 82, 76 mg) and Mg^{2+} stearate (3, 4, or 7 mg) [379]. The semaglutide formulation containing 300 mg SNAC had F_{ABS} of 0.63 % following oral administration in Beagle dogs, furthermore a dose dependency was observed for 5 mg (F_{ABS}: 0.33%), 10 mg (F_{ABS}: 0.63 %), 15 mg (F_{ABS}: 1.2%) and 20 mg (F_{ABS}: 1.4%).

5-CNAC has also been evaluated for the oral delivery of sCT [380] and PTH [381] under licence with Nordic Bioscience (Denmark), and partnered with Novartis. Like SNAC, 5-CNAC forms a lipophilic complex with peptides to improve intestinal permeation [382]. Interaction between 5-CNAC and sCT is likely to prominently involve HIP because sCT has a high isoelectric point (pI 7) and a higher proportion of amino acid side chains will be positively charged at pH 7 compared to insulin (pI 5.5). An insoluble complex is initially formed between sCT and 5-CNAC at low pH due to the higher proportion of cationic functional groups, but the complex is not stable at physiological pH in the small intestine.

The dose of sCT (0.8 mg) in oral formulation with 5-CNAC (200 mg) was higher than the nasal formulation (200 IU or 33 µg), and accordingly C_{MAX} was significantly higher in oral (145 pg/mL) versus nasal (11.4 pg/mL) [383]. Despite promising clinical performance of sCT formulations containing 5-CNAC [380, 384-388], these formulations did not reach primary endpoints in two phase III trials [389]. Publication of the trial data by Nordic Biosciences has assisted development of oral peptide formulation [390]. For example, administration of sCT:5-CNAC with 50 mL of water resulted in a three-fold increase in absorption compared to that obtained with 200 mL [385], while a significant food effect was also observed [391]. Nordic also evaluated

the most effective time of the day to administer oral sCT in post-menopausal women, where administration 1 h before dinner (5 pm) was more effective than after overnight fasting (8 am) or 4 h after an evening meal (10 pm) [391].

Case 14: Bridgelock™, Macrosol™ and Axcess™

Cortecs Ltd (UK) was one of the first companies to apply oily formulations in oral delivery of peptides. Bridgelock™ was an oily peptide dispersion formed by spraying a w/o emulsion onto sodium carboxymethyl cellulose followed by evaporation [78]. The dehydrated aqueous phase contained sCT, aprotinin, CA, polyoxyl 40 stearate, hydroxyproyl cellulose, NaCl, PC, and phosphatidyl glycerol, while the oil phase contained lecithin, monoolein, polysorbate 80, cholesterol and oleic acid [392]. A number of these agents can act as PEs, in particular oleic acid (Table S1). Intra-jejunal delivery of Bridgelock™ improved absorption of sCT in pigs as measured by reduction in plasma Ca^{2+} [392]. An iteration led to Macrosol™, an isotropic lipid based peptide dispersion that is formed by reconstitution of an anhydrous peptide-amphiphile mix in oil. The surfactant forms a sheath around the peptide by interaction with its hydrophilic head group; a process facilitated by lyophilisation of an ad-mixed solution [78]. When the anhydrous mix is dispersed in oil, it forms a molecular dispersion; which distinguishes it from particulates in oil. However, unlike Macrosol™, not all complexed peptides are soluble in lipid vehicles, rather form particulates in oil (solid/oil systems). For example, improved oral delivery of hGH in rats was measured from an oily suspension of protein complexed with sucrose erucidate dispersed in soybean oil [393].

Macrosol™ is part of the portfolio of Proxima (UK) (Axcess™) and has been licenced for oral delivery of insulin (Capsulin™; Diabetology, UK), calcitonin (Capsitonin™; Bone Medical, Australia) and PTH (CaPTHymone™, Bone Medical, Australia). In Capsulin™, insulin is mixed with sodium ursodeoxycholate, dispersed in benzyl alcohol, and inserted to size 4 soft gelatin capsules, which lowered blood sugar following oral delivery in pigs [394]. Repeated oral administration of Capsulin™ to diabetic patients 30 min prior to breakfast and an evening meal reduced HBA1c levels by 0.2%, while the percentage of patients remaining below the recommended excursion level increased from 10% to 36 % [395]. The oral Capsitonin™ and CaPTHymone™ formulations had comparable effects to their

respective injectable forms (Miacalcin® and Forteo®) in lowering of plasma C-terminal telopeptide (CTX-1) and plasma Ca^{2+} [396]. Many of the excipients used in Axcess™ formulations are listed in national compendia, for example phenoxy ethanol, benzyl alcohol, butylated hydroxyanisole and propyl gallate are commonly used preservatives [397, 398]. However, the quantities used in oral peptide delivery are significantly higher than those listed in the FDA Inactive Ingredients list.

4.4 NON-SURFACTANT PEs

Aside from the new generation of paracellular PEs, several non-surfactant PEs have been evaluated in pre-clinical and pilot clinical testing (Table I, Table S1). A number of PEs in this category have not progressed in recent years, such as sodium taurodihydrofusidate (STDHF) [399], salicylates and enamines [400]. Likewise, a cohort of this PE group have low clinical potential because of active pharmacology (e.g. salicylate [401]) and sodium nitroprusside [402]) or known systemic toxicity (p-chloromercurylphenyl sulphate [403]).

Case 15: salicylates and enamines

Studies on the action of sodium salicylate and 5-methoxy salicylate have contributed significantly to the potential application of PEs as vehicles to improve transmucosal peptide delivery, in particular across the rectal mucosa (Table S1). However, as salicylate is the active form of aspirin, its application in oral peptide delivery is limited by its anti-inflammatory and antiplatelet actions. Rectal suppositories of insulin (5-50 IU) containing triglyceride (700 mg), lecithin (70 mg) and sodium salicylate (300 mg) reduced plasma glucose in healthy dogs [404]. Moreover, absorption of hGH from a suspension of sodium salicylate in mineral oil exceeded enhancement from an aqueous solution in rat intestinal instillations [405]. In clinical testing of 10 T2D patients and 4 healthy volunteers, suppositories containing insulin (100 IU), hard fat (Witepsol H15) and sodium salicylate (200 mg) lowered plasma glucose by 28% over 2 h in T2D, after which blood sugar returned to basal level [406]. In another clinical evaluation, suppositories containing insulin in CA (0.5 M) and salicylate (300mg) improved rectal insulin absorption compared to the suppository base alone (Witepsol® H15) [407]. The enamine D,L-phenylalanine ethyl acetoacetate had comparable effect on rectal absorption of insulin. This enamine is formed by reacting phenylalanine and the food additive ethyl acetoacetate, but which

is hydrolysed to phenylalanine and ethyl acetoacetate in the body; suggesting that safety might not be a significant consideration. A variety of enamines have been shown to improve intestinal permeability in pre-clinical models, but phenylalanine ethyl acetoacetate exhibited stronger enhancement than several other enamines [400], and improved rectal absorption of insulin from a suppository in diabetic dogs [408].

Case 16: chitosan and its derivatives

Chitosan is one of the most widely studied semi-synthetic polymers in the delivery field, and its capacity to improve intestinal permeability is well-known [92, 409-412]. However, despite its promising action as an intestinal PE *in vitro*, it has not been assessed to date in clinical trials for oral peptide delivery. It is a polymeric PE formed by deacetylation of chitin to form a heteropolymer of N-acetylglucosamine and D-glucosamine. The primary amine of glucosamine has an approximate pKa of 6.5 and is therefore protonated in acidic conditions to yield the soluble cationic form, which is responsible for enhancement action. Permeation enhancement across Caco-2 monolayers was more evident for high MW variants and those that have a higher proportion of D-glucosamine i.e. a low degree of acetylation (<35%) [413]. As enteric coating is a critical component of oral peptide delivery systems, the pKa of chitosan is problematic, as variable ionisation within the jejunum (pH 6.1-7.1) creates variability in the proportion of soluble chitosan, which ultimately contributes to low bioavailability. Different chitosan salts have different efficacy as intestinal PEs [411], and a number of these salts have been shown to improve the transmucosal flux of peptides (e.g. HCl [414] and glutamate [415]). For example, rat intestinal instillation of chitosan HCl (1.5%) improved F_{ABS} of buserelin to 5%, which if translated in man would be impressive, given that the marketed nasal formulation has an F_{ABS} of 3.3% [414]. However, while these salt forms are likely to improve dissolution from enteric-coated formulations, chitosan is sensitive to pH-dependent precipitation. Another drawback to native chitosan is the gelling properties at concentrations as low as 1.5% [410], which impacts optimal release and presentation of peptide and PE at the intestinal epithelium. An assessment of chitosan acetate as a tablet binder showed that inclusion of low concentrations (1, 2, or 3 % w/w) prolonged dissolution of theophyline from tablets to beyond 3 h, which contrasted to dissolution from tablets containing an established binder (PVP K30), where complete release was demonstrated within 1 h [416]. In assessment in compendial media, release from HCl

(0.1 N) occurred in 6 h and release from phosphate buffer (pH 6.8) reached 97% and 100% within 16 and 24 h [416]. Although inclusion of excipients (e.g. Kollidon® VA64 (BASF)) can alter the disintegration of tablets that contain chitosan (5% w/w) [417], it is not clear if timely dissolution can be achieved for the higher concentrations of chitosan that are required for enhancement action. In a recent clinical evaluation, an oral dispersion of chitosan HCl (1 g/150 mL) reduced absorption of the BCS Class III drug, acyclovir (200 mg) in 12 healthy volunteers [418]. It seems that oral delivery of chitosan in fluidic dispersions in man do not mirror the enhancement data observed in pre-clinical delivery models. The safety of chitosan has been extensively reviewed with the finding that it has the potential to be a safe pharmaceutical excipient [419], although damage intestinal epithelial cells has been recorded in cell culture models [413]. In general, the systemic toxicity of such polymeric PEs is predicted to be low as they are poorly absorbed.

The formulation and process attributes of chitosan (125 kDa, 91% deacetylation) in matrix tablets has been evaluated [420]. This chitosan variant suffered from poor particle size uniformity (44.7% ranged between 63-150 μm) and poor powder flowability and compressability, which influences weight uniformity in tablet production [420]. Increasing degrees of deacetylation (80%, 85% and 90%) impact both compressability/flowability and uniformity [421]. Inclusion of a glidant and restriction of particle size limits to >212 μm were effective in controlling weight variability [420]. Chitosan had tablet and tableting properties that were similar to microcrystalline cellulose and was considered appropriate for tableting [422]. However, the compression force used in tableting resulted in slight melting of chitosan and the formation of hard tablets (300 N) that are likely to exhibit slow release. The mean dissolution time (MDT) of isoniazid from chitosan tablets was 7.65 min, a value that increase to 30 min upon inclusion of citric acid (8%); not surprising as CA increased the solubility of chitosan leading to the formation of an interfacial gel layer that restricts interfacial migration of the drug into the bulk of the phase [420].

Several analogues have been developed to address chitosan precipitation at small intestinal pH values including trimethylation (TMC) [423] (Table I), triethylation [424] and combinations therein [424] to form quaternary ammonium compounds that are charged at intestinal physiological pH values. Other derivation strategies have

involved the formation of mono-N-carboxymethyl chitosan which gives rise to ampholytic variants that are soluble at physiological pH [425]. The most comprehensively tested chitosan derivative is TMC, which has high aqueous solubility even at low acetylation (<10%) and lower intrinsic viscosity [426]. However, higher concentrations of TMC (>10%) used to improve oral octreotide delivery in pigs formed a gel and exhibited comparable viscosity to chitosan HCl [77]. While this gel improved oral octreotide bioavailability in pigs (F_{ABS}: 1.7% versus F_{ABS}: 24.8%), the rheological behaviour of TMC remains problematic for oral peptide delivery [59]. Dissolution of tablets containing TMC was also found to be challenging in that they did not dissolve in water even when formulated with disintegrants [427]. To address this problem, DDAVP (0.05-0.1 mg) and TMC (7.5-15 mg) were wet-granulated with microcrystalline cellulose 10-15% (Avicel® PH-101, FMC Biopolymers, USA), and subsequently mixed with a super-disintegrant (Ac-Di-Sol®, FMC Biopolymers) prior to tableting in to mini-tablets (3 mm). While dissolution of DDAVP was not impeded, only 50% of the TMC was released from each mini-tablet after 2 h, lower concentrations than those needed to improve oral octreotide delivery [77]. This formulation was adapted for oral delivery of octreotide in pigs, where mini-tablets of octreotide and TMC were loaded in enteric-coated capsules [410], but no improvement in bioavailability was observed [410].

Thiolated chitosan derivatives are a family of thiolated polymers or thiomers thatare reported to alter intestinal permeability to improve oral peptide delivery. The enhancement action of thiomers is typically not as efficacious as the leading surfactant-based PEs, but certain analogues can combine enhancement action with mucoadhesion, peptidase inhibition, and efflux pump inhibition [428, 429]. Chitosan-thioglycolic acid (TGA) improved permeability of leuprolide in isolated rat intestinal mucosae by 4-fold [430]. In oral delivery to rats, the F_{ABS} of leuprolide was increased by 3.8-fold from a gel formulation containing chitosan-TGA (8 mg/mL) [430]. Research effort has focused on the physical behaviour of thiomers in oral solid dosage forms. Several thiomers sustain drug release from tablets [431] and mini-tablets [67, 432]. Given that thiomers are mucoadhesive and can inhibit peptidase activity, it is not surprising that they retain the ability to improve intestinal permeability, although whether this is directly due to permeation enhancement or whether such enhancement can effectively translate to man is unknown.

Another thiomer, chitosan 4-thiobutylamidine (TBA) improved permeability of acyclovir across Caco-2 monolayers and isolated rat intestinal mucosa [432]. In the same study, release of acyclovir from mini-tablets (30 mg) containing chitosan 4-TBA was MW-dependent in the order of 9.4 kDa >150 kDa>600 kDa, yielding dissolution values of 90%, 60% and 40% after 1 h. Oral delivery of the decapeptide, antide, in a matrix tablet (2 × 500 mg) containing 400 mg of chitosan 4-TBA per tablet improved oral bioavailability of the peptide in pigs from an undetectable level to F_{ABS} of 1.1% (and R_{REL} of 3.2%) [431]. In a similar study, an enteric-coated oral tablet (10mg) containing insulin (2.8 mg) chitosan TBA (5mg) and two peptide inhibitor conjugates of chitosan (chitosan-BBI (0.75 mg) and chitosan-elastatinal (0.75 mg), as well as CA (0.1 M) was tested in rats [433]. This formulation sustained insulin release over at least 8 h, which was mirrored by lowering of blood sugar in rats; however, given the large dose of insulin, both F_{ABS} (0.7%) and F_{REL} (1.7%) were low. Similar results were observed from an oral formulation (5 mg) containing sCT (50 µg), chitosan TBA (3.75 mg) and a chitosan- peptidase inhibitor conjugate, although that formulation was not enteric coated [434].

Other thiomers also improve peptide delivery from oral solid dosage forms. Oral delivery of mini-tablets (30 mg) containing insulin and chitosan 6-MNA (6-mercaptonicotinic acid, 20 kDa, 1:4 ratio) improved oral absorption of insulin in rats (F_{ABS}: 0.73%) compared with control tablets containing chitosan alone (F_{ABS}: 0.15%) [67]. The rate of release of insulin from mini-tablets containing chitosan 6-MNA was comparable to native chitosan (60-80% after 2 h) despite a 5-fold increase in bioavailability. It is noteworthy that the dissolution media contained 30%v/v DMSO, which has a likely impact on the release kinetics in man. One of the reasons for this difference was attributed to the >80-fold increase in mucoadhesion for chitosan 6-MNA, which highlights the importance of regional retention and localisation in achieving efficient intestinal permeation enhancement. On a note of caution, it is important to reiterate that mucoadhesion is clearly limited by the rate of mucous turnover in the gastrointestinal tract, which is species specific [435]. All of the thiomers outlined above are in the portfolio of Thiomatrix (Austria), however evidence of the clinical effectiveness of thiomers in oral peptide delivery has yet to be reported.

Case 17: CPPs

Cell penetrating peptides (CPPs) are a group of peptides that can improve intestinal peptide permeability [436]. Research in CPPs is built around three sequence types: protein-derived CPPs (e.g. HIV transactivator of transcription (*tat*) peptide and penetratin (Table I)), chimeric peptides (e.g. transportan [437]) and designed/synthetic peptides (octa-arginine) [438] (Table S1). Exactly how CPPs increase intestinal permeability is related to their capacity to initiate endocytosis, direct translocation and to formation of channels within the cell membrane at high concentrations [439]. In many cases the therapeutic peptide is fused with the CPP or loaded into microparticles or nanoparticulates coated with CPP to improve translocation efficiency. For example, Enteris Pharma (NJ, USA) disclosed a membrane translocator fusion peptide derived from *tat* to improve oral delivery of sCT [440]. Intestinal instillation of the sCT-membrane translocator (4.5 mg / 43 mg CA) led to an improvement in F_{ABS} from <1% to 21% in rats [441]. The enhancement action of unconjugated CPPs is often observed at concentrations that are 2 log orders lower than conventional surfactants. For example, transportan (10 µM) reduced TEER in Caco-2 monolayers [442], far lower than the concentration of 10-13 mM required for C_{10} [89]. CPPs also improve intestinal epithelial permeation of associated antidiabetic peptides. Intestinal instillation of L-penetratin (0.6 mg) in rats improved absorption of GLP-1 (0.1 mg/kg) from 0.89% to 11% [443]. The ability to engineer structural analogues of CPPs permits optimisation of both enhancement action and safety. SAR analysis of a panel of penetratin analogues led to development of a more efficacious stable analogue, PenetraMax™ [444]. Absorption of insulin in rat instillations was improved in the order of D-PenetraMax (F_{REL}: 26%) > L-PenetraMax (19%) > L-Penetratin (12%) > octa-arginine (4%) [445]. A number of these analogues have been disclosed in patents registered by Toray Pharmaceutical Inc. (Japan), who have performed additional pre-clinical evaluation [446]. Further analysis of the potential of CPPs in oral peptide delivery is covered by Giralt *et al* (this Issue).

4.5 MULTIPLE MODES OF ENHANCEMENT ACTION

Many of the PEs that act primarily via the transcellular route have also been shown to alter paracellular permeability. In some reports, a clear concentration dependency is observed between paracellular and transcellular actions, but others report paracellular

enhancement at concentrations that more closely align with transcellular perturbation. However, a drop in TEER across Caco-2 monolayers is sometimes assumed to be an increase in "paracellular permeability", but any physical or chemical insult can non-specifically increase conductance across epithelial monolayers. More complex measurements using impedance spectroscopy have been reported [447], but such models require strict microscopic verification that the PE does not lead to intracellular uptake of a paracellular dye (i.e. transcellular perturbation), and applications that fail to do so can overestimate the contribution of the paracellular route [448]. In some cases, a paracellular mode can be uncoupled from transcellular enhancement in reductionist models, although the lower concentrations that avoid transcellular action may not be representative of the effective concentrations *in vivo*. While pharmacological inhibitors of enzymes, receptors, and signalling molecules have been shown to attenuate enhancement action of selected PEs, their actions are not comprehensively understood nor are they effective at higher concentrations that are necessary for enhancement in animal models.

There is a desire amongst investigators to research PEs that exclusively alter paracellular permeability, primarily due to perceptions of safety issues relating to transcellular perturbation. This is one of the reasons why specialist delivery companies favour delivery technologies that have a history of safe use in man or have been designated as GRAS. A wide range of tools have been used to evaluate detergent interaction with the plasma membrane of intestinal epithelial cells including cell integrity assays, BBMV and more recently, high content image analysis. A greater contribution from the paracellular route is observed at low and intermediate concentrations of membrane perturbants [449] owing to unpredictable actions ranging from (i) modulation of intracellular mediators (e.g. Ca^{2+} and ATP [202]) (ii) receptor activation (e.g. phospholipase C (PLC) [129]), (iii) selective removal of TJ proteins from fluidic regions of the membrane (e.g. claudin [450]) and (iv) cellular repair mechanisms. These diverse actions are most commonly observed with surfactants such as medium chain fatty acids, acyl carnitines and sucrose esters (Table I, Table S1).

There are a number of signalling molecules that, if depleted by transcellular perturbation, could lead to alteration in TJ structure. For example, an alteration in

membrane fluidity can lead to leakage of intracellular mediators like ATP, and its depletion has been associated with Ca^{2+}-dependent alteration in TJs [451]. A group of PEs deplete intracellular ATP including C_{10} [129] and acyl carnitines [202]. These actions are lost above a threshold concentration of PE, because the perturbation caused by the surfactant deactivates the cell, and in such a case, cells that have survived the initial perturbation by the surfactant PE stimulate intracellular signalling processes that are involved in mucosal repair. This begins with disbandment of TJs, spreading and protection of exposed surface, and is concluded by resealing [452].

Ca^{2+} is another intracellular signalling molecule associated with multimodal PEs, but the nature of its role has not been fully elucidated. Electron micrographs of Caco-2 monolayers treated with 10 mM C_{10} revealed dilation of 42% of TJs in one study [453] and transcellular perturbation in another [149]. *In vitro* experiments with C_{10} on monolayers and tissue mucosae are performed in the absence of extracellular Ca^{2+} on the apical side, so increases in intracellular Ca^{2+} are caused by release from intracellular stores, but whether alterations in barrier function are due to a defined signalling mechanism or perturbation of organelles involved in intracellular storage is not clear. At lower concentrations, C_{10} (2.5 mM) increased intracellular Ca^{2+} in Caco-2 cells before other cytotoxicity metrics could be observed using high content image analysis (e.g. plasma membrane permeability changes at 8.5 mM) [150]. In cases where alteration to intracellular Ca^{2+} can be dissociated from plasma membrane perturbation, the PE has only modest effect on TEER at such concentration (40% drop in TEER after 60 min). At widely studied concentrations *in vitro*, C_{10} (8.5-13 mM) altered plasma membrane and mitochondrial membrane integrity. Most mechanism of action studies with C_{10} are however performed at higher concentrations (10-13 mM). C_{10} (13 mM) reduced localisation of ZO-1 and occludin in Caco-2, and its effect on permeability could be reversed with several pharmacological inhibitors of cellular signalling molecules including PLC, calmodulin, diacylglycerol (DAG), inositol-3-phosphate (IP3), and Ca^{2+} [129] as well as MLCK [146]. This led to the theory that C_{10} increases intracellular Ca^{2+} through the activation of PLC, which activates calmodulin-dependent phosphorylation of MLCK, in turn phosphorylating MLC, leading to cytoskeletal contraction and disbandment of TJs [89].

The elucidation of mode of action is complicated by the capacity of certain surfactants to concurrently remove TJ proteins from regions of the plasma membrane that are more sensitive to detergents. Thus, C_{10} displaced claudin 4 and 5 from lipid rafts in MDCK cells, showing the importance of protein solubilisation [450]. Likewise, the interaction of surfactants with the plasma membrane can indirectly modulate the activity of membrane proteins by exposing the receptor to ligands. The transcellular route should therefore be considered in assessment of specific intracellular signalling mechanisms. Given that C_{10} alters multiple cellular metrics at 13 mM, it is not correct to consider it an exclusive paracellular PE, as its complete spectrum of actions cannot be dissociated from those of transcellular perturbation. Superficially, the paracellular mode of action appears to be less relevant *in vivo* as the dose of C_{10} used in oral peptide animal testing and those tested in oral dosage forms in man are far in excess of those used *in vitro*. However, concentrations at the intestinal epithelium could still be relatively low *in vivo* when dissolution, spreading and dilution are taken into account. It is noteworthy that pharmacological inhibitors associated with paracellular action are also effective in tissue models and pre-clinical animal testing, and so while transcellular enhancement is the predominant mode of action in animal models, a significant paracellular contribution is also possible. For example, a calmodulin inhibitor (W7) attenuated the enhancement action (TEER and FD4 flux) of both C_{10} and LCC in isolated rat and human colonic mucosae [454]. Likewise, W7 also attenuated enhancement action of C_{10} in rat rectal delivery, but while the authors conclude that C_{10} acts through calmodulin-dependent cytoskeletal contraction, the mechanism is far from clear since each suppository had 39 mg of C_{10}, which if dissolved in 0.3-1 mL rectal volume would reach a concentration of 200-670 mM [455].

A mechanistic study in Caco-2 cells performed on 51 PEs from 11 structural groups found that PEs can exploit both the paracellular and transcellular routes *in vitro* [449]. The screen involved calculation of K values (relative contribution of paracellular route) which measured from 0 (transcellular) to 1 (paracellular) from the formula K = EP-LP/EP where EP is enhancement potential ($EP = [100\% — TEER_{TEST}] \div [100\% —TEER_{TRITION\ X\text{-}100}]$) and LP is LDH Potential (LDH_{TEST}/LDH_{MAX}). The model assumes that LDH is an acceptable marker for transcellular enhancement, although its sole use could overestimate the contribution of the paracellular route, as the absence

of LDH release is not necessarily an optimal marker of cell perturbation. This point is emphasised when the IC_{50} of Triton X-100 was compared in different cytotoxicity assays: LDH (80µM) > MTT (44µM) > Neutral red (31µM) > ATP (43µM) [456]. High concentrations of PE surfactants including SLS (0), Triton® X-100 (K = 0.06), sodium oleate (K = 0.18), PCC (K = 0) and sodium deoxycholate (K = 0) exhibit transcellular enhancement action, but at lower concentrations, the contribution of some PE was associated with the paracellular route (e.g. sodium deoxycholate (K = 0.71) and sodium oleate (K = 0.96)). The action of established paracellular PEs like EDTA was comparable to literature observations (K = 0.72), but others like sodium salicylate (K = 0.8) had an unexpected paracellular action. The separation of paracellular from transcellular PEs allowed the discovery of a linear relationship between Log P and transcellular enhancement, and an inverse relationship between Log P and paracellular enhancement. A linear effect between Log P and enhancement was not observed for surfactant-like PEs, as specific criteria relating to HLB and CMC are more important than outright lipophilicity. Further, quantitative SAR using this dataset and an additional panel of physicochemical properties permitted further prediction around mechanism of action [457]).

5. SAFETY AND REGULATION OF PEs

Toxicity has long been cited as a potential drawback to the application of PEs in oral peptide delivery [458]. Each PE has specific attributes that must be considered in an overall risk benefit analysis and generalisation on toxicity is not appropriate. Enhancement action reported for the most clinically advanced PEs was often accompanied by evidence of regional toxicity in reductionist models, such as loss of cell viability in cytotoxicity assays, or focal and superficial mucosal injury in histological analysis of intestinal tissue. However, to our knowledge there have been no significant adverse events reported for any of the leading PEs tested in clinical trials to date. Given the fast rate of intestinal transit, spreading, dilution and absorption (Fig. 1), it is improbable that the intestinal epithelium will be exposed to PEs at high concentrations for prolonged periods locally, as was observed in some pre-clinical delivery models. Inferences relating to PE safety based on cytotoxicity measurements in static cell culture models are therefore not reflective of the dynamic *in vivo* environment or the capacity of the GI tract to repair from superficial mucosal injury.

5.1 TRANSCELLULAR ENHANCERS AND MEMBRANE PERTURBATION

Surfactants fulfil a wide array of applications in a variety of fields from household cleaning products, to stabilisation of cosmetics to heavy industrial applications. There are concerns relating to the application of strong detergents such as those used in industrial applications, but there are no studies advocating use of strong detergents in oral peptide delivery. Rather, the clinically-advanced PEs have established safety profiles, for instance SNAC is designated GRAS, C_{10} has Food Additive Status and sucrose laurate is an allowed excipient in the USA. Furthermore, no clinical evidence has been presented to date that suggest formulations containing PEs cause serious mucosal damage. Surfactants are widely used in formulation of both enteral and injectable dosage forms [215]. These excipients have been shown to alter barrier integrity, and relevant examples include polysorbate 20, polyethoxylated castor oil, PEG-8 glycerides, long chain fatty acids and medium chain monoglycerides. Some alteration in barrier integrity and cell viability is anticipated when surfactant excipients are used in oral formulation. The dose of Cremophor® EL (up to 600 mg) and Cremophor® RH40 (up to 405 mg) used in oral formulations suggests that the concentration of these additives could reach levels that alter intestinal epithelial cell viability (EL: 5 mg/mL and RH40: 10 mg/mL) [459].

Surfactants are also licensed without evidence of serious side effects. Sodium docusate is a stool softener used on a daily basis in the treatment of constipation. The intestinal permeation enhancement action of docusate has been reported (Table S1). Bile salts like ursodeoxycholate (Ursofalk®, DrFalk Pharma, Germany) are also used for the dissolution of gallstones (750 mg/day) or bile replacement in primary biliary cirrhosis (up to 1.75 g/day). Jejunal instillation of ursodeoxycholate (10 mg) increased F_{ABS} of octreotide from 0.3% to 4.9% in rats [90]. Enhancement was associated with a time and concentration dependent release of LDH in Caco-2 suggesting a transcellular mechanism. Chenodeoxycholate has also been used in dissolution of gallstones, and this bile acid increased absorption of octreotide in healthy human volunteers at a dose as low as 100 mg [90]. The most widely cited side effect of these bile acids is diarrhoea. Therefore, administration of selected molecules that alter barrier integrity can be administered in man without manifestation of toxicity.

It is tempting to justify the use of PEs by citing precedence in the routine use of substances capable of altering barrier integrity. However, the capacity of aspirin, alcohol or other substance to reversibly perturb the intestinal mucosae does not set precedent for approval of medicines containing PEs. In the US, the regulatory agency does not provide an approval path for excipients, but as part of the submission of a product formulation. Once an excipient is used in an approved oral drug formulation, it is more likely that it will be approved in other ones, but this will depend on API-specific aspects, doses of PE, and acute or chronic administration needs [460]. There is however, value in understanding how abrasive substances interact with the intestinal epithelium to predict how PEs might behave in man.

At one extreme, mild mucosal damage occurs in 40-50% of patients taking low dose aspirin, and these patients are at an increased risk of GI bleeding [460], although the risk of bleeding is multi-factorial [461]. There is no evidence to suggest that PEs inhibit cyclooxygenase, but perturbation has the potential to impact cell viability. The response of the intestinal epithelium to transcellular PEs that cause mild mucosal perturbation has been studied in pre-clinical delivery models, which has given insight into the rate and extent of cell and tissue recovery prior to repeat dosing. The most relevant example to date was observed following histological assessment of rectal mucosae of patients administered Doktacillin™ suppositories containing C_{10} [83]. Mild and reversible histological damage to the mucosae was seen in patients, and there was association between enhancement and mucosal damage in the rectum, which the authors ascribed to a combination of C_{10}, the suppository base, and hyperosmolar conditions. Pre-clinical data has highlighted how exposure time and concentration impact epithelial integrity and viability, and also the extent to which the barrier can repair following removal of the PE. Studies reveal such information for bile salts [71, 462-464], ethoxylates [465] medium chain fatty acids [149], monoglycerides [71] and SDS [71].

Treatment of Caco-2 monolayers with C_{10} (8.5 mM) for 15, 30 or 60 min led to a reduction in TEER to 15%, 5% and 0% of initial respectively, but upon removal of the PE, TEER recovered to 100% after 2 h, 4 h and 7 h, respectively [149]. A number of toxicity metrics were evaluated during the recovery from the 60 min treatment with C_{10}. In high content image analysis, there was no change in cell number between

treatment and 24 h recovery, and there was a progressive recovery in a range of cytotoxicity metrics to control levels including intracellular Ca^{2+} and plasma membrane permeability. Electron microscopic analysis showed that damaged epithelium recovered after 4 and 24 h, and there was a time-dependent modulation in expression of inflammatory markers. The most noteworthy change was observed for interleukin-8 (IL-8) where expression changed by +11 (1 h), +26 (4 h), +3 (8 h) and –3 (24 h) recovery. Increased IL-8 expression was observed in inflamed mucosa of patients with IBD [466], and incubation of colonic epithelial cells with IL-8 was associated with recruitment of neutrophils and an increase in resealing of the mucosal barrier [467-469].

Compared to monolayers, recovery of intestinal epithelial integrity is better established in isolated intestinal tissue mucosae and in animal models. Short term exposure of isolated guinea pig mucosae to Triton® X-100 (0.06% w/v) led to denudation at the tips of 86% of ileal villi, which reversed after 60 min [465]. The perfusion of rat intestine for 15 min caused denuding of both enterocytes and goblet cells in the order of sodium deoxycholate (5 mM) > SLS (5 mM) > EDTA (25mM) > PEG 400 (50%) [470]. While extensive loss of epithelial cells (80%) recovered to only 5% after 2 h, complete recovery was noted after 24 h. Damage to rat intestinal mucosa was also observed following instillation of C_{10} (100 mM) and oral delivery of SDS (1-2%), and repair was noted by light microscopy between 30-60 min for C_{10} [62] and 1 h for SDS [75]. Pre-treatment of rat colonic mucosae with misoprostol reduced C_{10}-induced mucosal damage through stimulation of mucous secretion [149]. Misoprostol also attenuated C_{10}-induced damage to Caco-2 monolayers in a mechanism involving Ca^{2+} homeostasis and production of phospholipids to reinforce the plasma membrane [149]. The capacity of the intestinal mucosa to repair following injury has been studied in response to endogenous detergents [471], dietary agents, and xenobiotics [472], where prostaglandins [473], nitric oxide [471] and growth factors [474], play a protective role (reviewed in [452]). Injured intestinal epithelial cells are detached from the basement membrane and from cells that shouldering the injury, which leads to sloughing. The response to protect exposure of the basement membrane to luminal constituents involves villus contraction, epithelial restitution and resealing of complexes at the lateral membrane.

5.2 THE BYSTANDER ABSORPTION ARGUMENT

A concern regarding the use of PEs in oral peptide formulations is the possible absorption of bystanders (such as toxins, bacteria, viruses and allergens) during the period of temporary epithelial permeability enhancement. There have been no adverse local or systemic immunological responses reported in the scientific literature or clinical studies with any of the lead candidate PE-containing delivery systems. A relatively small number of pre-clinical studies have evaluated the uptake of potentially harmful substances during an enhancement window. C_{10} (10-100 mM) did not permit translocation of *E. coli* across Caco-2 monolayers because the surfactant exhibits strong antimicrobial activity [89], whereas translocation of *E. coli* across monolayers was increased in the presence of Triton® X-100. In isolated ileal mucosae, C_{10} reduced adhesion of *S. typhimurium* [475].

Concern also relates to induced permeability to inflammatory molecules that may lead to intestinal inflammation, which in turn may propagate more serious permeability alterations associated with IBD. In principle, this is a valid concern as chemical inducers of murine colitis including dextran sodium sulphate (DSS) are believed to damage the colonic epithelium resulting in uptake of bacterial products to the underlying immune cell-rich sub-mucosa. However, PEs have not been shown to cause the extensive mucosal damage associated with ingestion of large quantities of DSS in rodents. On the contrary, even the PEs in clinical trials have only a modest effect on permeability of peptides so they are unlikely to increase permeability to large bacterial toxins or endotoxin. Chitosan nanoparticles opened TJs and increased intestinal permeability of insulin, but co-administration with LPS (5 mg/kg) to rats for 7 days did not lead to an increase in hepatic necrosis [476]. In a similar study, co-administration of penetratin (5mM) or C_{10} (154 mM) with LPS for 7 days to mice had no effect on biomarkers of hepatic necrosis, however taurodeoxycholate (96 mM) increased plasma levels of aspartate transaminase and alanine transaminase [477]. It is noteworthy that uptake of LPS into the hepatic portal vein is not pathological as low level uptake is associated with maintaining a normal responsive kupffer cell population, but excessive levels may lead to hepatic dysfunction [478].

The necessity of intimate contact of PE and payload at the mucosal surface to cause temporary and reversible membrane permeability provides an additional argument

that bystander molecules present will not have their permeation assisted, since they are dilute and not in direct association with the formulation.

It has been proposed [479] that surfactants derived from lipid digestion (including monoglycerides and free fatty acids [463, 480]) can impair barrier integrity following ingestion of a high fat meal. Clinical manifestations can be diarrhoea or constipation. In more extreme cases, both endogenous and exogenous substances that alter barrier integrity are considered environmental contributors to intestinal diseases that are characterised by an over active immune response. A strong link is established between abnormal intestinal permeability and mucosal inflammation in IBD [481]. While there is no evidence to suggest that PEs increase regional antigen permeation, they should not be used patients with IBD. Most toxicological concerns relating to the use of PEs relate to transcellular perturbation, but the effects of paracellular PEs are not free from concerns relating to barrier integrity. Altered expression of claudin is associated with ulcerative colitis (claudin-1,2,3,4,7), Crohn's disease (claudin-2,3,5,8), celiac disease (claudin-2,3,5,7,15), irritable bowel disease (claudin-1,2,4) and various infectious diseases (reviewed in [482]).

5.3 ARE PARACELLULAR PEs SAFER THAN TRANSCELLULAR PEs?

One of the driving forces for development of paracellular PEs is their apparent safety advantage relative to those that act transcellularly, but these agents are new chemical entities whose development comes with far more risk than PEs with established safety in man. The most advanced paracellular candidate is EDTA, but there are significant restrictions on this chelator that could impact the dose required to facilitate oral peptide delivery [483]. The maximum amount of EDTA in the FDA Inactive Ingredients List is 4 mg, significantly lower than the quantities used in pre-clinical testing and in oral peptide clinical trials. The discovery of ZoT for example was associated with elucidation of the toxicological actions of virulent strains of *V. cholera*. The native forms of ZoT, *C. perfringens* enterotoxin, and melittin are mainly tools to elucidate the structure and function of the TJ, but their analogues might be more suitable as PE candidates in oral peptide formulations. The FDA and International Council for Harmonisation (ICH) provides guidance documents for short, medium and long term pre-clinical safety testing of candidate pharmaceutical excipients. In the event that a candidate excipient is found to be pharmacologically

active or where previous studies have reported toxicity, this can influence further development.

The difference in mode of action for 1st and 2nd generation TJ modulators tends to favour development of 2nd generation from a safety perspective. There is less likelihood that a 2nd generation short peptide sequence will be absorbed at sufficiently high concentration to reach a threshold concentration to alter TJs at other epithelia. This contrasts with first generation TJ modulators that target ubiquitous cell processes in opening of TJs (e.g. PKC [484]). The consequence of absorption of first generation molecules is far more difficult to predict in different tissues, organs and systems, and they therefore represent a significant development risk due to potential systemic toxicity. Nevertheless, regardless of the general consensus that 2nd generation PEs are less likely to have off-target toxicity, there have been cases where peptides that alter CAR sequences can increase permeability at other epithelia (e.g. kidney epithelia) and endothelia (e.g. blood brain barrier [485]) and, if the peptides are not metabolised prior to excretion, there is the possibility that they could be concentrated within the bladder to act on TJs. The Claudin binder, C-CPE, distributed to liver (24%) and kidney (9.5%) 10 min post i.v. injection in mice [486]. Hepatic levels returned to 3.2% after 3 h, but renal levels increased to 47% after 6 h. A C-CPE mutant that lacked the ability to bind claudin had lower distribution in the liver but similar distribution in kidney. Likewise, C-CPE increased biomarkers of hepatic injury. This PE class therefore still poses a development risk that justifies more detailed safety assessment.

To date there have been no disclosed clinical assessments involving specific paracellular PEs, which indicates that TJ modulators are not yet viewed as lead candidates. There is the argument that there is little incentive to develop innovative excipients and delivery systems due to the risk involved. In order to avoid the requirement of supplementary safety testing, delivery companies are prioritising development of PEs that have established safety record in man. Others have invested in pre-clinical safety testing that enables them to formally request GRAS status and/or provide regulators with safety information to mitigate risk [368].

6. PE DEVELOPABILITY CLASSIFICATION SYSTEM

The effectiveness of a PE for oral peptide delivery can be simplified to three key criteria (i) onset time, (ii) extent of enhancement and (iii) recoverability/safety. These criteria were first used to categorise PEs as Class I (strong and fast reactivity with fast recovery, e.g. C_{10}, acylcarnitines), Class II (moderate and fast reactivity with fast recovery e.g. salicylate) and Class III (strong reactivity with slow recovery e.g. EDTA and CA) [206]. Here, we offer a PE Developability Classification System (PEDCS) to assist identification of PEs that demonstrate adequate enhancement for further assessment in oral formulation (Table III). The metrics were selected based on performance of C_{10} in (i) Caco-2 monolayers (TEER, P_{app} of [^{14}C-mannitol), (ii) isolated rat colonic mucosae (TEER, P_{app} of [^{14}C]-mannitol, histology score [487], and (iii) colonic instillation (F_{ABS} of FD4, enhancement kinetics, histology score). We also reviewed Compendium information and literature. A PE is required to obtain a score of >2 of 3 in order to be assigned "*Fast Enhancement*" status; >2 of 3 under to gain "*strong enhancement*" and >3 of 4 to gain "*recovery/safety*" criteria. A fast onset of enhancement action is required to ensure permeation of peptides under normal GI transit. It is therefore optimal that a rapid decrease in TEER be recorded in cell monolayer and tissue mucosae models (Table III). The most effective rate metric is a short T_{MAX} of FD4 in rat intestinal instillations. An optimal extent is measured by an increase in P_{APP} of FD4 to $>1 \times 10^{-5}$ cm/s; a level predictive of high permeability within the BCS. In instillation, F_{ABS} of FD4 should be >20% in rats.

Safety is measured at effective concentrations by (i) tissue damage observed in 2 h incubation in isolated intestinal tissue mounted in Ussing chambers, (ii) tissue damage and histological recovery after 2 h in rat intestinal instillations, (iii) the capacity of the GI to recover from a transport induced state as determined by F_{ABS} of FD4 measured 2-4 h post administration of PE, and (iv) overall safety assessment is a global rating that must be substantiated with assessment of five metrics (excipient status, additive status, GRAS status, mode of enhancement action, and published systemic safety data). The ideal PE is designated Class 1 due to (i) fast enhancement to facilitate rapid uptake of peptide during GI transit, (ii) exhibition of potent and efficacious enhancement and (iii) demonstration of good safety/recovery. Class 2 PEs are likely to have strong enhancement action, but overall regional safety via oral delivery is a key consideration; although other routes may be possible. Class 3 PEs have modest enhancement and could be more effective in assisting permeation of selected small

molecule drugs. Class 4 PEs have strong enhancement action but their slow rate of onset limits their application to alternative routes of administration, such as topical buccal or rectal. Class 5 PEs are slow to act, but despite strong enhancement action, their use is limited by safety concerns. Class 6 PEs have good safety, but this is accompanied by slow onset and modest to low enhancement action, which renders them unsuitable for oral peptide delivery.

7. CONCLUSIONS

Over 250 PEs have improved intestinal permeability of poorly absorbed drugs including peptides in every conceivable pre-clinical drug delivery model. Yet there is a relatively poor translation of PE-based delivery systems for oral peptides. The majority of pre-clinical data was achieved in epithelial monolayer cultures, isolated tissue and intestinal instillations, and, while these delivery models identify PEs that alter barrier integrity and improve flux/bioavailability of peptides, such systems do not address formulation considerations. Clinical evaluation has largely been limited to a small group of PEs that can be formulated into solid-dose formulations and which have established safety in man. Even then, bioavailability typically remains low and variable, leading to new strategies to formulate peptides, such as PEs in lipid-based systems and the application of nano-encapsulation strategies with PEs. Novel TJ modulators are a promising group of candidate PEs, but none have yet progressed to clinical testing. Convergence between delivery and formulation sciences will facilitate better understanding of the hurdles to translation between oral peptide delivery systems and optimal dosage forms for man.

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FIGURE LEGENDS

Fig. 1. Optimal liberation of peptide and PE from an oral solid dosage is required to maximise enhancement of oral bioavailability. High regional concentration of peptide and PE improves intestinal flux through the creation of a diffusion gradient and by enabling the PE to reach a threshold concentration for flux enhancement.

Fig. 2. Modes of PE action. Paracellular PEs are divided into two classes. 1st Generation paracellular PEs increase intestinal permeability by targeting cell signalling pathways involved in disbandment of TJs. 2nd generation directly target the physical disruption of TJ by interfering in intercellular homophilic interactions. Transcellular PEs act via alteration to the integrity of the cell plasma membrane or via hydrophobisation of the target therapeutic peptide. Selected permeation PEs exhibit both paracellular and transcellular enhancement action in a concentration and/or time dependent fashion, and these PEs are referred to multimodal PEs.

FIGURES

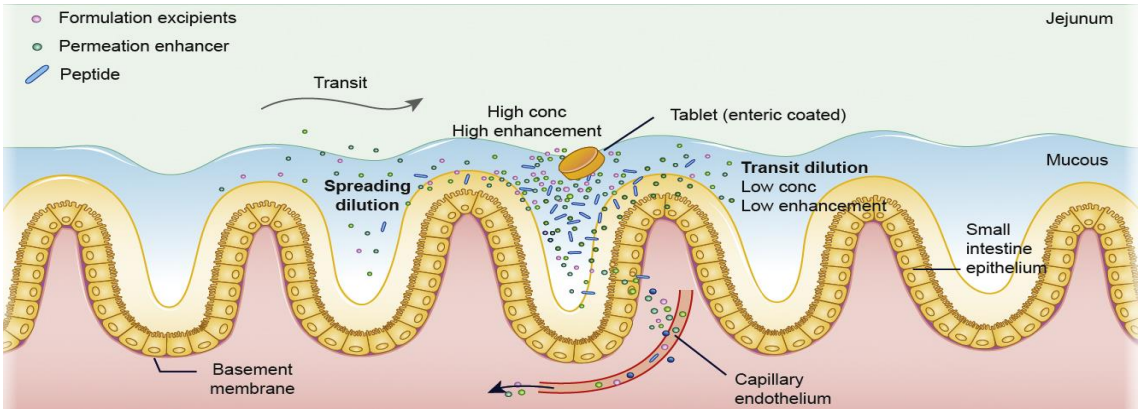

Fig. 1:

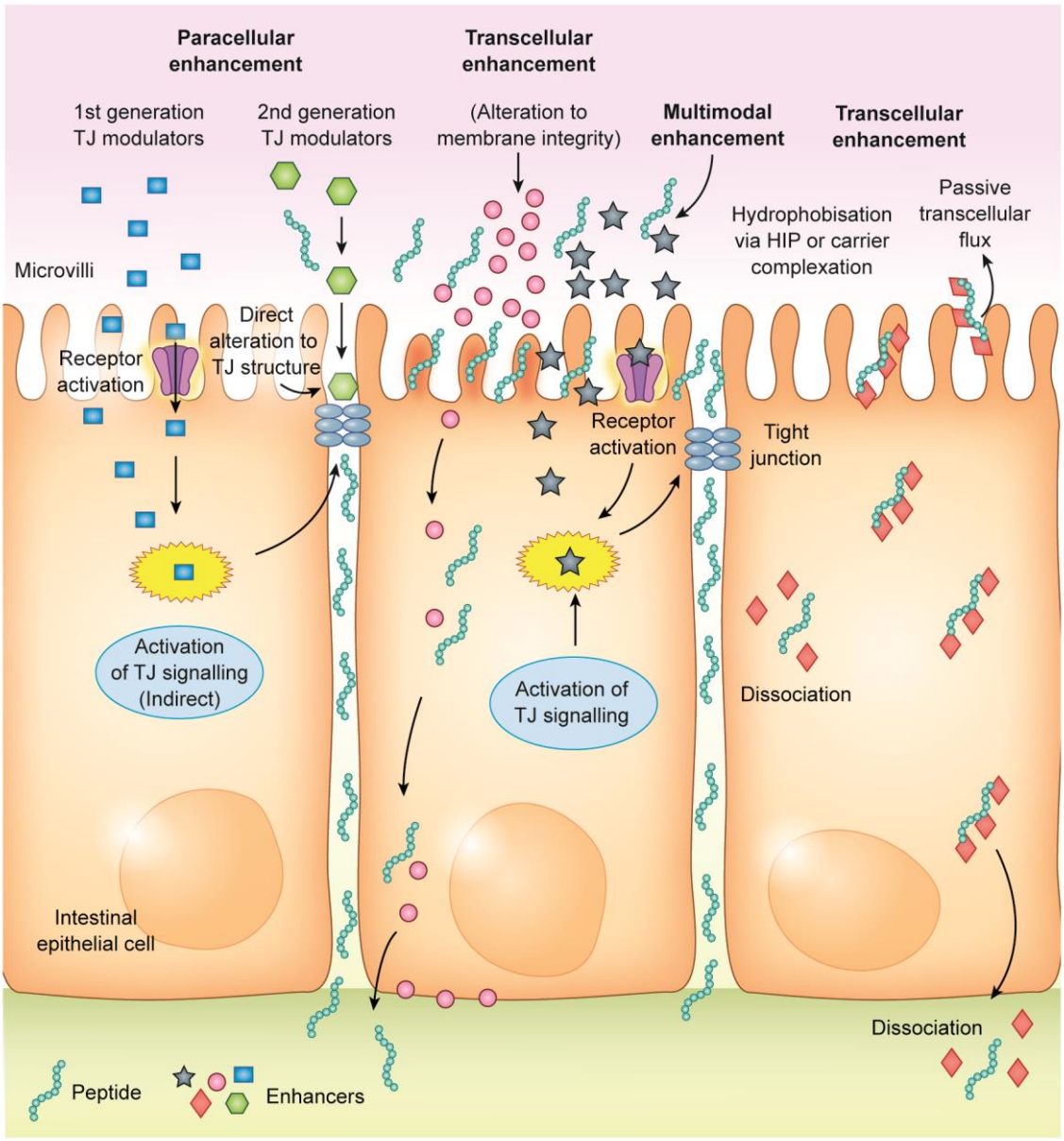

Fig. 2:

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Table I. Leading PEs tested in oral delivery of poorly permeable drugs and transport markers

ENHANCER	MODE	ACTIONS	MODEL	REPRESENTATIVE PEPTIDE/METRIC	CONCENTRATION & DOSE	ENHANCEMENT	REF
$C_{12}E_9$	Transcellular	Membrane fluidity	*In situ* (rat): intestinal loop *In vitro*: Caco-2 *Ex vivo* (rabbit): Ussing *In vivo* (rabbit):rectal instillation *In vivo* (dog): suppository *In vivo* (rat): gavage *In situ* (rat): perfusion *In vivo* (dog):suppository *In situ* (rat): rectal instillation *In situ* (rat): rectal *In vivo* (dog): suppository *In vivo* (rat): suppository	*In situ*: flux (fosfomycin) *In vitro*: flux (FD-10) *Ex vivo* (rabbit): flux (insulin) *In vivo* (rabbit): AUC (insulin) *In vivo*: RH (insulin) *In vivo*: PK/PD (heparin) *In situ*: flux (PABA) *In vivo*: PK/PD, flux (insulin) *In situ*: PK/PD (insulin) *In situ*: PK/PD (calcitonin) *In vivo*: flux, PK/PD (insulin) *In vivo*: PK/PD (insulin)	*In situ*: 1% *In vitro*: 0.1% *Ex vivo*: 5% *In vivo*: 1% *In vivo*: 1% *In vivo*: 500 mg/kg *In situ*: 1% *In vivo*: 3% w/w *In situ*: 5% *In situ*: 0.5% *In vivo*: 3% w/w *In vivo*: 3%	*In situ*: — *In vitro*: 520-fold *Ex vivo*: — *In vivo*: — *In vivo*: RH = 55% *In vivo*: — *In situ*: 19-fold *In vivo*: — *In situ*: 3-fold *In situ*: — *In vivo*: — *In vivo*: 118-fold	[209] [488] [489] [489] [211] [203] [490] [408] [208] [212] [210] [491]
Caprylocaproyl PEG 8 glycerides	Transcellular	Fluidic dispersion Membrane fluidity	*In vitro*: Caco-2 *Ex vivo* (rat): ileum *In situ* (rat): closed loop *In situ* (rat): colon *In situ* (rat): jejunal patches *In situ* (rat): jejunal *In situ* (rat): duodenal *In situ* (rat): duodenal *In situ* (rat): colon *In situ* (rat): Ileal *In situ* (rat): jejunal *In situ* (rat): intraduodenal *In vivo* (Dog): oral	*In vitro*: TEER, flux (mannitol) *Ex vivo*: flux (LY) *In situ*: F (rhodamine123) *In situ*: F (insulin) *In situ*: AUC,F (erythropoietin) *In situ*: AUC, F (erythropoietin) *In situ*: F (lansoprazole) *In situ*: AUC (LMWH) *In situ*: F (gentamicin) *In situ*: AUC (vancomycin) *In situ*: AUC (LMWH) *In situ*: AUC (LMWH) *In vivo*: F (gentamicin)	*In vitro*: 1% w/v *Ex vivo*: 0.1% v/v *In situ*: 0.1% v/v *In situ*: — *In situ*: 94 mg/kg *In situ*: 50 mg/kg *In situ*: 170 mg *In situ*: 30 mg/kg *In situ*: 1 mL/kg *In situ*: 1.06 g/kg *In situ*: 50 mg/kg *In situ*: 30 mg/kg *In vivo*: 0.6 mL	*In vitro*: 34-fold *Ex vivo*: no effect *In situ*: F = 22.82% *In situ*: F = 0.25% *In situ*: 21-fold *In situ*: 12-fold *In situ*: F = 28.1% *In situ*: 4-fold *In situ*: F = 55.3% *In situ*: — *In situ*: — *In situ*: — *In vivo*: F = 22.4%	[283] [492] [492] [287] [284] [288] [493] [161] [494] [495] [279] [161] [496]
Citric acid	Paracellular	Intracellular ATP	*In vitro*: Caco-2 *In vivo* (rat): oral *Ex vivo* (rat): Ussing	*In vitro*: flux (insulin) *In vivo*: PK/PD, F (sCT) *Ex vivo*: flux (FD-4)	*In vitro* 5 mg *In vivo*: 10 mg *Ex vivo*:	*In vitro*: no effect *In vivo*: F = 1.8% *Ex vivo*: —	[25] [201] [202]
Dodecyl-β-D-maltopyranoside (DDM)	Multimodal	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *Ex vivo* (rat): perfusion *Ex vivo* (rat): Ussing *Ex vivo* (rat): colon *In situ* (rat,) loop *In situ* (rat): loop *In situ* (rat): colon *In situ* (rat): closed loop *In vivo* (dog): oral	*In vitro*: TEER, flux (mannitol) *In vitro*: TEER, flux (FD-4) *In vitro*: flux (tiludronate) *In vitro*: flux (ranitidine) *Ex vivo*: flux (tiludronate) *Ex vivo*: flux (Phenol red) *Ex vivo*: flux (ebiratide) *In situ*: AUC(phenol red) *In situ*: F (carboxyfluorescein) *In situ*: AUC, flux (azetirelin) *In situ*: PA (hCT) *In vivo*: F (azetirelin)	*In vitro*: 0.1% w/v *In vitro*: 0.1% w/v *In vitro*: 0.025% w/v *In vitro*: 0.1% w/v *Ex vivo*: 0.025% w/v *Ex vivo*: 20 mM *Ex vivo*: 20 mM *In situ*: 20 mM *In situ*: 20 mM *In situ*: 2.5 mM *In situ*: 10 mM *In vivo*: 2.5 mM	*In vitro*: 9-fold *In vitro*: 26-fold *In vitro*: 3-fold *In vitro*: 19-fold *Ex vivo*: 7-fold *Ex vivo*: no effect *Ex vivo*: 7-fold *In situ*: 4-fold *In situ*: F = 57% *In situ*: 9-fold *In situ*: 4-fold *In vivo*: F = 43.5%	[227] [227] [497] [498] [498] [499] [245] [500] [501] [86] [231] [241]
EDTA	Paracellular	Ca^{2+} chelation PKC activation	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *Ex vivo* (rat): colon *In situ* (rat,) loop method	*In vitro*: flux (FD-4) *In vitro*: flux (FD-4) *In vitro*: flux (FD-4) *In vitro*: flux (PEG 4000) *Ex vivo*: flux (inulin) *In situ*: AUC (phenol red)	*In vitro*: 1 mM *In vitro*: 1 mM *In vitro*: 0.25% *In vitro*: 0.25% *Ex vivo*: 50 mM *In situ*: 20 mM	*In vitro*: 6-fold *In vitro*: 6-fold *In vitro*: 53-fold *In vitro*: 29-fold *Ex vivo*: — *In situ*: 2-fold	[502] [503] [136] [88] [504] [500]

			In situ (rat): ligated loop, colon *In vivo* (rabbit): oral *In vivo* (rat): rectal, microenema *In vivo* (rat): jejunum *In situ* (rat): rectal	*In situ*: AUC (insulin) *In vivo*: AUC,(norfloxacin) *In vivo*: flux (trypan blue) *In vivo*: flux (fosfomycin) *In situ*: PK/PD (insulin)	*In situ*: 1% w/v *In vivo*: 1:1 *In vivo*: — *In vivo*: 1% w/v *In situ*: —	*In situ*: 55-fold *In vivo*: no effect *In vivo*: — *In vivo*: — *In situ*: 3-fold	[20] [505] [265] [209] [208]
Glyceryl monocaprate	Transcellular	Membrane fluidity	*In situ* (rat): rectal *In situ* (rat): rectal loop *In vitro*: Caco-2	*In situ*: F (cefmetazole) *In situ*: AUC (insulin) *In vitro*: P_{app} (mannitol)	*In situ*: 0.25 mL/kg *In situ*: 50 mM *In vitro*: 1 mM	*In situ*: F = 18.2% *In situ*: 15-fold *In vitro*-	[266] [185] [257]
Laurylocarnitine	Multimodal	Membrane fluidity — Decrease Claudin level Increase Ca^{2+} levels Decrease ATP levels	*Ex vivo* (rat): BBM *In vivo* (rat): rectal *Ex vivo* (rats): S-G diffusion cell *Ex vivo* (rats): S-G diffusion cell *In vivo* (rat):oral (microcapsule) *In vivo* (dog): oral (EC capsule) *In vitro*: Caco-2 *Ex vivo* (rat) Ussing *Ex vivo* (rat) Ussing	*Ex vivo*: S (DPH) *In vivo*: F (cefoxitin) *Ex vivo*: flux (LY) *Ex vivo*: TEER *In vivo*: F (DMP 728) *In vivo*: F (DMP 728) *In vitro*: TEER, flux (FD-40) *Ex vivo*: I sc (Cl) *Ex vivo*: flux (FD-4)	*Ex vivo*: — *In vivo*: — *Ex vivo*: 10 mM *Ex vivo*: 2 mM *In vivo*: 8 mg/kg *In vivo*: 2 mg/kg *In vitro*: 100 µM *Ex vivo*: 0.5% *Ex vivo*: 0.5%	*Ex vivo*: — *In vivo*: 26-fold *Ex vivo*: 20-fold *Ex vivo*: — *In vivo*: F = 6.9% *In vivo*: F = 17% *In vitro*: — *Ex vivo*: — *Ex vivo*: —	[506] [506] [73] [73] [188] [188] [507] [202] [202]
n-Tetradecyl β-D-maltopyranoside (TDM)	Transcellular	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2 *Ex vivo* (rat): Ussing (jejunal) *Ex vivo* (rat): Ussing (jejunal)	*In vitro*: TEER, flux (mannitol) *In vitro*: TEER, flux (FD-4) *Ex vivo*: flux (mannitol) *Ex vivo*: flux (FD-4)	*In vitro*: 0.1% w/v *In vitro*: 0.1% w/v *In vitro*: 0.1% w/v *In vitro*: 0.1% w/v	*In vitro*: 143-fold *In vitro*: 153-fold *Ex vivo*: 9-fold *Ex vivo*: 20-fold	[227] [227] [508] [508]
N-Trimethylated chitosan	Multimodal	Membrane fluidity TJ alteration via PKC	*In vitro*: Caco-2 *In situ* (rat): instillation *In situ* (rat): intubation *In vivo* (pig): oral (EC capsule)	*In vitro*: flux (FD-4) *In situ*: F (buserelin) *In situ*: F (octreotide) *In vivo*: F (octreotide)	*In vitro*: 2.5% w/v *In situ*: 1% w/v *In situ*: 10% w/v *In vivo*: 40% (70 mg)	*In vitro*: 363-fold *In situ*: 16-fold *In vivo*: 15-fold *In vivo*: F = 0.5%	[509] [510] [77] [427]
Palmitoylcarnitine	Multimodal	Ca^{2+} level ATP levels Membrane fluidity Claudin modulation	*Ex vivo* (rat): Ussing *Ex vivo* (rat): Ussing *Ex vivo* (rat): BBM *In vivo* (rat): rectal *Ex vivo* (rats): S-G diffusion cell *Ex vivo* (rats): S-G diffusion cell *In vitro*: Caco-2 *In vitro*: Caco-2 *In vivo* (rat): oral (microcapsule) *In vivo* (dog): oral (capsule) *In vitro*: Caco-2 *In vitro*: Caco-2 *Ex vivo* (rat): Ussing *Ex vivo* (human): Ussing *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2	*Ex vivo*: I_{sc} (Cl^-) *Ex vivo*: flux (FD-4) *Ex vivo*: fluorescence polarization *In vivo*: F (cefoxitin) *Ex vivo*: flux (LY) *Ex vivo*: TEER *In vitro*: TEER, flux (ruthenium red) *In vitro*: flux (PEG 4000) *In vivo*: F (DMP 728) *In vivo*: F (DMP 728) *In vitro*: TEER, flux (mannitol) *In vitro*: flux (mannitol, Ca^{2+}) *Ex vivo*: flux (FD-4) *Ex vivo*: flux (FD-4) *In vitro*: TEER, flux (mannitol) *In vitro*: TEER, flux (FD-40) *In vitro*: TEER, flux (fluorescein)	*Ex vivo*: 0.5% *Ex vivo*: 0.5% *Ex vivo*: — *In vivo*: — *Ex vivo*: 5 mM *Ex vivo*: 1 mM *In vitro*: 0.2 mM *In vitro*: 0.2 mM *In vivo*: 8 mg/kg *In vivo*: 2 mg/kg *In vitro*: 500 µM *In vitro*: 100 µM *Ex vivo*: 0.5% *Ex vivo*: 0.5% *In vitro*: 0.75 mM *In vitro*: 100 µM *In vitro*: 200 µM	*Ex vivo*: — *Ex vivo*: — *Ex vivo*: — *In vivo*: 34-fold *Ex vivo*: 18-fold *Ex vivo*: — *In vitro*: 20-fold *In vitro*: no effect *In vivo*: F = 14.6% (6-fold) *In vivo*: F = 20.5% (2-fold) *In vitro*: 10-fold *In vitro*: — *Ex vivo*: 13-fold *Ex vivo*: 8-fold *In vitro*: TEER <10% *In vitro*: — *In vitro*: —	[202] [202] [506] [506] [73] [73] [511] [511] [188] [188] [503] [512] [454] [454] [513] [507] [514]
Penetratin (D- penetratin)	Transcellular	Carrier	*In vivo* (rat): oral *In vitro*: Caco-2 *In situ* (rat): perfusion *In situ* (rat): perfusion *In vitro*: Caco-2 *In vivo* (rat): instillation	*In vivo*: PA (insulin) *In vitro*: flux (insulin) *In situ*: AUC (GLP-1) *In situ*: AUC (extendin-4) *In vitro*: flux (insulin) *In vivo*: F (insulin)	*In vivo*: 5 mM *In vitro*: 60 µM *In situ*: 0.5 mM *In situ*: 0.5 mM *In vitro*: — *In vivo*: —	*In vivo*: 79-fold *In vitro*: — *In situ*: no effect *In situ*: 2-fold *In vitro*: 3-fold *In vivo*: F = 3.1%	[515] [516] [443] [443] [517] [517]
SNAC	Transcellular	Carrier	*In vivo* (human): oral *In vivo* (human): oral *In vitro*: Caco-2	*In vivo*: AUC (GLP-1) *In vivo*: AUC (PYY^{3-36}) *In vitro*: TEER, flux (mannitol)	*In vivo*: 2 mg *In vivo*: 1 mg *In vitro*: 50 mg/mL	*In vivo*: 2-fold *In vivo*: 2-fold *In vitro*: 27-fold	[372] [372] [360]

			In situ (rat): oral *In vitro*: Caco-2 *In vivo* (rabbit): oral *In vitro*: Caco-2	*In situ*: PK/PD (heparin) *In vitro*: TEER, flux (insulin) *In vivo*: flux (heparin) *In vitro*: TEER, flux (mannitol)	*In situ*: 300 mg/kg *In vitro*: 55 mM *In vivo*: 120 mg/kg *In vitro*: 50 mg/mL	*In situ*: — *In vitro*: 10-fold *In vitro*: no effect *In vitro*: 2-fold	[361] [363] [518] [360]
sodium caprate (C_{10})	Multimodal	PLC MLCK Membrane fluidity	*In vivo* (rat): suppository *In vitro*: Caco-2 *In situ* (rat): instillation *In situ* (rat): colon *In vivo* (rat): oral *In vivo* (dog) oral	*In vivo*: AUC (sodium ampicillin) *In vitro*: flux (mannitol) *In situ*: flux (FD-4) *In situ*: AUC (cefmetazole) *In vivo*: AUC, F (DMP 728) *In vivo*: F (DMP 728)	*In vivo*: 20 µmol/kg *In vitro*: 10 mM *In situ*: 100 mM *In situ*: 0.25% w/v *In vivo*: 40.2% *In vivo*: 40.2%	*In vivo*: 6-fold *In vitro*: 8-fold *In situ*: 14-fold *In situ*: 10-fold *In vivo*: F = 6% (3-fold) *In vivo*: F = 17.7%	[166] [453] [70] [177] [188] [188]
sodium caprylate (C_8)	Multimodal	Membrane fluidity Affinity for Ca^{2+}	*In vivo* (rat): suppository *In vitro*: Caco-2 *In vivo* (human): suppository *In situ* (rat): suppository *In vivo*: (rabbit) suppository *Ex vivo* (rat): inverted sac *Ex vivo* (rat): everted colon *In situ* (rat): colon) *In situ* (rat): rectal loop *In situ* (rat): colon *In vivo* (rat): jejunal *Ex vivo* (rat): colon *In vitro*: Caco-2 *In situ* (rat): duodenum	*In vivo*: AUC (sodium ampicillin) *In vitro*: flux (mannitol) *In vivo*: F (cefoxitin sodium) *In situ*: AUC (acyclovir) *In vivo*: flux (gentamicin) *Ex vivo*: flux (CsA) *Ex vivo*: flux (inulin) *In situ*: flux (inulin) *In situ*: AUC (insulin) *In situ*: AUC (cefmetazole) *In vivo*: flux (fosfomycin) *Ex vivo*: flux (urea) *In vitro*: TEER, flux (mannitol) *In situ*: AUC (LMWH)	*In vivo*: 20 µmol/kg *In vitro*: 120 mM *In vivo*: 0.5 g *In situ*: 4% *In vivo*: 0.18 mM EX vivo: 1% w/v *Ex vivo*: 0.25% *In situ*: 0.25% *In situ*: 50 mM *In situ*: 0.25% w/v *In vivo*: 1% w/v *Ex vivo*: 0.25% *In vitro*: — *In situ*: 30 mg/kg	*In vivo*: 5-fold *In vitro*: 2-fold *In vivo*: 2.5-fold *In situ*: 2-fold *In vivo*: — *Ex vivo*: 9-fold *Ex vivo*: — *In situ*: — *In situ*: no effect *In situ*: 2-fold *In vivo*: — *Ex vivo*: no effect *In vitro*: — *In situ*: —	[166] [453] [334] [519] [273] [158] [520] [520] [185] [177] [209] [179] [150] [161]
sodium cholate	Multimodal	Membrane Fluidity Tight junction	*In situ* (rat); perfusion *In situ* (rat): perfusion *In vivo* (rat): suppository *In vivo* (human): microenema *Ex vivo* (rat): everted sac *In vivo* (rabbit):(EC capsule) *In vivo* (dog): rectal suppository *In situ* (dog): EC microtablet *In situ* (dog): non-EC capsule *In vivo* (dog): oral EC microtablet *In situ* (rat): intestinal loop *In vivo* (rat): instillation *In vivo* (rat): enema	*In situ*: flux (cefazolin) *In situ*: flux (cefazolin) *In vivo*: PK/PD (insulin) *In vivo*: PK/PD (insulin) *Ex vivo*: P_{app} (cefotaxime) *In vivo*: RH (insulin) *In vivo*: RH (insulin) *In situ*: flux, PK/PD (insulin) *In situ*: flux, PK/PD (insulin) *In vivo*: flux, PK/PD (insulin) *In situ*: flux (insulin, RNase) *In vivo*: PK/PD (insulin) *In vivo*: PK/PD (insulin)	*In situ*: 10 mM *In situ*: 10 mM *In vivo*: 2% *In vivo*: 20 mg/mL *Ex vivo*: 1%w/v *In vivo*: 50 mg *In vivo*: 50 mg *In situ*: 12% w/w *In situ*: 100 mg *In vivo*: 12% w/w *In situ*: 10 mg/mL *In vivo*: 10 mg/mL *In vivo*: 2 mg/mL	*In situ*: no effect *In situ*: no effect *In vivo*: — *In vivo*: — *Ex vivo*: 3-fold *In vivo*: no effect *In vivo*: RH = 40% *In situ*: — *In situ*: — *In vivo*: — *In situ*: 4-fold, 9-fold *In vivo*: — *In vivo*: —	[521] [282] [491] [251] [158] [522] [523] [524] [524] [524] [37] [37] [240]
sodium deoxycholate	Multimodal	Membrane Fluidity Tight junction	*In vitro*: Caco-2 *In vitro*: Caco-2 *In situ* (rat): Instillation *In vitro*: Caco-2 *In situ* (rat): everted sac *In vitro*: Caco-2 *In situ* (rat): instillation *Ex vivo* (rat): everted sac *In situ* (rat): perfusion *In vivo* (rat): oral *Ex vivo* (rabbit): Ussing *In vivo* (rabbit): rectal *In situ* (rat): perfusion *In vivo* (rat): rectal Perfusion	*In vitro*: TEER, flux (FD-4) *In vitro*: TEER, flux (Rh 123) *In situ*: PK/PD (insulin) *In vitro*: flux (epirubicin) *In situ*: flux (epirubicin) *In vitro*: Ca^{2+} (Fura-2-AM) *In situ*: AUC (phenol red) *Ex vivo*: P_{app} (cefotaxime) *In situ*: flux (cefotaxime) *In vivo*: F *Ex vivo*: flux (insulin) *In vivo*: AUC (insulin) *In situ*: flux (oxalate, urea) *In vivo*: AUC (sulfanilic acid)	*In vitro*: 0.05% *In vitro*: 0.05% *In situ*: 2 mg *In vitro*: 1.2 mM *In situ*: 12 mM *In vitro*: 0.05%w/v *In situ*: 20 mM *Ex vivo*: 1%w/v *In situ*: 1% w/v *In vivo*: 1%w/v *Ex vivo*: 1% *In vivo*: 1% *In situ*: 5 mM *In vivo*: 5 mM	*In vitro*: 33-fold l vitro: 12-fold *In situ*: — *In vitro*: — *In situ*: — *In vitro*: no effect *In situ*: 9-fold *Ex vivo*: 15-fold *In situ*: 4-fold *In vivo*: F = 33% *Ex vivo*: — *In vivo*: — *In situ*: 10-fold, 7-fold *In vivo*: —	[525] [525] [526] [527] [527] [528] [501] [158] [529] [529] [489] [489] [530] [470]

			In vivo (rat): gavage	*In vivo*: flux, PK/PD (heparin)	*In vivo*: 500 mg/kg	*In vivo*: —	[203]
			In situ (rat): intestinal loop	*In situ*: PK/PD (calcitonin)	*In situ*: 10 mM	*In situ*: 7-fold	[241]
			In vivo (human): oral (capsule)	*In vivo*: PK/PD (PSP)	*In vivo*: 300 mg	*In vivo*: no effect	[531]
			In vivo (dog): suppository	*In vivo*: RH (insulin)	*In vivo*: 150 mg	*In vivo*: 35% RH	[523]
			In vivo (hamster): perfusion	*In vivo*: PK/PD (inulin)	*In vivo*: 5 mM	*In vivo*: 50-fold	[532]
			In vivo (hamster): perfusion	*In vivo*: PK/PD (albumin)	*In vivo*: 5 mM	*In vivo*: 9-fold	[532]
			In vitro: Caco-2	*In vitro*: flux (VLPVP)	*In vitro*: 100 µmol/L	*In vitro*: —	[533]
			Ex vivo (rat): everted gut	*Ex vivo*: flux (phenol red)	*Ex vivo*: 100 mM	*Ex vivo*: 8-fold	[534]
			In situ (rat): instillation	*In situ*: flux (phenol red)	*In situ*: 100 mM	*In situ*: 12-fold	[534]
			In vivo (rat): gastric	*In vivo*: PK/PD (phenol red)	*In vivo*: 150 µmol	*In vivo*: 3-fold	[534]
			In vivo (rat): enema	*In vivo*: PK/PD (insulin)	*In vivo*: 5 mg/mL	*In vivo*: —	[240]
sodium dodecyl sulphate	Transcellular	Membrane fluidity Oxidative phosphorylation ATP depletion	*In vitro*: Caco-2	*In vitro*: flux (mannitol)	*In vitro*: 2 mM	*In vitro*: >139-fold	[216]
			In vitro: Caco-2	*In vitro*: flux	*In vitro*: 2 mM	*In vitro*: 142-fold	[216]
			In vitro: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 0.4 mM	*In vitro*: 20-fold	[255]
			Ex vivo (rat): jejunum	*Ex vivo*: flux (amoxicillin)	*Ex vivo*: 0.2 mg/mL	*Ex vivo*: 2-fold	[535]
			In vivo (rat): oral gavage	*In vivo*: AUC (phenol red)	*In vivo*: 2% w/v	*In vivo*: 6-fold	[75]
			In situ (rat):	*In situ*: flux (Cefazolin)	*In situ*: 10 mM	*In situ*: 5-fold	[282]
			In situ (rat): colon	*In situ*: TEER, flux (LY)	*In situ*: 5 mM	*In situ*: 24-fold	[73]
			In situ (rat): rectal perfusion	*In situ*: AUC (sulfanilic acid)	*In situ*: 5 mM	*In situ*: —	[470]
			In situ (rat): intestine	*In situ*: flux (BTDS)	*In situ*: 0.1% w/v	*In situ*: 2-fold	[536]
sodium taurocholate	Multimodal	—	*In situ* (rat): perfusion	*In situ*: flux (cefazolin)	*In situ*: 10 mM	*In situ*: no effect	[521]
			In situ (rat): loop	— AUC (cefmetazole)	— 0.25%	— 2-fold	[177]
			Ex vivo (rat): everted sac	*Ex vivo*: flux (inulin)	*Ex vivo*: 0.25%	*Ex vivo*: —	[520]
			In situ (rat): colon loop	*In situ*: flux (inulin)	*In situ*: 0.25%	*In situ*: —	[520]
			In vitro: Caco-2	*In vitro*: TEER, flux (FD-4, EMD)	*In vitro*: 15 mM	*In vitro*: —	[242]
			In situ (rat): instillation	*In situ*: AUC (Phenol red)	*In situ*: 20 mM	*In situ*: 3-fold	[501]
			In vitro: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 50 mM	*In vitro*: >403-fold	[216]
			In vitro: Caco-2	*In vitro*: TEER, flux	*In vitro*: 50 mM	*In vitro*: >663-fold	[216]
			In vitro: Caco-2	*In vitro*: TEER, flux (heparin)	*In vitro*: 0.1%	*In vitro*: 4 -fold	[537]
			In vitro: Caco-2	*In vitro*: flux (CRC 220, SR 101)	*In vitro*: 40 mM	*In vitro*: 280-fold, 530-fold	[538]
			In vivo (dogs): suppository	*In vivo*: RH (insulin)	*In vivo*: 100 mg	*In vivo*: RH = 50%	[523]
			In situ (rat): perfusion	*In situ*: flux (sulfaguanidine)	*In situ*: 20 mM	*In situ*: 2-fold	[539]
			In situ (rat): perfusion	*In situ*: flux (cefazolin)	*In situ*: 10 mM	*In situ*: —	[282]
			In situ (rat): perfusion	*In situ*: flux (ACDB, phenol red)	*In situ*: 40 mM	*In situ*: —	[540]
			In situ (rat): perfusion	*In situ*: flux (sulfanilamide)	in situ: 40 mM	*In situ*: no effect	[540]
			Ex vivo (rabbit): Ussing	*Ex vivo*: flux (insulin)	*Ex vivo*: 1%	*Ex vivo*: —	[489]
			In situ (rat): perfusion	*In situ*: flux (oxalate)	*In situ*: 5 mM	*In situ*: no effect	[530]
			In situ (rat): perfusion	*In situ*: flux (sulfaguanidine)	*In situ*: 20 mM	*In situ*: 3-fold	[541]
			In situ (rat): perfusion	*In situ*: flux (sulfanilic acid)	*In situ*: 20 mM	*In situ*: 4-fold	[541]
Sucrose monolaurate	Paracellular	Increase pore radius	*In situ* (rat): rectal loop	*In situ*: AUC (insulin)	*In situ*: 3%	*In situ*: 17-fold	[185]
			Ex vivo (pig): intestinal	*Ex vivo*: flux (cyclosporin A)	*Ex vivo*: 10%	*Ex vivo*: 6-fold	[542]

Table II. Marketed therapeutic peptides (arbitrary MW cut-off: 9 kDa)

Generic name	Trade name®	Manufacturer	Delivery	MW	Classification/Application
Eptifibatide	Integrilin	GSK	IV	832	Anti-platelet drug
Octreotide	Sandostatin	Novartis	SC, IV	1019	Somatostatin analogue
Desmopressin	DDAVP	Ferring	Oral	1069	Synthetic vasopressin analogue
Vasopressin	Pitressin	Goldshield	SC, IM	1084	Antidiuretic peptide
Lanreotide	Somatuline LA	Ipsen	IM	1096	Somatostatin analogue
GnRH	HRF	Intrapharm	SC	1182	Peptide hormone
Cyclosporin	Neoral	Novartis	Oral	1203	Immunosuppressant peptide
Leuprorelin / Leuprolide acetate	Prostap	Takeda	SC, IM	1209	GnRH agonist
Terlipressin	Glypressin	Ferring	IV	1227	Synthetic vasopressin analogue
Mifamurtide	Mepact	Takeda	IV	1238	Osteosarcoma
Buserelin	Suprefact	Sanofi-Aventis	SC, Nasal	1239	GnRH agonist
Goserelin	Zoladex	AstraZeneca	Implant	1269	GnRH super agonist
Icatibant	Firazyr	Shire HGT	SC	1305	Hereditary angioedema
Triptorelin	Decapeptyl SR	Ipsen	IM	1312	GnRH agonist
Nafarelin	Synarel	Pharmacia	Nasal	1322	GnRH agonist
Histrelin	Vantas	Orion	Implant	1324	GnRH agonist
Abarelix	Plenaxis	Speciality European Pharma Ltd		1416	Prostate cancer
Cetrorelix	Cetrotide	Merck Sorono	SC	1431	GnRH antagonist
Vancomycin	Vancocin matrigel	Flynn Pharma	Oral (local),	1486	Antibiotic peptide
Linaclotide	Linzess	Ironwood Pharma	Oral (local)	1527	IBS
Degarelix	Firmagon	Ferring	SC	1631	GnRH antagonist
Bivalirudin	Angiox	The Medicines Company	IV	2180	Anticoagulant
Tetracoactide	Synacthen	Alliance	SC	2933	ACTH analogue
Tetracosactide	Synacthen	Alliance	IM, IV	2933	Corticotrophin analogue
Salmon calcitonin	Miacalcic	Novartis	SC, IV, Nasal	3432	Anti-osteoporotic peptide
Nesiritide	Natrecor	Scios Inc	IV	3464	human B-type natriuretic peptide
Glucagon	Glucagen	Novo Nordisk	SC, IM, IV	3483	Antidiabetic peptide
Liraglutide	Victoza	Novo Nordisk	SC	3751	GLP-1 analogue agonist peptide
Teduglutide	Gattex/Nycomed	NPS Pharma	SC	3752	GLP-2 analogue agonist peptide
Pramlintide	Symlin	AstraZeneca	SC	3951	Analogue of Amylin
teriparatide	Forsteo	Lilly	SC	4118	rh parathyroid hormone (analogue)
Exenatide	Byetta	Lilly/Amylin	SC	4187	Exendin-4
Enfuvirtide	Fuzeon	Roche	SC	4492	Antiviral peptide
rh Insulin	Actrapid	Novo Nordisk	SC	5808	Antidiabetic peptide
rh Insulin	Insuman rapid	Sanofi Aventis	SC	5808	Antidiabetic peptide
rh Insulin	Humulin S	Lilly	SC	5808	Antidiabetic peptide
Insulin lispro	Humalog	Lilly	SC	5808	Analogue of rh insulin

Insulin glulisine	Apidra	Sanofi Aventis	SC	5823	Analogue of rh insulin
Insulin aspart	NovoRapid	Novo Nordisk	SC	5826	Analogue of rh insulin
Insulin detemir	Levemir	Novo Nordisk	SC	591	Analogue of rh insulin
Insulin glargine	Lantus	Sanofi Aventis	SC	6063	Analogue of rh insulin
Glatiramer acetate	Copaxone	Teva Pharma	SC	6400	Immunomodulator peptide
Ecallantide	Kalbitor	Dyax	SC	7054	Hereditary angioedema
Mecasermin	Increlax	Ipsen	SC	7649	rh insulin like growth factor-I
rh PTH	Preotact	Nycomed	SC	9000	Anti-osteoporotic peptide

MW = molecular weight; SC = subcutaneous injection; IM = intramuscular injection;
IV = Intravenous injection or infusion; rh = recombinant human

Table S1. PEs tested in oral delivery of poorly permeable drugs and transport markers

ENHANCER	MODE	ACTIONS	MODEL	REPRESENTATIVE PEPTIDE/METRIC	CONCENTRATION & DOSE	ENHANCEMENT RATIO	REF
12-hydroxy $C_{18}E_{12}$ (Kolliphor HS15)	Transcellular	Membrane fluidity	*In vitro*: Caco-2 *Ex vivo*: Ussing	*In vitro*: TEER, flux (FD-4) *Ex vivo*: TEER, flux (FD-4)	*In vitro*: 1 mM *Ex vivo*: 1 mM	*In vitro*: 2-fold *Ex vivo*: 3-fold	[543] [543]
2-Hydroxydecanoic acid	—	Ca^{2+} chelation	*In situ* (rat): intestinal loop	*In situ*: PK/PD (PSP)	*In situ*: 100 µmol/kg	*In situ*: 14-fold	[544]
3,5-Diiodosalicylate sodium (DIS)	—	—	*In situ* (rat): rectal	*In situ*: flux (insulin)	*In situ*: 0.15 M	*In situ*: —	[545]
3-alkoxy-2-alkylamido propylphosphocholine	Paracellular	Alteration of ZO-1	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 0.14 mM	*In vitro*: 10-fold	[546]
3-Amino-1-hydroxypropylidene-1,1-diphosphonate	—	—	*In vivo* (rat): rectal infusion	*In vivo*: F (cefoxitin)	*In vivo*: 4% (w/v)	*In vivo*: F = 85%	[547]
3-Hydroxydecanoic acid	—	Ca^{2+} chelation	*In situ* (rat): intestinal loop	*In situ*: PK/PD (PSP)	*In situ*: 100 µmol/kg	*In situ*: 2-fold	[544]
3-Methoxysalicylate	—	—	*In situ* (rat): intestinal perfusion	*In situ*: flux (cefmetazole)	*In situ*: 0.5%	*In situ*: 2-fold	[548]
3-nitrocoumarin	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 600 µM	*In vitro*: 3-fold	[503]
4'-Ethynyl-2-fluoro-2'-deoxyadenosine	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (EFdA)	*In vitro*: 400 µM	*In vitro*: —	[549]
N-[8-(2-hydroxy-4-methoxy)bensoyl]amino caprylic acid (4-MOAC)	Transcellular	Carrier function	*In vivo* (monkey): oral gavage	*In vivo*: F (PTH)	*In vivo*: 200 mg/kg	*In vivo*: F = 2.1%	[550]
5-Methoxysalicylate	Transcellular	—	*In situ* (rat): intestinal *In situ* (rat): rectal microenema *In vivo* (dog): suppository *In vivo* (dog): suppository *In vivo* (rat): microenema *In vivo* (rat): microenema *In vivo* (dog): suppository *In situ* (rat): rectal *In situ* (rat): intestinal	*In situ*: flux (insulin) *In situ:* F (pentagastrin) *In vivo*: flux (insulin) *In vivo*: F (penicillin G) *In vivo*: F (theophylline) *In vivo*: PK/PD (insulin) *In vivo*: AUC (cefoxitin) *In situ*: flux (insulin) *In situ*: flux (insulin)	*In situ*: <15 mg/rat *In situ*: 125 µg/kg *In vivo*: 300 mg *In vivo*: 100 mg *In vivo*: 15 mg/kg *In vivo*: 17 mg/kg *In vivo*: 300 mg *In situ*: 0.75 M *In situ*: 60 mg	*In situ*: 4-fold *In situ*: 6-fold (F = 33%) *In vivo*: — *In vivo*: F = 1.04 *In vivo* F = 28.4% *In vivo*: — *In vivo*: 26-fold *In situ*: — *In situ*: 41-fold	[551] [552] [401] [553] [548] [554] [555] [545] [556]
Amino acid enamines of ethylacetoacetate	—	Chelation	*In vivo* (rat): microenema *In vivo* (rat): suppository *In vivo* (dog): microenema *In vivo* (dog): suppository *In vivo* (rabbit): suppository *In vivo* (dog): microenema *In vivo* (dog): suppository *In vivo* (human): suppository *In vivo* (dog): suppository	*In vivo*: PK/PD, flux (insulin) *In vivo*: PK/PD, flux (insulin) *In vivo*: PK/PD, flux (insulin) *In vivo*: flux (insulin) *In vivo*: PK/PD, flux (insulin) *In vivo*: PK/PD, flux (insulin) *In vivo*: PK/PD, flux (insulin) *In vivo*: PK/PD, IRI (insulin) *In vivo*: PK/PD,IRI (insulin)	*In vivo*: 100 mg/mL *In vivo*: 100 mg/g *In vivo*: 5 mg/kg *In vivo*: 5 mg/kg *In vivo*: 100 mg *In vivo*: 400 mg *In vivo*: 150 mg/g *In vivo*: 50 mg *In vivo*: 5%	*In vivo*: — *In vivo*: — *In vivo*: — *In vivo*: — *In vivo*: — *In vivo*: — *In vivo*: — *In vivo*: — *In vivo*: —	[557] [557] [557] [557] [558] [558] [558] [407] [408]
Acetyl carnitine	—	Membrane fluidity	*In vivo* (rat) : rectal	*In vivo*: F (cefoxitine)	*In vivo*: —	*In vivo*: 3-fold	[506]
Alkyl aryl sulphate	Transcellular	Ca^{2+}/Mg^{2+}chelation	*In situ* (rat): instillation	*In situ:* PK/PD (heparin)	*In situ*: 38 mg/kg	*In situ*: —	[559]
Aloe Vera	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (insulin)	*In vitro*: 5% (w/v)	*In vitro*: 3-fold	[560]
Amantidine	—	—	*In vitro*: Caco-2	*In vitro*: flux (FD-10)	*In vitro*: 10 mM	*In vitro*: 178-fold	[488]
Amidosulfobetain-16 (PPS)	—	—	*In vitro*: Caco-2 *In situ* (rat): instillation	*In vitro*: flux (FITC-insulin) *In situ*: F (calcitonin)	*In vitro*: 0.03% *In situ*: 1% (w/v)	*In vitro*: 2.3 fold *In situ*: 32-fold	[164] [164]
AT1002	Paracellular	ZO-1↓	*In vivo* (rat): pulmonary *In situ* (rat): duodenal *In vitro*: Caco-2 *In vitro*: Caco-2	*In vivo*: AUC (sCT) *In situ*: AUC (CsA) *In vitro*: flux (CsA) *In vitro*: TEER, flux (LY)	*In vivo*: 1000 µg/mL *In situ*: 5 mM *In vitro*: 5 mM *In vitro*: 5 mg/mL	*In vivo*: 5-fold *In situ*: no effect *In vitro*: no effect *In vitro*: 40-fold	[111] [115] [115] [111]
Bacteroides fragilis enterotoxin	Paracellular	E-cadherin↓	*In vitro*: T84	*In vitro*: TEER	*In vitro*: 100 ng/mL	*in vitro*: —	[561]
Benzethonium chloride	—	—	*In vitro*: Caco-2	*In vitro*: flux (FD-10)	*In vitro*: 1 mM	*In vitro*: 3-fold	[488]
$C_{12}E_2$ lauryl ether sulphate	Transcellular	Membrane fluidity	*In vivo* (rat): suppository	*In vivo*: PK/PD (insulin)	*In vivo*: 3%	*In vivo*: —	[491]
$C_{12}E_{10}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): Ussing *In vivo* (rat): gavage *In situ* (rat): perfusion	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin) *In situ*: flux (cefazolin)	*Ex vivo*: 1% *In vivo*: 500 mg/kg *In situ*: 10 mM	*Ex vivo*: 16-fold *In vivo*: — *In situ*: 2-fold	[205] [203] [282]
Polyoxyethylene 20: sorbitol monolaurate	Transcellular	Membrane fluidity	*In situ* (rat): perfusion	*In situ*: flux (PABA)	*In situ*: 0.1%	*In situ*: 7-fold	[490]

$C_{12}E_{23}$	Transcellular	Membrane fluidity	*In vivo* (rabbit): oral (granule) *Ex vivo* (rabbit): Ussing *In vivo* (rat): gavage	*In vivo*: PK/PD (insulin) *Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin)	*In vivo*: — *Ex vivo*: 1% *In vivo*: 500 mg/kg	*In vivo*: — *Ex vivo*: 7-fold *In vivo*: —	[562] [205] [203]
$C_{12}E_{25}$	Transcellular	Membrane fluidity	*In situ* (rat): perfusion	*In situ*: flux (PABA)	*In situ*: 1%	*In situ*: 15-fold	[490]
$C_{12}E_{4}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): Ussing *In vivo* (rat): gavage	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin)	*Ex vivo*: 0.001% *In vivo*: 500 mg/kg	*Ex vivo*: 3-fold *In vivo*: —	[205] [203]
C14-OP_{90-103} peptide	Paracellular	Cell adhesion recognition	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 75 µM	*In vitro*: 35-fold	[101]
$C_{16}E_{10}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath *In vivo* (rat): gavage	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin)	*Ex vivo*:1% *In vivo*: 500 mg/kg	*Ex vivo*: 6-fold *In vivo*: —	[205] [203]
$C_{16}E_{10}$PPG4	Transcellular	Membrane fluidity	*In situ* (rat): perfusion	*In situ*: flux	*In situ*: 2%	*In situ* (rat): 5-fold	[521]
$C_{16}E_{14}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath	*Ex vivo*: flux (paraquat)	*Ex vivo*:1%	*Ex vivo*: 4-fold	[205]
$C_{16}E_{2}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath *In vivo* (rat): gavage	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin)	*Ex vivo*: 0.001% *In vivo*: 500 mg/kg	*Ex vivo*: 1-fold *In vivo*: —	[205] [203]
$C_{16}E_{20}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath *In vivo* (rat): gavage *In situ* (rat): instillation *In vivo* (rabbit): oral (granule) *In vivo* (rat): suppository *In vivo* (dog): gastric pouch	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin) *In situ*: PK/PD (insulin) *In vivo*: PK/PD (insulin) *In vivo*: PK/PD (Gly-α-ACTH) *In vivo*: flux (cephaloridine)	*Ex vivo*:1% *In vivo*: 500 mg/kg *In situ*: 88 Mg/0.5 Ml *In vivo*: — *In vivo*: 2% *In vivo*: 0.5% (w/v)	*Ex vivo*: 4-fold *In vivo*: — in situ: 5-fold *In vivo*: — *In vivo*: 15-fold *In vivo*: 36-fold	[205] [203] [521] [562] [563] [564]
$C_{16}E_{6}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue chamber	*Ex vivo*: flux (paraquat)	*Ex vivo*:1%	*Ex vivo*: 1-fold	[205]
$C_{16}E_{60}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue chamber	*Ex vivo*: flux (paraquat)	*Ex vivo*:1%	*Ex vivo*: 3-fold	[205]
$C_{16}E_{7}$	Transcellular	Membrane fluidity	*In vivo* (rat): suppository	*In vivo*: PK/PD(Gly-α-ACTH)	*In vivo*: 2%	*In vivo*: 24-fold	[563]
$C_{18:1}E_{10}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath *In vivo* (rat): gavage	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin)	*Ex vivo*:1% *In vivo*: 500 mg/kg	*Ex vivo*: 9-fold *In vivo*: —	[205] [203]
$C_{18:1}E_{2}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath *In vivo* (rat): gavage	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin)	*Ex vivo*: 0.001% *In vivo*: 500 mg/kg	*Ex vivo*: 3-fold *In vivo*: —	[205] [203]
$C_{18:1}E_{20}$	Transcellular	Membrane fluidity	*In vivo* (dog): oral (capsule) *Ex vivo* (rabbit): Ussing *In vivo* (rat): gavage *In vivo* (rabbit): oral (granule) *In vivo* (dog): Gastric pouch	*In vivo*: PK/PD (insulin) *Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin) *In vivo*: PK/PD (insulin) *In vivo*: flux (cephalothin)	*In vivo*: 200 mg *Ex vivo*: 0.1% *In vivo*: 500 mg/kg *In vivo*: — *In vivo*: 0.5% (w/v)	*In vivo*: — *Ex vivo*: 8-fold *In vivo*: — *In vivo*: — *In vivo*: 24-fold	[565] [205] [203] [562] [564]
$C_{18}E_{10}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): Ussing *In vivo* (rat): gavage *In situ* (rat): perfusion *In vivo* (dog): gastric pouch	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin) *In situ*: flux (PABA) *In vivo*: flux (cephaloridine)	*Ex vivo*: 0.1% *In vivo*: 500 mg/kg *In situ*: 0.1% *In vivo*: 0.5% (w/v)	*Ex vivo*: 5-fold *In vivo*: — *In situ*: 4-fold *In vivo*: 15-fold	[205] [203] [490] [564]
$C_{18}E_{2}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): Ussing *In vivo* (rat): gavage *In vivo* (dog): Gastric pouch	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin) *In vivo*: flux (cephalothin)	*Ex vivo*: 0.0001% *In vivo*: 500 mg/kg *In vivo*: 0.5% (w/v)	*Ex vivo*: 3-fold *In vivo*: — *In vivo*: no effect	[205] [203] [564]
$C_{18}E_{20}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): Ussing *In vivo* (rat): gavage *In vivo* (dog): gastric pouch	*Ex vivo*: flux (paraquat) *In vivo*: PK/PD (heparin) *In vivo*: flux (cephaloridine)	*Ex vivo*: 0.1% *In vivo*: 500 mg/kg *In vivo*: 0.5% (w/v)	*Ex vivo*: 8-fold *In vivo*: — *In vivo*: 10-fold	[205] [203] [564]
$C_{18}E_{40}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath *In situ* (rat): perfusion	*Ex vivo*: flux (paraquat) *In situ*: flux (PABA)	*Ex vivo*: 0.0001% *In situ*: 0.01%	*Ex vivo*: 1-fold *In situ*: 5-fold	[205] [490]
C1C2	Paracellular	Claudin modulation	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: MDCK-II *In vitro*: MDCK-II	*In vitro*: TEER, flux (LY) *In vitro*: TEER, flux (FD-10) *In vitro*: TEER, flux (LY) *In vitro*: TEER, flux (FD-10)	*In vitro*: 200 µM *In vitro*: 200 µM *In vitro*: 200 µM *In vitro*: 200 µM	*In vitro*: 20-fold *In vitro*: 37-fold *In vitro*: 42-fold *In vitro*: 30-fold	[124] [124] [124] [124]
$C_{7\text{-}9}E_{10}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath	*Ex vivo*: flux (paraquat)	*Ex vivo*:1%	*Ex vivo*: 1-fold	[205]
$C_{7\text{-}9}E_{20}$	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): tissue bath	*Ex vivo*: flux (paraquat)	*Ex vivo*: 0.001%	*Ex vivo*: 2-fold	[205]
Calmidazolium	—	—	*In vitro*: Caco-2	*In vitro*: flux (FD-10)	*In vitro*: 400 µM	*In vitro*: —	[488]

Calyculin A	Paracellular	Phosphatase↓	*In vitro*: Caco-2	*In vitro*: TEER, flux (FD-40)	*In vitro*: 400nM	*In vitro*: 39-fold	[566]
Eligen® carriers	Transcellular	Eligen® Carriers	*In vivo* (primate): oral	*In vivo*: flux (IFN)	*In vivo*: 500 mg/kg	*In vitro*: —	[567]
N-phenylsulfonyl-L-serine sodium salt (E37).			*Ex vivo* (rabbit): duodenum	*Ex vivo*: flux (hGH)	*Ex vivo*: 150 mg/mL	*Ex vivo*: No effect	[568]
amino butyric acid,sodium salt (E277)			*Ex vivo* (rabbit): duodenum	*Ex vivo*: flux (hGH)	*Ex vivo*: 150 mg/mL	*Ex vivo*: No effect	[568]
butyric acid, sodium salt (E198)			*Ex vivo* (rabbit): duodenum	*Ex vivo*: flux (hGH)	*Ex vivo*: 150 mg/mL	*Ex vivo*: 2-fold	[568]
4-MOAC			*In vivo* (primate): oral	*In vivo*: F (PTH)	*In vivo*: 200 mg/kg	*In vivo*: F = 2.1%	[550]
Propylene glycol			*In vivo*: (monkey): oral	*In vivo*: Cmax (hGH)	*In vivo*: —	*In vivo*: —	[569]
SABA (4-[4-(2-hydroxybenzoyl)aminophenyl]butyric acid)			*In vivo* (rat): oral	*In vivo*: PK/PD (heparin)	*In vivo*: —	*In vivo*: —	[569]
Carrier E352			*In vitro*: Caco-2	*In vitro*: flux (hGH)	*In vitro*: 25 mg/mL	*In vitro*: 9-fold	[570]
Carrier E414			*In vitro*: Caco-2	*In vitro*: flux (hGH)	*In vitro*: 37.5 mg/mL	*In vitro*: 12-fold	[570]
Carrier E352			*in vitro*: Caco-2	*In vitro*: flux (hGH)	*In vitro*: 25 mg/mL	*In vitro*: —	[382]
Carrier E414			*in vitro*: Caco-2	*In vitro*: flux (hGH)	*In vitro*: 37.5 mg/mL	*In vitro*: —	[382]
Hydroxybenzoylaminophenyl butyric acid			*In vivo* (rat): oral gavage	*In vivo*: flux (hGH)	*In vivo*: 500 mg/kg	*In vivo*: —	[358]
Carrier E-94			*In situ* (rat): intraduodenal	*In situ*: PD (insulin)	*In situ*: 600 mg/kg	*In situ*: —	[571]
Carrier E-94			*In vivo* (rat): oral gavage	*In vivo*: PD (insulin)	*In vivo*: 1200 mg/kg	*In vivo*: —	[571]
Carrier E-94			*Ex vivo*: (rabbit) intestinal	*Ex vivo*: (hGH)	*Ex vivo*: —	*Ex vivo*: —	[571]
	—	—	*In vitro*: Caco-2	*In vitro*: flux (SR 101)	*In vitro*: 1 mM	*In vitro*: 12-fold	[488]
Cetomacrogol	Transcellular	Membrane fluidity	*In situ* (rat): instillation	*In situ*: PK/PD (insulin)	*In situ*: 175.4 mg/mL	in situ: 5-fold	[572]
			In vivo (rat): microenema	*In vivo*: PK/PD (insulin)	*In vivo*: 1 mg/mL	*In vivo*: —	[573]
			In vivo: enema	*In vivo*: flux (gentamicin)	*In vivo*: 1000 mg	*In vivo*: 8-fold	[574]
			In vivo: oral/gastric cannula	*In vivo*: flux (gentamicin)	*In vivo*: 200 mg	*In vivo*: —	[574]
			In vivo: oral/gastric cannula	*In vivo*: AUC (amikacin)	*In vivo*: 200 mg	*In vivo*: 9-fold	[574]
			In vivo (rat): microenema	*In vivo*: PK/PD (heparin)	*In vivo*: 0.8 g	*In vivo*: —	[575]
Chitosan glutamate	Multimodal	Membrane fluidity	*In vitro*: Caco-2	*In vitro*: flux (PEG 4000)	*In vitro*: 1.5% (w/v)	*In vitro*: 11-fold	[426]
		Unspecified TJ	*In vitro*: Caco-2	*In vitro*: flux (mannitol)	*In vitro*: 1.5% (w/v)	*In vitro*: 11-fold	[576]
Chitosan hydrochloride	Multimodal	Membrane fluidity	*In vitro*: Caco-2	*In vitro*: flux (PEG 4000)	*In vitro*: 1.5% (w/v)	*In vitro*: 48-fold	[426]
		Unspecified TJ	*In situ* (rat): intraduodenal	*In situ*: F (buserelin)	*In situ*: 1.5% (w/v)	*In situ*: F =5.1%	[414]
			In situ (rat): duodenal	*In situ*: AUC (buserelin)	*In situ*: 500 µg	*In situ*: 2-fold	[510]
Cholylsarcosine	—	—	*In vitro*: Caco-2	*In vitro*: flux (desmopressin)	*In vitro*: 20 mM	*In vitro*: no effect	[577]
			In vitro: Caco-2	*In vitro*: flux (octreotide)	*In vitro*: 20 mM	*In vitro*: 2-fold	[577]
			In situ (rat): instillation	*In situ*: flux (desmopressin)	*In situ*: 20 mM	*In situ*: 15-fold	[577]
			In situ (rat): instillation	*In situ*: flux (octreotide)	*In situ*: 20 mM	*In situ*: 14-fold	[577]
Cholyltaurine	—	—	*In vitro*: Caco-2	*In vitro*: flux (desmopressin)	*In vitro*: 20 mM	*In vitro*: 3-fold	[577]
			In vitro: Caco-2	*In vitro*: flux (octreotide)	*In vitro*: 20 mM	*In vitro*: 4-fold	[577]
			In situ (rat): instillation	*In situ*: flux (desmopressin)	*In situ*: 20 mM	*In situ*: 14-fold	[577]
			In situ (rat): instillation	*In situ*: flux (octreotide)	*In situ*: 20 mM	*In situ*: 13-fold	[577]
Claudin-1_{53-80} Peptide	Paracellular	Cell adhesion recognition	*In vitro*: T84	*In vitro*: TEER, flux	*In vitro* 200 µM	*In vitro*: —	[123]
			In vivo (rat): oral gavage	*In vivo*: flux (disaccharide)	*In vivo*: 0.1-1 mg/kg	*In vivo*: 2-fold	[123]
Clostridium botulinum toxin C3	Paracellular	TJ signalling	*In vitro*: T84	*In vitro*: TEER, flux (FD-4)	*In vitro*: 1.6 µg/mL	*In vitro*: —	[578]
Clostridium difficile toxin A	Paracellular	Cytoskeleton	*In vitro*: T84	*In vitro*: flux (insulin)	*In vitro*: 7 x 10^{-1} µg/mL	*In vitro*: 3-fold	[579]
			In vitro: T84	*In vitro*: flux (mannitol)	*In vitro*: 240 ng	*In vitro*-	[580]
Clostridium difficile toxin B	Paracellular	Cytoskeleton	*In vitro*: T84	*In vitro*: flux (mannitol)	*In vitro*: 80 ng/mL	*In vitro*: —	[580]
CPE (C-CPE)	Paracellular	Claudin modulation	*in vitro*: MDCK-I	*In vitro*: TEER, flux (FD-4)	*In vitro*: 2.5 µg/mL	*In vitro*: 2-fold	[580]
C-terminal of CPE184	Paracellular	Claudin modulation	*In situ* (rat): colonic loop	*In situ*: AUC (FD-4)	*In situ*: 0.1 mg/mL	*In situ*: 24-fold	[93]
			In situ (rat): jejunum	*In situ*: flux (hPTH)	*In situ*: 5 µg	*In situ*: 8-fold	[126]
Cytochalasin B	Paracellular	PKC, MLCK-P	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 5 µg/mL	*In vitro*: —	[145]
Cytochalasin D	Paracellular	PKC, MLCK-P	*In vitro*: Caco-2	*In vitro*: TEER, flux (SR101)	*In vitro*: 100 µM	*In vitro*: 3-fold	[488]
			Ex vivo (pig)	*Ex vivo*: flux (sodium)	*Ex vivo*: 10 µg/mL	*Ex vivo*: 2-fold	[581]
			Ex vivo (pig)	*Ex vivo*: TEER	*Ex vivo*: 5 µg/mL	*Ex vivo*: —	[581]

			Ex vivo (pig): Ussing *In situ* (rat): enteral	*Ex vivo*: TEER, flux (Inulin) *In situ*: F (insulin)	*Ex vivo*: 10 µg/mL *In situ*: 10% (w/v)	*Ex vivo*: — *In situ*: F = 5.63%	[96] [582]
Decanoylcarnitine	Multimodal	Ca^{2+} modulation	*In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: TEER, flux (FD-4) *In vitro*: TEER, flux (FD-4)	*In vitro*: 0.25% *In vitro*: 0.25%	*In vitro*: 2.8-fold *In vitro*: 3-fold	[136] [583]
Decanoyl-N-methylglucamide	—	—	*In situ* (rat): buccal	*In situ*: potency *vs.* I.M.(insulin)	*In situ*: 0.14 M	*In situ*: 33-fold	[225]
Decyl β-D-glucopyranoside	—	—	*In situ* (rat): jejunal instillation	*In situ*: F (sCT)	*In situ*: 0.2% (w/v)	*In situ*: 2.7-fold	[584]
Dibucaine	—	—	*In vitro*: Caco-2	*In vitro*: flux (FD-10)	*In vitro*: 10 mM	*In vitro*: 33-fold	[488]
Diclofenac	—	—	*In situ* (rat): rectal perfusion	*In situ*: PK/PD (insulin)	*In situ*: 10 mM	*In situ*: 11-fold	[585]
Diethyl ethoxymethylene malonate	—	—	*In situ* (rat): rectal loop *In vivo* (rat) : microenema *In situ* (rat): colonic loop *In situ* (rat): colonic loop *Ex vivo* (rat): rectal sac *In vivo* (rabbit): suppository	*In situ*: flux (cefmetazole) *In vivo*: AUC (calcitonin) *In situ*: AUC (cefmetazole) *In situ*: AUC (calcitonin) *Ex vivo*: flux (inulin) *In vivo*: PK/PD (insulin)	*In situ*: 0.17 M *In vivo*: 0.17 M *In situ*: 50 mM *In situ*: 50 mM *Ex vivo*: 2% *In vivo*: 1%	*In situ*: 12-fold *In vivo*: 186-fold *In situ*: 11-fold *In situ*: — *Ex vivo*: — *In vivo*: —	[586] [586] [587] [587] [403] [403]
Diethyl maleate	Transcellular	—	*Ex vivo:* Ussing *In situ* (rat): loop instillation *In vivo* (rat): microenema *In vivo* (rat): microenema *In situ* (rat): instillation *In situ* (rat): instillation	*Ex vivo*: flux (phenol red) *In situ*: AUC (phenol red) *In vivo*: AUC (cefmetazole) *In vivo*: PK/PD (cefmetazole) *In situ*: flux (phenol red) *In situ*: flux (phenol red)	*Ex vivo*: 20 mM *In situ*: 20 mM *In vivo*: 100 µmol/kg *In vivo*: 3.75 mM *In situ*: 20 mM *In situ*: 20 mM	*Ex vivo*: 2-fold *In situ*: — *In vivo*: 8-fold *In vivo*: — *In situ*: no effect *In situ*: no effect	[499] [499] [588] [265] [501] [500]
Diethylmethyl chitosan	Multimodal	—	*Ex vivo* (rat): everted sac *In vivo* (rat): instillation	*Ex vivo*: flux (Brilliant Blue) *In vivo*: PK/PD	*Ex vivo*: 1% *In vivo*: 1%	*Ex vivo*: — *In vivo*: —	[589] [589]
Difructose anhydride III	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (LY) —	*In vitro*: 100 mmol/L	*In vitro*: —	[590]
Dimethyl-β-cyclodextrin	Transcellular	Lipid extraction	*In situ* (rat): rectal loop) *In vivo* (rabbit): suppository	*In situ*: flux (insulin), Cmax *In vivo*: AUC (insulin)	*In situ*: 5% (w/w) *In vivo*: 30 mg	*In situ*: — *In vivo*: 8-fold	[591] [592]
Dioctyl sulphosuccinate (DOSS)	Transcellular	Membrane fluidity	*In vitro*: Caco-2 *In situ* (rat): intestinal loop *In vivo* (rat): gastric intubation *Ex vivo* (hamster): everted sac *In vivo* (human): oral (solution)	*In vitro*: TEER, flux (mannitol) *In situ*: flux (PS) *In vivo*: flux (PS) *Ex vivo*: flux (water) *In vivo*: PK/PD (PS)	*In vitro*: 1.6 mM *In situ*: 1% (w/v) *In vivo*: 30 mg *Ex vivo*: 0.5 mM *In vivo*: 500 mg	*In vitro*: 88-fold *In situ*: 49-fold *In vivo*: 2-fold *Ex vivo*: — *In vivo*: 2-fold	[216] [593] [593] [532] [594]
Dipotassium glycyrrhizinate	Paracellular	—	*In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: TEER, flux (heparin) *In vitro*: TEER, flux (FD-4)	*In vitro*: 2% *In vitro*: 0.5%	*In vitro*: 9-fold *In vitro*: —	[537] [525]
Discodermin A	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (LY)	*In vitro* 4 µM	*In vitro*: —	[566]
Disodium D,L-glycerophosphate	—	—	*In situ* (rat): rectal loop *In vivo* (rat): microenema *In vivo* (dog): suppository	*In situ*: AUC (cefoxitin) *In vivo*: F (cefoxitin) *In vivo*: F (cefoxitin)	*In situ*: 205 mM *In vivo*: 515 µmol/kg *In vivo*: 1 mmol/animal	*In situ*: < 9-fold *In vivo*: F = 42.6% (21-fold) *In vivo*: F = 19% (5-fold)	[595] [595] [595]
Glycrrhetinic acid 3-β-O-monohemiphthalate	Multimodal	—	*In situ* (rat): rectal	*In situ*: F(ampicillin)	*In situ*: 1.5%	*In situ*: 21-fold	[596]
Docosahexanoic acid (DHA)	—	—	*In situ* (rat): rectal and colon loop *In vivo* (rat): rectal	*In situ*: AUC, PK/PD (insulin) *In vivo*:% PA (insulin)	*In situ*: 2% *In vivo*: 2%	*In situ*:— *In vivo*: 28-fold	[316] [316]
Dodecyl phosphatidyl choline (DPC)	Paracellular	—	*In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: flux (mannitol) *In vitro*: flux (LY)	*In vitro*: 0.75 mM *In vitro*: 0.75 mM	*In vitro*: 12-fold IN vitro: 16-fold	[513] [513]
E. Coli Cytotoxic necrotizing factor	Paracellular	MLCK-P	*In vitro*: Caco-2	*In vitro*: TEER, flux (FD-4)	*In vitro*: 100ng/mL	*In vitro*: —	[597]
EGTA	Paracellular	—	*In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: flux (mannitol) *In vitro*: flux (atenolol)	*In vitro*: 1.5 mM *In vitro*: 2.5 mM	*In vitro*: 125-fold *In vitro*: 7-fold	[598] [133]
Eicosapentaenoic acid (EPA)	—	—	*In situ* (rat): rectal and colon loop	*In situ*: AUC, PK/PD (insulin)	*In situ*: 2%	*In situ*:—	[316]
Ethanol	Multimodal	MLCK	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 7.5%	*In vitro*: —	[87]
Ethoxyethyl acetoacetate phenylglycine enamine	—	—	*Ex vivo* (rat) :rectal sac *In vivo* (dogs, rabbits):rectal	*Ex vivo*: flux (Inulin) *In vivo*: PK/PD (insulin)	*Ex vivo*: 2% *In vivo*: 1%	*Ex vivo*: — *In vivo*: —	[403] [403]

Ethyl acetoacetate phenylalanine enamine	Multimodal	—	*In vivo* (rabbit): suppository *In situ* (rat): rectal loop *In vivo* (rat): microenema	*In vivo*: PK/PD(heparin, lysozyme) *In situ*: flux (cefmetazole) *In vivo*: AUC (ECT)	*In vivo*: 5% (w/w) *In situ*: 0.30 M *In vivo*: 0.33 M	*In vivo*: — *In situ*: 11-fold *In vivo*: 214-fold	[599] [586] [586]
Ethyl acetoacetate phenylglycine enamine	Multimodal	—	*Ex vivo* (rat) :rectal sac *In vivo* (dogs, rabbits):Rectal *In vivo* (rabbit): suppository	*Ex vivo*: flux (Inulin) *In vivo*: PK/PD (insulin) PK/PD(Hep.), flux (lysozyme)	*Ex vivo*: 2% *In vivo*: 20% *In vivo*: 5%(w/w)	*Ex vivo*: — *In vivo*: — *In vivo*: —	[403] [403] [599]
Glyceryl esters of acetoacetic acid	—	Ca^{2+} chelation	*Ex vivo* (rat): rectal sac *In vivo* (rabbit): suppository	*Ex vivo*: flux (Inulin) *In vivo*: flux, PK/PD (insulin)	*Ex vivo*: 10% *In vivo*: 50 mg	*Ex vivo*: — *In vivo*: —	[600] [600]
Glyceryl monocaprylate	Transcellular	Membrane fluidity	*In situ* (rat): rectal *In vivo*: (rabbits) suppository	*In situ*: F (cefmetazole) *In vivo*: flux (gentamicin)	*In situ*: 0.25 mL/kg *In vivo*: 0.18 mmol	*In situ*: F =37.2% *In vivo*: —	[266] [273]
Glyceryl monolaurate	Transcellular	Membrane fluidity	*In situ* (rat): rectal *In situ* (rat): rectal loop	*In situ*: F (cefmetazole) *In situ*: AUC (insulin)	*In situ*: 0.25 mL/kg *In situ*: 50 mM	*In situ*: F =13.8% *In situ*: No effect	[266] [185]
Glyceryl monooleate	Transcellular	Membrane fluidity	*In situ* (rat): rectal loop *In situ* (rat): colon *In situ* (rat): colon *In situ* (rat): colon *In situ* (rat): colon	*In situ*: AUC (insulin) *In situ*: AUC (hCT) *In situ*: AUC (PEG 4000) *In situ*: AUC (HRP) *In situ*: flux (PABA)	*In situ*: 50 mM *In situ*: 40 mM *In situ*: 40 mM *In situ*: 40 mM *In situ*: 0.1%	*In situ*: 5-fold *In situ*: 12-fold *In situ*: 6-fold *In situ*: 9-fold *In situ*: 5-fold	[185] [239] [239] [239] [490]
Glyceryl monopalmitate	Transcellular	Membrane fluidity	*In vitro*: Caco-2	*In vitro*: TEER, flux (FD-10)	*In vitro*: 1 mM	*In vitro*: 104-fold	[488]
Glycyrrhizin	—	—	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *Ex vivo* (rat): Ussing *In vivo* (rat): oral gavage *In vivo* (rat): oral gavage	*In vitro*: TEER, flux (FS) *In vitro*: flux (FD-4) *In vitro*: flux (Rh-123) *In vitro*: TEER, flux (heparin) *In vitro*: flux (LMWH) *Ex vivo*: flux (LMWH) *In vivo*: F (LMWH) *In vivo*: F (LMWH)	*In vitro*: 0.5% *In vitro*: 0.2% *In vitro*: 0.2% *In vitro*: 2% *In vitro*: 0.02% *Ex vivo*: 50µg *In vivo*: 50 µg/kg *In vivo*: 50 µg/kg	*In vitro*: ↑ TEER, no effect *In vitro*: no effect *In vitro*: no effect *In vitro*: 9-fold *In vitro*: 7-fold *Ex vivo*: — *In vivo*: F = 13.29% (2-fold) *In vivo*: F = 4.01% (2-fold)	[525] [525] [525] [537] [601] [601] [601] [601]
Haemagglutinin	Paracellular	Occludin↓	*In vitro*: Caco-2	*In vitro*: TEER, flux (R Red)	*In vitro*: 500 nM	*In vitro*: —	[602]
Hexadecylphosphocholine	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 250 µM	*In vitro*: 4-fold	[503]
Hexadecyltrimethyl ammonium bromide	—	—	*In vitro*: Caco-2	*In vitro*: flux (FD-10)	*In vitro*: 1 mM	*In vitro*: —	[488]
Hexanoyl carnitine	—	—	*Ex vivo* (rat): BBMV *In vivo* (rat): rectal	*Ex vivo*: S (DPH) *In vivo*: F (cefoxitin)	*Ex vivo*: — *In vivo*: —	*Ex vivo*: — *In vivo*: no effect	[506] [506]
Hexyl-D-Glucopyranoside	Paracellular	—	*In situ* (rat): buccal	*In situ*: potency *vs.* I.M.(insulin)	*In situ*: 0.19 M	*In situ*: 10-fold	[225]
HIV TAT	Transcellular	Carrier	*In vitro*: Caco-2	*In vitro*: P eff (insulin-FITC))	*In vitro*: 0.45 µM	*In vitro*: 8-fold	[603]
Homoharringtonine	Paracellular	Claudin modulation	*In vitro*: Caco-2	*In vitro*: TEER, flux (FD-4)	*In vitro*: 10 µM	*In vitro*: —	[604]
Homovanilate	—	Membrane fluidity	*In vivo* (rat): microenema	*In vivo*: F (theophylline)	*In vivo*: 15 mg/kg	*In vivo*: F = 119.7%	[548]
Hyaluronic acid 202 kDa)	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (acyclovir)	*In vitro*: 0.4%(w/v)	*In vitro*: —	[605]
Hydroxybenzoate	—	Altered membrane protein	*In vitro*: artificial membrane *Ex vivo* (rat): rectal BBMV	*In vitro*: flux (cefoxitin) *Ex vivo*: flux (cefoxitin)	*In vitro*: 20 mM *Ex vivo*: 20 mM	*In vitro*: — *Ex vivo*: —	[606] [606]
Hydroxypropyl-β-cyclodextrin		Lipid extraction	*Ex vivo* (rat): jejunal segments) *In vivo* (rabbit) suppository	*Ex vivo*: flux (CSA) *In vivo*: AUC (insulin)	*Ex vivo*: 1.8% (w/v) *In vivo*: 30 mg	*Ex vivo*: 27-fold *In vivo*: 5-fold	[158] [592]
IFN-γ	Paracellular	ZO-1	*In vitro*: T84 *In vitro*: T84 *In vitro*: T84 *In vitro*: T84 *In vitro*: T84	*In vitro*: TEER, flux (mannitol) *In vitro*: TEER, flux (mannitol) *In vitro*: TEER, flux (Inulin) *In vitro*: TEER, flux (FD-3) *In vitro*: TEER, flux (HRP)	*In vitro*: 100 ng/mL *In vitro*: 1,000 U/mL *In vitro*: 1,000 U/mL *In vitro*: 100 U/mL *In vitro*: 100 U/mL	*In vitro*: 10-fold *In vitro*: 7-fold *In vitro*: 5-fold *In vitro*: 18-fold *In vitro*: no effect	[607] [608] [608] [609] [610]
IL-13	—	—	*Ex vivo* (mice): Ussing	*Ex vivo*: TEER, flux (glucose)	*Ex vivo*: 10 µg	*Ex vivo*: —	[611]
IL-1β	Paracellular	Occludin MLCK	*In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: TEER, flux (Inulin) *In vitro*: TEER, flux (Inulin)	*In vitro*: 10 ng/mL *In vitro*: 10 ng/mL	*In vitro*: — *In vitro*: 20-fold	[612] [613]

			In vitro: Caco-2	*In vitro*: TEER, flux (HRP)	*In vitro*: 0.5 ng/mL	*In vitro*: 26-fold	[614]
IL-4	Multimodal	—	*In vitro*: T84 *In vitro*: T84	*In vitro*: TEER, flux (HRP) *In vitro*: TEER, flux (HRP)	*In vitro*: 10 ng/mL *In vitro*: 10 ng/mL	*In vitro*: 3-fold *In vitro*-	[615] [616]
IL-6	Multimodal	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 10 ng/mL	*In vitro*: —	[617]
Indomethacin	—	—	*In situ* (rat):rectal perfusion	*In situ*: AUCR (insulin)	*In situ*: 5 mM	*In situ*: 14-fold	[585]
L-arginine	—	NO donor	*In vitro*: Caco-2 *Ex vivo* (rat):Ussing *In vivo* (rat): gavage	*In vitro*: TEER, flux (LMWH) *Ex vivo*: flux (LMWH) *In vivo*: PK/PD (LMWH)	*In vitro*: 2% *Ex vivo*:250 mg/kg *In vivo*: 250 mg/kg	*In vitro*: 3-fold *Ex vivo*: — *In vivo*: 2-fold	[618] [618] [618]
Laurocapram (Azone)	—	Membrane fluidity	*In situ* (rat): closed loop	*In situ*: AUC (CF)	*In situ*: 20 mM	*In situ*: 44-fold	[619]
Lauroylcholine	Transcellular	Membrane fluidity	*In vitro*: Caco-2	*In vitro*: flux (SR 101)	*In vitro*: 1 mM	*In vitro*: —	[488]
Lecithin	Transcellular	Membrane fluidity	*In vitro*: Caco-2	*In vitro*: flux (FD-10)	*In vitro*: 10 mM	*In vitro*: 1174-fold	[488]
Linoleic acid ($C_{18:2}$)	Transcellular	Membrane fluidity	*In situ* (rat): instillation *Ex vivo* (rat): colon	*In situ*: AUC (CF) *Ex vivo*: flux (adenylyl cyclase)	*In situ*: 30 mM *Ex vivo*: 2 mM	*In situ*: 18-fold *Ex vivo*: 3-fold	[620] [621]
Linolenic acid ($C_{18:3}$)	Transcellular	Membrane fluidity	*In situ* (rat): perfusion *In situ* (rat): Perfusion	*In situ*: flux (water, Na^+, K^+) *In situ*: flux (oxalate)	*In situ*: 2 mM *In situ*: —	*In situ*: ↓ absorption *In situ*: —	[621] [530]
Lysophosphatidylcholine	Paracellular	PKC	*In vitro*: Caco-2 *In vitro*: Caco-2 *In situ* (rat): loop instillation	*In vitro*: TEER *In vitro*: flux (DDAVP) *In situ*: flux (FD-10, FD-70)	*In vitro*: 0.1 mM *In vitro*: 0.5% (w/v) *In situ*: 20 mM	*In vitro*: *In vitro*: 33-fold *In situ*: —	[622] [623] [624]
Medium chain glycerides	Transcellular	Membrane fluidity	*Ex vivo* (rabbit): Ussing *In vivo* (dog): rectal *In vivo* (rat): oral *In situ* (rat): intestinal *In vivo* (rat): rectal	*Ex vivo*: flux (cephalexin) *In vivo*: F (CMZ) *In vivo*: F (dDAVP) *In situ*: flux (BTB) *In vivo*: F (cefoxitin)	*Ex vivo*: 1% *In vivo*: 1750 mg *In vivo*: — *In situ*: 10% *In vivo*: 53% (w/w)	*Ex vivo*: 29-fold *In vivo*: F = 21% *In vivo*: 10-fold *In situ*: — *In vivo*: F = 84%	[625] [626] [627] [258] [267]
Melittin	Multimodal	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2 *Ex vivo* (rat): Ussing *Ex vivo* (human): Ussing *In situ* (rat): instillation	*In vitro*: flux (FD-10) *In vitro*: flux (mannitol) *Ex vivo*: flux (mannitol) *Ex vivo*: flux (mannitol) *In situ*: F (FD-4)	*In vitro*: 50 µM *In vitro*: 1.5 µM *Ex vivo*: 10 µM *Ex vivo*: 10 µM *In situ*: 50 µM	*In vitro*: 157-fold *In vitro*: 4-fold *Ex vivo*: 5-fold *Ex vivo*: 2-fold *In situ*: 8-fold	[488] [628] [629] [119] [118]
Methyl-β-cyclodextrin	Paracellular	Cholesterol leakage	*In vitro*: Caco-2	*In vitro*: TEER, flux (FD-4)	*In vitro*: 100 mM	*In vitro*: 7-fold	[507]
Monocarboxymethylchitosan	Multimodal	Membrane fluidity Unspecified TJ disruption	*In situ* (rat): instillation *In vitro*: Caco-2	*In situ*: AUC (LMWH) *In vitro*: flux (LMWH)	*In situ*: 3% (w/v) *In vitro*: 3% (w/v)	*In situ*: 7-fold *In vitro*: 87-fold	[425] [425]
Monodesmosides (saponin)	—	—	*In situ* (rat): rectal loop *In vivo* (rat): suppository	*In situ*: flux (ampicillin) *In vivo*: flux (ampicillin)	*In situ*: 0.08% (w/v) *In vivo*: 0.1% (w/w)	*In situ*: — *In vivo*: —	[630] [630]
Mycalolide B	Paracellular	F-actin	*In vitro*: Caco-2	*In vitro*: TEER, flux (LY)	*In vitro*: 400nM	*In vitro*: —	[566]
Myristoylcarnitine	Transcellular	Membrane fluidity	*Ex vivo* (rat): BBMV *In vivo* (rat): rectal *Ex vivo* (rats): S-G diffusion cell	*Ex vivo*: S (DPH) *In vivo*: F (cefoxitin) *Ex vivo*: TEER	*Ex vivo*: — *In vivo*: — *Ex vivo*: 1 mM	*Ex vivo*: — *In vivo*: 14-fold *Ex vivo*: —	[506] [506] [73]
N-acyl derivative of collagen (C18)	—	—	*In situ* (rat): rectal loop *In situ* (rat): Rectal perfusion *In vivo* (rabbit): suppository	*In situ*: Cmax (ampicillin) *In situ*: plasma (ampicillin) *In vivo*: plasma (ampicillin)	*In situ*: 5% *In situ*: 0.1% *In vivo*: 5%	*In situ*: — *In situ*: — *In vivo*: —	[631] [631] [631]
N-butyrylphenylalanine		Carrier	*In situ* (rat): rectal loop	*In situ*: flux (sodium ampicillin)	*In situ*: 20 mM	in situ: no effect	[632]
NC 1059	Paracellular	Channel opening	*In vitro*: T84	*In vitro*: TEER, flux 10 kDa)	*In vitro*: 200 µM	*In vitro*: 4-fold	[633]
N-caproylphenylalanine	Transcellular	Carrier	*In situ* (rat): rectal loop	*In situ*: flux (sodium ampicillin)	*In situ*: 20 mM	*In situ*: no effect	[632]
N-carboxymethyl chitosan (MCC)	Paracellular		*In vitro*: Caco-2 *In situ*: Rat	*In vitro*: flux (LMWH) *In situ*: AUC (LMWH)	*In vitro*: 3% (w/v) *In situ*: 3% (w/v)	*In vitro*: 87-fold *In situ*: 7-fold	[425] [425]
N-decanoylalanine	Transcellular	Carrier	*In situ* (rat): rectal loop	*In situ*: flux (sodium ampicillin)	*In situ*: 20 mM	*In situ*: 31-fold	[632]
N-decanoylphenylalanine	Transcellular	Carrier	*In situ* (rat): rectal loop	*In situ*: flux (sodium ampicillin)	*In situ*: 10 mM	*In situ*: 151-fold	[632]
n-Decyl-β-D-Maltopyranoside	Multimodal	—	*In situ* (rat): buccal	*In situ*: potency *vs.* I.M.(insulin)	*In situ*: 0.1 M (5%)	*In situ*: 38-fold	[225]

N-ethyl maleimide	—	—	*Ex vivo* (rat): everted intestine *In situ* (rat): perfusion	*Ex vivo*: flux (CF) in situ: flux (CF)	*Ex vivo*: 10 mM *In situ*: 0.1 mM	*Ex vivo*: 10 mM *In situ*: 0.1 mM	[634]
N-lauroylalanine	Transcellular	Carrier	*In situ* (rat): rectal loop	*In situ*: flux (sodium ampicillin)	*In situ*: 15 mM	*In situ*: 146-fold	[632]
N-laurylphenylalanine	Transcellular	Carrier	*In situ* (rat): rectal loop	*In situ*: flux (sodium ampicillin)	*In situ*: 10 mM	*In situ*: 171-fold	[632]
NOC12	Paracellular	NO Donor	*Ex vivo* (rat): Ussing *Ex vivo* (rat): colon *In situ* (rat): loop instillation	*Ex vivo*: TEER, flux (CF) *Ex vivo*: PA (insulin) *In situ*: F (CF)	*Ex vivo*: 0.1 mM *Ex vivo* 0.1 mM *In situ*: 5 nM	*Ex vivo*: — *Ex vivo*: 8-fold *In situ*: 4-fold	[99] [635] [636]
NOC5	Paracellular	NO Donor	*In situ* (rat): jejunal loop *Ex vivo* (rat): colon *Ex vivo* (rat): Ussing	*In situ*: F (CF) *Ex vivo*: PA (insulin) *Ex vivo*: TEER, flux (CF)	*In situ*: 5 nM *Ex vivo* 0.1 mM *Ex vivo* 0.1 mM	*In situ*: 2-fold *Ex vivo*: 2-fold *Ex vivo*: —	[636] [635] [99]
N-octanoylphenylalanine	Transcellular	Carrier	*In situ* (rat): rectal loop	*In situ*: flux (sodium ampicillin)	*In situ*: 20 mM	*In situ*: 89-fold	[632]
Nonyl D-Glucopyranoside	Paracellular	—	*In situ* (rat): buccal	*In situ*: PK/PD (insulin)	*In situ*: 0.16 M	*In situ*: 3-fold	[225]
Nonylphenoxypolyoxyethylene		—	*In situ* (rat): intestinal perfusion	*In situ*: flux (phenol red)	*In situ*: 1% (w/v)	*In situ*: 89-fold	[214]
NOR1	Paracellular	NO Donor	*In vivo* (rabbit): suppository	*In vivo*: flux (insulin)	*In vivo*:4.2 Mg	*In vivo*: 4-fold	[637]
NOR4	Paracellular	NO Donor	*In vivo* (rabbit): suppository	*In vivo*: flux (insulin)	*In vivo*: 5.6 Mg; 18µM	*In vivo*: 3-fold	[637]
N-palmitoylalanine	—	Carrier	*In situ* (rat): rectal loop	*In situ*: flux (sodium ampicillin)	*In situ*: 20 mM	*In situ*: 196-fold	[632]
N-sulfanto N,O-carboxymethylchitosan	Paracellular	—	*In vitro*: Caco-2 *In situ* (rat): instillation	*In vitro*: P_{app} flux (mannitol) *In situ*: AUC (LMWH)	*In vitro*: 5% (w/v) *In situ*: 3% (w/v)	*In vitro*: 31-fold *In situ*: 19-fold	[638] [638]
N-α-deoxycholyl-L-lysine-methylester	Transcellular	Carrier	*In vitro*: Caco-2 *In vivo* (rat): oral	*In vitro*: flux (insulin) *In vivo*: AUC (insulin)	*In vitro*: 0.5 mg/mL *In vivo*: 1.5 mg/kg	*In vitro*: 5-fold *In vivo*: 8-fold	[639] [346]
Ocatanoyl carnitine	—	Membrane fluidity	*In vivo* (rat) : rectal	*In vivo*: F (cefoxitin)	*In vivo*: —	*In vivo*: 4-fold	[506]
Ochratoxin A	Paracellular	Claudin	*In vitro*: HT-29-D4 *In vitro*: Caco-2	*In vitro*: flux (L-Serine) *in vitro*: flux (FITC-dextran)	*In vitro*: 100 µM *In vitro*: 100 µM	*In vitro*: 3-fold *In vitro*:	[640] [641]
Octyl D-Glucopyranoside	Paracellular	—	*In situ* (rat): buccal *Ex vivo* (rat): jejunum	*In situ*: F (insulin) *Ex vivo*: flux (ebiratide)	*In situ*: 0.19 M (5%) *Ex vivo*: 20 mM	*In situ*: 26-fold *Ex vivo*: 2-fold	[225] [245]
Octylthioglucoside	Paracellular	—	*In situ* (rat): buccal	*In situ*: potency *vs.* I.M.(insulin)	*In situ*: 0.16 M (5%)	*In situ*: 16-fold	[225]
Okadaic acid	Paracellular	Phosphatase	*In vitro*: Caco-2	*In vitro*: TEER, flux (LY)	*In vitro* 2 µM	*In vitro*: —	[566]
Oleic acid (C18:1)	Transcellular	Membrane fluidity	*Ex vivo* (rat): colon *In situ* (rat): loop *In situ* (rat): rectal suppository *In situ* (rat): closed loop	*Ex vivo*: flux (adenylyl cyclase) *In situ*: AUC (CF) *In situ*: AUC (insulin) *In situ*: AUC (CF)	*Ex vivo*: 2 mM *In situ*: 30 mM *In situ*: 50 mM *In situ*: 32 mM	*Ex vivo*: 2-fold *In situ*: 16-fold *In situ*: 4-fold *In situ*: 4-fold	[621] [198] [185] [642]
Pancreozymin	—	—	*In vitro*: Caco-2	*In vitro*: flux (FD-10)	*In vitro*: 100 units/mL	*In vitro*: —	[488]
Patulin	Paracellular	ZO-1 and occludin	*In vitro*: Caco-2 *Ex vivo* (rat) Ussing	*In vitro*: TEER, flux (FD-4) *Ex vivo*: flux (mannitol)	*In vitro*: 100 µM *Ex vivo*: 500 µM	*In vitro*: 12-fold *Ex vivo*: 3-fold	[643] [644]
P-chloromercuryl phenyl sulphate	—	Ca^{2+} chelation	*In situ* (rat): rectal loop *In vivo* (rat): rectal	*In situ*: flux (cefmetazole) *In vivo*: AUC (calcitonin)	*In situ*: 0.05 M *In vivo*: 0.05 M	*In situ*: 11-fold *In vivo*: 22-fold	[586] [586]
PEG 10: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): everted sac	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG 10: octylphenyl ether	Transcellular	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In situ* (rat): intestinal *In situ* (rat): intestinal	*In vitro*: flux (heparin) *In vitro*: TEER, flux (SR101) *In vitro*: TEER, flux (FD-10) *In situ*: AUC (FD-4) *In situ*: AUC (sCT)	*In vitro*: 1% (w/v) *In vitro*: 0.1% *In vitro*: 0.1% *In situ*: 5% (w/v) *In situ*: 1 mg	*In vitro*: 151-fold *In vitro*: 157-fold *In vitro*: 126-fold *In situ*: 2-fold *In situ*: 2-fold	[537] [488] [488] [43] [44]
PEG 10.5: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): everted sac	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG 100: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): everted sac	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG 15: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): everted sac	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG 16: lanolin	Transcellular	Membrane fluidity	*In Vitro*: Caco-2	*In vitro*: TEER, flux (cmetformin)	*In vitro*: 0.5%	*In vitro*: —	[217]
PEG 20: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): everted sac	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG 20: sorbitan monolaurate	Transcellular	Membrane fluidity	*In situ* (rat): perfusion	*In situ*: flux (PABA)	*In situ*: 1%	*In situ*: 5-fold	[490]

(polysorbate 20)			*In vitro*: Caco-2 *In situ* (rat): perfusion	*In vitro*: TEER, flux (metformin) *In situ*: flux (sulfanilic acid)	*In vitro*: 5% *In situ*: 5% (w/v)	*In vitro*: — *In situ*: 34-fold	[217] [218]
PEG 20: sorbitan monolpalmitate (polysorbate 40)	Transcellular	Membrane fluidity	I*n situ* (rat): perfusion *In vivo* (rat, rabbit): enema *In situ* (rat): perfusion	*In situ*: flux (PABA) *In vivo*: PK/PD (insulin) *In situ*: flux (sulfanilic acid)	*In situ*: 1% *In vivo*: 33% (w/w) *In situ*: 5% (w/v)	*In situ*: 5-fold *In vivo*: — *In situ*: 20-fold	[490] [646] [218]
PEG 20: sorbitan monooleate (polysorbate 80)	Multimodal	Membrane fluidity	*In vitro*: Caco-2 *In vivo* (rat): gastric intubation *In situ* (rat): rectal loop *Ex vivo* (rat): intestinal tract *In situ* (rat): perfusion *In situ* (rat): perfusion *In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: flux [AcF (N-Mef)2 NH2] *In vivo*: PK/PD (phenol red) *In situ*: PK/PD (calcitonin) *Ex vivo*: flux (BTDS) *In situ*: flux (sulfanilic acid) *In situ*: flux (quinine) *In vitro*: TEER, flux (mannitol) *In vitro*: TEER, flux (PEG)	*In vitro*: 1% *In vivo*: 10% *In situ*: 0.1% *Ex vivo*: 0.5% (w/v) *In situ*: 5% (w/v) *In situ*: — *In vitro*: 3.8 mM *In vitro*: 3.8 mM	*In vitro*: 2-fold *In vivo*: no effect *In situ*: — *Ex vivo*: — *In situ*: 16-fold *In situ*: — *In vitro*: 4-fold *In vitro*: 3-fold	[281] [647] [212] [536] [218] [648] [216] [216]
PEG 20: sorbitan monostearate (polysorbate 60)	Transcellular	Membrane fluidity	*In situ* (rat): perfusion *In vitro*: Caco-2 *In vivo* (rabbit): suppository *In situ* (rat): perfusion	*In situ*: flux (PABA) *In vitro*: TEER, flux (metformin) *In vivo*: PK/PD (insulin) *In situ*: flux (sulfanilic acid)	*In situ*: 1% *In vitro*: 5% *In vivo*: 2% *In situ*: 5% (w/v)	*In situ*: 5-fold *In vitro*: — *In vivo*: — *In situ*: 16-fold	[490] [217] [219] [218]
PEG 20: sorbitan trioleate (polysorbate 85)	Transcellular	Membrane fluidity	*In vivo* (rat, rabbit): microenema *In situ* (rat): perfusion	*In vivo*: PK/PD (insulin) *In situ*: flux (sulfanilic acid)	*In vivo*: 33% (w/w) *In situ*: 5% (w/v)	*In vivo*: — *In situ*: 12-fold	[646] [218]
PEG 24: cholesterol	Transcellular	Membrane fluidity	*In situ* (rat): intra-jejunal *In situ* (rat): intra-jejunal	*In situ*: F (octreotide) in situ: F (NBD-octreotide)	*In situ*: 1% (w/v) *In situ*: 1% (w/v)	*In situ*: F = 6.9% *In situ*: F = 4.9%	[649] [649]
PEG 24: Cholesteryl	Transcellular	Membrane fluidity	*In vitro*: Caco-2 *In vivo* (dog): gastric pouch	*In vitro*: TEER, flux (metformin) *In vivo*: flux (streptomycin)	*In vitro*: 0.5% *In vivo*: 0.5%(w/v)	*In vitro*:— *In vivo*: 14-fold	[217] [564]
PEG 30: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): instillation	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG 32: lauroyl glycerides	Transcellular	Membrane fluidity	*In situ* (rat): duodenum	*In situ*: PK/PD (LMWH)	*In situ*: 30 mg/kg	*In situ*: —	[161]
PEG 35: castor oil	Transcellular	Membrane fluidity	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 0.1% (w/v)	*In vitro*: no effect	[281]
PEG 4: sorbitan monolaurate (polysorbate 21)	Transcellular	Membrane fluidity	*In vivo* (rat, rabbit): enema *In situ* (rat): perfusion	*In vivo*: PK/PD (insulin) *In situ*: flux (sulfanilic acid)	*In vivo*: 33% (w/w) *In situ*: 5% (w/v)	*In vivo*: — *In situ*: 14-fold	[646] [218]
PEG 40: hydrogenated castor oil	Transcellular	Membrane fluidity	*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol, PEG)	*In vitro*: 7.1 mM	*In vitro*: —	[216]
PEG 40: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): rectal instillation	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG 5: oleylamine	Transcellular	Membrane fluidity	*In vivo* (rat): suppository	*In vivo*: PK/PD (insulin)	*In vivo*: 3%	*In vivo*: 98-fold	[491]
PEG 5: sorbitan monooleate (polysorbate 81)	Transcellular	Membrane fluidity	*In vivo* (rat, rabbit): microenema	*In vivo*: PK/PD (insulin)	*In vivo*: 33% (w/w)	*In vivo*: —	[646]
PEG 60: castor oil	Transcellular	Membrane fluidity	*In situ* (rat): perfusion *In vivo* (rat): oral	*In situ*: flux (cefazolin) *In vivo*: F (CsA)	*In situ* 5 mM *In vivo*: 8%	*In situ* 2-fold *In vivo*: F = 27.3%	[282] [650]
PEG 65: sorbitan monolaurate (polysorbate 65)	Transcellular	Membrane fluidity	*In vivo* (rat, rabbit): microenema	*In vivo*: PK/PD (insulin)	*In vivo*: 33%(w/w)	*In vivo*: —	[646]
PEG 7.5: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): instillation	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG 9: nonylphenylether	Transcellular	Membrane fluidity	*Ex vivo* (rat): instillation	*Ex vivo*: flux (leuprolide)	*Ex vivo*: 10 mg/mL	*Ex vivo*: —	[645]
PEG ethers (PEG 32) Cholesteryl ether	—	—	*In vivo* (dog): gastric pouch	*In vivo*: flux (cephaloridine)	*In vivo*: 0.5% (w/v)	*In vivo*: 12-fold	[564]
PEG400	—	Membrane fluidity	*In vivo* (rat): rectal	*In vivo*: AUC (sulfanilic acid)	*In vivo*: 50%	*In vivo*: —	[470]
Peptide inhibitor of phosphatase 250	Paracellular	MLCK-P	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vivo* (rat): ILI injection DEFINE	*In vitro*: TEER, flux (FD-4) *In vitro*: TEER, flux (FD-70) *In vivo*: PK/PD (insulin)	*In vitro*:10 mM *In vitro*: 20 mM *In vivo*: 10 mM	*In vitro*: 3-fold *In vitro*: 6-fold *In vivo*: —	[147] [147] [147]
Peptide inhibitor of phosphatase 640	Paracellular	MLCK-P	*In vitro*: Caco-2 *In vivo* (rat): ILI Injection DEFINE	*In vitro*: TEER, flux (FD-4) *In vivo*: PK/PD (insulin)	*In vitro*: 20 mM *In vivo*: 20 mM	*In vitro*: 3-fold *In vivo*: —	[147] [147]
Phenylbutazone	—	—	*In situ* (rat): rectal perfusion	*In situ*: AUCR (insulin)	*In situ*: 10 mM	*In situ*: 7-fold	[585]
Phorbol myristate acetate	Paracellular	PKC	*In vitro*: IEC-18 *Ex vivo* (rat): colon *In vitro*: T84 *In vitro*: T84 *In vitro*: Caco-2	*In vitro*: TEER, flux (PEG) *Ex vivo*: Clearance(EDTA) *In vitro*: TEER, flux (mannitol) *In vitro*: TEER, flux (inulin) *In vitro*: TEER, flux (mannitol)	*In vitro*: 10^{-7} M) *Ex vivo*: 800 µg *in vitro*: 10^{-7} M) *in vitro*: 10^{-7} M) *In vitro*: 50 nM	*In vitro*: 2-fold *Ex vivo*: — *In vitro*: 8-fold *In vitro*: 8-fold *In vitro*: 3-fold	[651] [652] [653] [653] [654]

Phosphatidyl choline (Soybean)	Transcellular		*In situ* (rat): perfusion *In vitro*: Caco-2 *In vitro*: Caco-2	*In situ*: flux (hexarelin) *In vitro*: flux (mannitol) *In vitro*: flux (fragmin)	*In situ*: 15 mM *in vitro*: 8 mM *In vitro*: 8 mM	*In situ*: 20-fold *in vitro*: 20-fold *In vitro*: 8-fold	[655] [655] [655]
Phospholipase A2 (PLA2)	—	—	*In vitro*: Caco-2	*In vitro*: flux (FD-10)	*In vitro*: 100 µM	*In vitro*: —	[488]
Piperine	—	—	*In situ* (rat): intestinal perfusion *In vivo* (rat): oral (suspension)	*In situ*: flux (CsA) *In vivo*: F (CsA)	*In situ*: 0.004% (w/v) *In situ*: 0.004% (w/v)	*In situ*: 2-fold *In vivo*: F = 33.10%	[529] [529]
PN159	Paracellular	Claudin modulation	*In vivo* (rabbit): nasal	*In vivo*: AUC (PYY)	in vivo: 50 µM	*In vivo*: 46-fold	[53]
Polyarganine	Transcellular	Carrier	*Ex vivo* (rat): ileal membranes *In situ* (rat): intestinal loop *In situ* (rat): intestinal loop	*Ex vivo*: flux (leuprolide) *In situ*: PK/PD (leuprolide) *In situ*: AUC (insulin)	*Ex vivo*: 200 µM *In situ*: 200 µM *In situ*: 25.0 mg/kg	*Ex vivo*: no effect In situ: no effect *In situ*: F = 16% (37-fold)	[656] [656] [657]
Polyarginine	—	—	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In situ* (rat): intestinal loop *In situ* (rat): intestinal loop	*In vitro*: flux (SR 101) *In vitro*: flux (SR 101) *In vitro*: flux (SR 101) *In situ*: F (insulin) *In situ*: F (FD-4)	*In vitro*: 50 µM *In vitro*: 100 µM *In vitro*: 10 µM *In situ*: 25 mg/kg *In situ*: 0.5%	*In vitro*: — *In vitro*: — *In vitro*: — *In situ*: F = 15.7% (39-fold) *In situ*: F = 24% (14-fold)	[488] [488] [488] [657] [658]
Polyethylene imine	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (inulin)	*In vitro*: 0.002% (w/v)	*In vitro*:—	[659]
Polylysine	—	—	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: flux (SR 101) *In vitro*: flux (SR 101) *In vitro*: flux (SR 101) *In vitro*: flux (SR 101) *In vitro*: flux (SR 101)	*In vitro*: 50 µM *In vitro*: 10 µM *In vitro*: 20 µM *In vitro*: 20 µM *In vitro*: 20 µM	*In vitro*: — *In vitro*: — *In vitro*: — *In vitro*: — *In vitro*: —	[488] [488] [488] [488] [488]
Pz-peptide	Paracellular	Na^+ channel	*In vitro*: Caco-2 *Ex vivo* (rabbit): Ussing	*In vitro*: flux (mannitol) *Ex vivo*: flux (mannitol)	*In vitro*: 5 mM *Ex vivo*: 5 mM	*In vitro*: — *Ex vivo*: —	[660] [660]
Quillayasaponin	Multimodal		*In vitro*: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: 0.2% (w/v)	*In vitro*: —	[661]
Rhamnolipids	Multimodal	—	*In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: flux (phenol red) *In vitro*: flux (propranolol)	*In vitro*: 150 mg/L *In vitro*: 150 mg/L	*In vitro*: 8-fold *In vitro*: 2-fold	[662] [662]
Ricinoleic acid 12-hydroxy-$C_{18:1}$)	—	—	*In situ* (rat): perfusion *In situ* (rat): Perfusion	*In situ*: flux (water, Na^+, K^+) *In situ*: flux (oxalate)	*In situ*: 2 mM *In situ*: 8 mM	*In situ*: ↓ absorption *In situ*: —	[621] [530]
Saponin	—	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (heparin)	*In vitro*: 0.05%	*In vitro*: 302-fold	[537]
S-nitroso-N-acetyl-D,L-penicillamine	Paracellular	NO Donor	*Ex vivo* (rat): colon *In situ* (rat): jejunal loop *In vivo* (rabbit): suppository	*Ex vivo*: PA (insulin) *In situ*: F (CF), PK/PD *In vivo*: AUC (insulin)	*Ex vivo* 0.1 mM *In situ*: 5nM *In vivo*: 0.25 mg	*Ex vivo*: 10-fold *In situ*: 5-fold *In vivo*: no effect	[635] [636] [637]
Sodium caproate (C_6)	Multimodal	—	*In vivo* (rat): suppository *In vivo*: (rabbits) suppository *In vitro*: Caco-2 *In situ* (rat): duodenum	*In vivo*: AUC (sodium ampicillin) *In vivo*: flux (gentamicin) *In vitro*: flux (mannitol) *In situ*: AUC (LMWH)	*In vivo*: 20 µmol/kg *In vivo*: 25 mg *In vitro*: 120 mM *In situ*: 30 mg/kg	*In vivo*: 2-fold *In vivo*: — *In vitro*: 2-fold *In situ*: —	[166] [273] [453] [161]
Sodium chenodeoxycholate	Paracellular	Membrane Fluidity	*In situ* (rat): perfusion *In vitro*: Caco-2 *In vitro*: Caco-2 *In situ* (rat): instillation	*In situ*: flux (oligonucleotide) *In vitro*: flux (octreotide) *In vitro*: flux (desmopressin) *In situ*: flux (octreotide)	*In situ*: 12 mM *In vitro*: 2.5 mM *In vitro*: 2.5 mM *In situ*: 20 mM	*In situ*: 16-fold *In vitro*: 5-fold *In vitro*: 5-fold *In situ*: 71-fold	[663] [577] [577] [577]
Sodium glycocholate			*In situ* (rat): rectal *In situ* (rat): intestinal loop *In situ* (rat): perfusion *In situ* (rat): perfusion *Ex vivo* (rat): Ussing *In situ* (rat): closed loop	*In situ*: PK/PD (insulin) *In situ*: PA% (calcitonin) *In situ*: flux (ACDB, phenol red) In Situ: flux (sulphanilamide) *Ex vivo*: flux (ebiratide) *In situ*: F (carboxyfluorescein)	*In situ*: 5% (w/v) *In situ*: 10 mM *In situ*: 40 mM *In situ*: 40 mM *Ex vivo*: 20 mM In situ: 20 mM	*In situ*: 2-fold *In situ*: 5-fold *In situ*: — *In situ*: no effect *Ex vivo*: 4-fold *In situ*: F = 38%	[664] [241] [540] [540] [245] [86]
Sodium glycodeoxycholate	—	—	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: flux (hEGF) *In vitro*: flux (cimetidine) *In vitro*: flux (inulin)	*In vitro*: 1% (w/v) *In vitro*: 1% (w/v) *In vitro*: 1% (w/v)	*In vitro*: 20-fold *In vitro*: 37-fold *In vitro*: 96-fold	[243] [243] [243]

Sodium laurate (C_{12})	Multimodal	Membrane fluidity	*In vitro*: Caco-2	*In vitro*: flux (hydrophilic subs.)	*In vitro*: 0.3 mM	*In vitro*: —	[665]
			In vitro: Caco-2	*In vitro*: flux (hydrophilic subs.)	*In vitro*: 0.75 mM	*In vitro*: 7-fold	[666]
			In vitro: Caco-2	*In vitro*: flux (mannitol)	*In vitro*: 0.75 mM	*In vitro*: 8-fold	[453]
			Ex vivo (rat): everted colon	*Ex vivo*: flux (inulin)	*Ex vivo*: 0.25%	*Ex vivo*: —	[520]
			In vivo (rat): suppository	*In vivo*: AUC (ampicillin)	*In vivo*: 20 µmol/kg	*In vivo*: 5-fold	[166]
			In vivo (rat): suppository	*In vivo*: AUC (rebamipide)	*In vivo*: 2 mg (10 µmol)	*In vivo*: 7-fold	[667]
			In situ (rat): suppository	*In situ*: AUC (acyclovir)	*In situ*: 4%	*In situ*: 2-fold	[519]
			In situ (rat): Intraduodenal	*In situ*: AUC (LMWH)	*In situ*: 30 mg/kg	*In situ*: —	[161]
			In situ (rat): rectal loop	*In situ*: AUC (insulin)	*In situ*: 50 mM	*In situ*: 10-fold	[185]
			In situ (rat): colon	*In situ*: flux (inulin)	*In situ*: 0.25%	*In situ*: —	[520]
			In situ (rat): colon	*In situ*: AUC (cefmetazole)	*In situ*: 0.25% (w/v)	*In situ*: 7-fold	[177]
			In vivo (rat): rectal infusion	*In vivo*: AUC (cefoxitin)	*In vivo*: 0.3 M)	*In vivo*: —	[333]
			In vivo (rat): Jejunal	*In vivo*: flux (fosfomycin)	*In vivo*: 1% (w/v)	*In vivo*: —	[209]
			In vitro: Caco-2	*In vitro*: TEER, flux (mannitol)	*In vitro*: —	*In vitro*: —	[150]
Sodium laurate with cetylalcohol	Transcellular	Membrane fluidity	*In vivo* (rat): oral (EC capsule)	*In vivo*: PK/PD, F (insulin)	*In vivo*: 20 mg	*In vivo*: F = 12.7%	[19]
Sodium lauryl sarcosinate	—	—	*In vivo* (rat): rectal instillation	*In vivo*: PK/PD 3GS)	*In vivo*: 30%	*In vivo*: 20-fold	[668]
Sodium lauryl sulfate (SLS)	—	Ca^{2+}/Mg^{2+}chelation	*In situ* (rat): intestinal instillation	*In situ*: PK/PD (heparin)	*In situ*: 14 mg/kg	*In situ*: —	[559]
Sodium lauryl sulfate (SLS)			*In situ* (dog): oral	*In situ*: PK/PD (heparin)	*In situ*: 25 mg/kg	*In situ*: —	[559]
Sodium lauryl sulfate (SLS)			*In vivo* (dog): oral (EC capsule)	*In situ*: PK/PD (heparin)	*In situ*: 25 mg/kg	*In situ*: —	[559]
Dioctyl sodium sulfosuccinate (DSS)			*In situ* (rat): instillation	*In situ*: PK/PD (heparin)	*In situ*: 31 mg/kg	*In situ*: —	[559]
Benzoate derivative of DSS (DSS-B)			*In vivo* (dog): oral (EC capsule)	*In vivo*: PK/PD (heparin)	*In vivo*: 25 mg/kg	*In vivo*: —	[559]
Sodium myristate (C_{14})	Multimodal	Membrane fluidity Affinity for Ca^{2+}	*In vitro*: Caco-2	*In vitro*: flux (mannitol)	*In vitro*: 0.15 mM	*In vitro*: 81-fold	[666]
			In situ (rat): duodenal	*In situ*: AUC (LMWH)	*In situ*: 30 mg/kg	*In situ*: —	[161]
			In vivo (rat): suppository	*In vivo*: AUC (Na ampicillin)	*In vivo*: 20 µmol/kg	*In vivo*: 2-fold	[166]
			In vitro: Caco-2	*In vitro*: flux (PcV)	*In vitro*: 0.1 mM	*In vitro*: —	[665]
Sodium N-decanoyl-L-phenylalaninate	Paracellular	—	*In vivo* (rat):suppository	*In vivo*: F (ampicillin)	*In vivo*: 20 µmol/kg	in vivo: 9-fold	[166]
Sodium nitroprusside	Paracellular	NO donor	*In situ* (rat): closed loop	*In situ*: AUC (FD-4)	*In situ*: 10 mg/kg	*In situ*: —	[402]
			In situ (rat): closed loop	*In situ*: AUC (carboxyfluorescein)	*In situ*: —	*In situ*: —	[402]
			In vitro: Caco-2 BBe	*In vitro*: flux (FD-4, FS)	*In vitro*: 1.25 mM	*In vitro*: 800-fold, 400-fold	[669]
Sodium N-lauroyl-N-methylglycinate	Paracellular	—	*In vivo* (rat):suppository	*In vivo*: F (ampicillin)	*In vivo*: 20 µmol/kg	in vivo: 5-fold	[166]
Sodium N-lauryl-L-glutamate	—	—	*In vivo* (rat): suppository	*In vivo*: PK/PD (insulin)	*In vivo*: 3%	*In vivo*: 109-fold	[491]
Sodium oleate ($C_{18:1}$)			*In situ* (rat): perfusion	*In situ*: flux (oxalate)	*In situ*: 20 mM	*In situ*: —	[530]
Sodium palmitate (C_{16})	—	—	*In situ* (rat): instillation	*In situ*: AUC (LMWH)	*In situ*: 30 mg/kg	*In situ*: 8-fold	[161]
Sodium pelargonate (C_9)	—	—	*In vitro*: Caco-2	*In vitro*: P app (hydrophilic marker)	*In vitro*: 40 mM	*In vitro*: 16-fold	[666]
			In vitro: Caco-2	*In vitro*: flux (PcV)	*In vitro*: 40 mM	*In vitro*: —	[665]
			In vitro: Caco-2	*In vitro*: P app (mannitol)	*In vitro*: 21 mM	*In vitro*: —	[150]
sodium p-n-pentylbenzoate	Paracellular	—	*In vivo* (rat):suppository	*In vivo*: F (ampicillin)	*In vivo*: 20 µmol/kg	*In vivo*: 7-fold	[166]
sodium salicylate	Multimodal	—	*In situ* (rat): rectal loop	*In situ*: AUC (calcitonin)	*In situ*: 0.6 M	*In situ*: 25-fold	[586]
			In situ (rat): large intestine	*In situ*: (phenol red)	*In situ*: 20 mM	*In situ*: no effect	[500]
			In situ (rat): closed loop	*In situ*: PA (hCT)	*In situ*: 10 mM	*In situ*: 2-fold	[241]
			In vivo: (dog) suppository	*In vivo*: AUC (insulin)	*In vivo*: 50 mg	*In vivo*: RH = 49%	[211]
			In vivo (human): suppository	*In vivo*: F (cefoxitin)	*In vivo*: 2.10 g	*In vivo*: F = 22%	[670]
			In situ (rat): loop	*In situ*: F (carboxyfluorescein)	*In situ*: 20 mM	*In situ*: F = 8%	[86]
			Ex vivo (rat): Ussing	*Ex vivo*: flux (phenol red)	*Ex vivo*: 20 mM	*Ex vivo*: 3-fold	[499]
			In vitro: Caco-2	*In vitro*: flux (FD-4)	*In vitro*: 1 mM	*In vitro*: 3-fold	[502]
			In vivo (human): suppository	*In vivo*: AUC (insulin)	*In vivo*: 100 mg	*In vivo*: —	[671]
			In vivo (human): suppository	*In vivo*: PK/PD (insulin)	*In vivo*: —	*In vivo*: —	[407]
			In vivo (human): suppository	*In vivo*: F (cefoxitin sodium)	*In vivo*: 1g	*In vivo*: F = 13%	[334]
			In vitro: Caco-2	*In vitro*: TEER, flux (FD-4)	*In vitro*: 173 mg	*In vitro*: 6-fold	[672]
			In vivo (dog): suppository	*In vivo*: AUC (gentamicin)	*In vivo*: 300 mg	*In vivo*: 87-fold	[555]

			In vivo (human): Suppository *In vivo* (rat): suppository *In vivo* (rat): microenema	*In vivo*: PK/PD(insulin) *In vivo*: AUC (met-hGH) *In vivo*: F (gentamicin)	*In vivo*: — *In vivo*: 7 mg *In vivo*: 2%	*In vivo*: — *In vivo*: 4-fold *In vivo*: F = 29% (2-fold)	[406] [673] [674]
sodium taurodihydrofusidate (STDHF)	Multimodal	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *Ex vivo* (rat): duodenum *In situ* (rat): rectal infusion *In situ* (rat): rectal bolus *In vivo* (rat): rectal	*In vitro*: flux (PEG) *In vitro*: flux (mannitol) *In vitro*: TEER, flux (FD-4) *In vitro*: TEER, flux (fluorescein) *Ex vivo*: flux (dDAVP) *In situ*: F (DGAVP) *In situ*: F (DGAVP) *In vivo*: F (insulin)	*In vitro*: 5 mM *In vitro*: 5 mM *In vitro*: 2.8 mM *In vitro*: 2.8 mM *Ex vivo*: 15 mM *In situ*: 4% (w/v) *In situ*: 4% (w/v) *In vivo*: 1% (w/v)	*In vitro*: 188-fold *In vitro*: >366-fold *In vitro*: 52-fold *In vitro*: 11-fold *Ex vivo*: — *In situ*: F = 47% *In situ*: F = 27% *In vivo*: 21-fold (F = 4.2%)	[216] [216] [672] [672] [675] [676] [676] [677]
sodium taurochenodeoxycholate	Multimodal	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2 *Ex vivo* (Rabbit): Ussing chamber *Ex vivo* (Rabbit): Ussing chamber	*In vitro*: flux (hEGF) *In vitro*: flux (cimetidine) *In vitro*: flux (inulin) *Ex vivo*: flux (sodium) *Ex vivo*: flux (albumin)	*In vitro*: 1% (w/v) *In vitro*: 1% (w/v) *In vitro*: 1% (w/v) *Ex vivo*: 4 mM *Ex vivo*: 4 mM	*In vitro*: 22-fold *In vitro*: 44-fold *In vitro*: 111-fold *Ex vivo*: 7-fold *Ex vivo*: 7-fold	[243] [243] [243] [678] [678]
sodium taurodeoxycholate	Multimodal	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2 *In situ* (rat): perfusion *In situ* (rat): perfusion *Ex vivo* (rat): tissue bath *In vivo* (dog): Oral (EC capsule) *Ex vivo* (rat): everted intestine	*In vitro*: TEER, flux (mannitol) *In vitro*: TEER, flux (PEG) *In situ*: flux (sulfaguanidine) *In situ*: flux (phenol red) *Ex vivo*: TEER, flux (calcitonin) *In vivo*: F (calcitonin) *Ex vivo*: flux (salicylate)	*In vitro*: 5 mM *In vitro*: 5 mM *In situ*: 20 mM *In situ*: 20 mM *Ex vivo*: 1% *In vivo*: — *Ex vivo*: 100 mM	*In vitro*: >208-fold *In vitro*: 332-fold *In situ*: 2-fold *In situ*: 2-fold *Ex vivo*: 14-fold *In vivo*: — *Ex vivo*: 2-fold	[216] [216] [539] [539] [82] [82] [679]
sodium tauroursodeoxycholate	Multimodal	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: TEER, flux (hEGF) *In vitro*: flux (inulin)	*In vitro*: 1% *In vitro*: 1%	*In vitro*: 2-fold *In vitro*: 4-fold	[243] [591]
sodium tridecanoate (C_{13})	—	—	*In vitro*: Caco-2 *In vitro*: Caco-2	*In vitro*: P_{app} (hydrophilic sub) *In vitro*: flux (PcV)	*In vitro*: 0.4 mM *In vitro*: 0.1 mM	*In vitro*: 81-fold *In vitro*: —	[666] [665]
sodium undecanoate (C_{11})	Multimodal	Membrane fluidity	*In situ* (rat): Instillation *In vitro*: Caco-2 *In vitro*: Caco-2 *In vitro*: Caco-2	*In situ*: flux (FD-4) *In vitro*: P_{app} (hydrophilic sub) *In vitro*: flux (PcV) *In vitro*: TEER, flux	*In situ*: 100 mM *In vitro*: 2.5 mM *In vitro*: 1.5 mM *In vitro*: 4 mM	*In situ*: 37-fold *In vitro*: 222-fold *In vitro*: — *In vitro*: —	[70] [666] [665] [150]
sodium undecylenate ($C_{11:1}$)	Multimodal	Membrane fluidity	*In vitro*: Caco-2 *In vitro*: Caco-2 *In situ* (rat): Instillation, colon *In situ* (rat): rectal loop	*In vitro*: flux (hydrophilic subs) *In vitro*: flux (hydrophilic subs) *In situ*: AUC (FD-4) *In situ*: AUC (insulin)	In vitro: 2.5 mM In vitro: 1.5 mM *In situ*: 100 mM *In situ*: 50 mM	*In vitro*: 222-fold *In vitro*: — *In situ*: 29-fold *In situ*: 24-fold	[666] [665] [70] [185]
sodium ursodeoxycholate	—	—	*In situ* (rat): Instillation	*In situ*: flux (interferon)	*In situ*: 4 mg	*In situ*: —	[244]
sodium vaccenate ($C_{18:1}$)	—	—	*In vivo* (rat): Rectal	*In vivo*: PK/PD (insulin)	*In vivo*: 50 mM	—	[185]
sodium α-bromocaprate	Paracellular	—	*In vivo* (rat):suppository	*In vivo*: F (ampicillin)	*In vivo*: 20 µmol/kg	in vivo: 5-fold	[166]
Sorbitan monolaurate	Paracellular		*In situ* (rat): Buccal	*In situ*: potency *vs.* I.M.(insulin)	*In situ*: 5%	*In situ*: 2-fold	[225]
Spermine	Paracellular	NO Donor	Ex Vivo (rat): Ussing *In vivo* (rat): oral *In vivo* (dog): oral	*Ex vivo*: TEER, flux (FD-4) *In vivo*: F (rebamipide) *In vivo*: F (rebamipide)	*Ex vivo*:10 mM *In vivo*: 10 mM *In vivo*: 25 mM	*Ex vivo*: — *In vivo*: 3-fold *In vivo*: no effect	[680] [681] [681]
Stearyl carnitine	—	—	*Ex vivo* (rat): BBMV	*Ex vivo*: S (DPH)	*Ex vivo*: —	*Ex vivo*: —	[506]

			In vivo (rat): rectal *Ex vivo* (rat): S-G diffusion cell	*In vivo*: F (cefoxitin) *Ex vivo*: TEER	*In vivo*: — *Ex vivo*: 2 mM	*In vivo*: 26-fold *Ex vivo*: —	[506] [73]
Sucrose monocaprate	Paracellular	Increase pore radius	*In situ* (rat): rectal loop	*In situ*: AUC (insulin)	*In situ*: 3%	*In situ*: 25-fold	[185]
Sucrose oleate	Paracellular	Increase pore radius	*In situ* (rat): rectal loop	*In situ*: AUC (insulin)	*In situ*: 3%	*In situ*: 2-fold	[185]
Sucrose palmitate	Paracellular	Increase pore radius	*In situ* (rat): rectal loop	*In situ*: AUC (insulin)	*In situ*: 3%	*In situ*: 8-fold	[185]
Tartaric acid	Paracellular	Intracellular ATP & PH	*Ex vivo* (rat): colon segment	*Ex vivo*: flux (FD-4)	*Ex vivo*: —	*Ex vivo*: —	[202]
Taurine	Paracellular	—	*In vitro*: Caco-2	*In vitro*: TEER, flux (heparin)	*In vitro*: 2%	*In vitro*: 83-fold	[537]
Thiolated chitosans 4-thio-butylamidine	Multimodal	Membrane fluidity Unspecified TJ disruption	*In vivo* (rat): oral (minitablet) *In vivo* (pig): oral (Tablet)	*In vivo* F (sCT) *In vivo* F (antide)	69% (w/w) 3.45 mg 80% (w/w) 800 mg	*In vivo*: F = 1.5% *In vivo*: F = 3.2%	[434] [431]
Thiolated polycarbophil	Paracellular		*In situ* (rat): intraduodenal	*In situ*: F (buserelin)	*In situ*: 0.5% (w/v)	*In situ*: F =1.9%	[414]
TJ modulating peptide (FDFWITP)	Paracellular	Claudin modulation	*In vitro*: MDCK	*In vitro*: TEER	*In vitro*: 500 μM	*In vitro*: —	[682]
TNF-α	Paracellular	MLCK	*In vitro*: Caco-2 *In vitro*: T84 *In vitro*: HT-29/B6 *In vitro*: HT29 cl.19A *In vitro*: Caco-2	*In vitro*: TEER, flux (inulin) *In vitro*: TEER, flux (FD-3) *In vitro*: TEC, flux 22Na+) *In vitro*: flux (HRP) n vitro: TEER, flux (mannitol)	*In vitro*: 10ng/mL *In vitro*: 10ng/mL *In vitro*: 100 ng/mL *In vitro*: 10 ng/mL/ *In vitro*: 10ng/mL	*In vitro*: — *In vitro*: — *In vitro*: 6-fold *In vitro*: 8-fold *In vitro*:	[683] [609] [684] [685] [686]
Transportan (L-Penetratin)	Transcellular	Membrane fluidity	*In vivo* (rat): oral *In situ* (rat): nasal	*In vivo*: PA (insulin) *In situ*: AUC (insulin)	*In vivo*: 2 mM *In situ*: 0.5 mM	*In vivo*: 16-fold *In situ*: 9-fold	[515] [687]
Triethylchitosan	Paracellular	Modulates F-actin, ZO-1	*Ex vivo* (rat): everted sac	*Ex vivo*: flux	*Ex vivo*: —	*Ex vivo*: —	[688]
Vacuolating toxin	Paracellular	$Na^+/K^+/2Cl^-$	*in vitro*: MDCK	*In vitro*: flux (Fe^{3+} & Ni^{2+})	*In vitro*:	*In vitro*: —	[689]
VP8	Paracellular	—	*In vitro*: MDCK *In vivo* (rat): oral	*in vitro*: flux (FD-4) *In vivo*: PK/PD (insulin)	*In vitro*: 4 mg/mL *In vivo*: 100 ug	*In vitro*: 25-fold *In vivo*: —	[120] [120]
Zonula occludens toxin (zot)	Paracellular	PKC	*Ex vivo* (rabbit): Ussing *In situ* (rat): Perfusion *In vivo* (rat): oral (gastric cannula)	*Ex vivo*: TEER, flux (insulin) *In situ*: flux (insulin) Oral: PK/PD (insulin)	*Ex vivo*: 5 ug *In situ*: 5 ug *In vivo*: 20 ug	*Ex vivo*: 2-fold *In situ*: 10-fold *In vivo*: 3-fold	[98] [98] [98]
α-Cyclodextrin	Transcellular	—	*In vivo* (rabbit): suppository *In vivo* (rabbit): suppository	*In vivo*: PK/PD (hCG) *In vivo*: PK/PD, AUC (insulin)	*In vivo*: 30 mg/kg *In vivo*: 30 mg	*In vivo*: — *In vivo*: 3-fold	[690] [592]
α-Cyprinol sulfate	—	—	*In situ* (rat): intestinal loop	*In situ*: flux (ampicillin)	*In situ*: 12.5 mM	*In situ*: —	[691]
Y-Cyclodextrin	Transcellular	Lipid extraction	*In vivo* (Rabbit): suppository	*In vivo*: AUC (insulin)	*In vivo*: 30 mg	*In vivo*: 4-fold	[592]

Table S2. Key oral insulin patents published in the last 30 years

PATENT NO	YEAR	AUTHOR	TITLE	INVENTION SUMMARY
US20140056953	2014	Foger FA, Makhlof A, Hoyer H (Novo Nordisk)	Fatty acid acetylated amino acids for oral peptide delivery	Peptide: antidiabetic peptides Embodiments Dispersion type: admixed format Dispersion additives: fatty acid acetylated amino acids
WO2014031874	2014	Mustata G, Pan D, Gschneider D	Phenoxy alkyl diethanolamine and diisopropanolamine compounds for delivering active agents	Peptide: insulin Embodiments Dispersion type: non-covalent complexation Dispersion additives: phenoxy alkyl diethanolamine and diisopropanolamine as complexing agents and permeation enhancers
CN102920664	2013	In Chinese	Preparation method of long term oral insulin sustained-release microspheres	Peptide: insulin Embodiments Dispersion type: microparticulate insulin (100-300 microns) Dispersion additives: Eudragit L30D-55 NOTE: a long acting oral insulin delivery vehicle. Insulin microparticles were coated in a side spray fluidised bed coating suspension granulator.
CA 2511530	2013	Goldberg M, Arbit E, (MG, EA, Emisphere)	Night-time oral insulin therapy	Peptide: insulin Embodiments Dispersion type: non covalent complexation (Eligen) Dispersion additives
CN 103169946	2013	In Chinese	Application of safenour cyclopeptide in oral insulin medicine for treating diabetes	Peptide: insulin Embodiments Dispersion type: admixed format Dispersion additive: cyclopeptide inhibitor of acidic and protease action, permeation enhancement
CN102120781	2013	In Chinese	Preparation and application of novel oral insulin nanoparticles	Peptide: insulin Embodiments Dispersion type: nanoparticles Dispersion additives: N-amino acid composition of chitosan NOTE: —
CN102908332	2013	In Chinese	Enteric coated capsules containing cationic nanoparticles for oral insulin delivery	Dosage form: enteric coated capsule Peptide: insulin Embodiments Dispersion type: nanoparticle (solid dosage form) Dispersion additives: polycationic/mucoadhesive/biodegradable polymer, pH sensitive polymer coating NOTE: system enhances paracellular permeability of insulin
CN103371973	2013	In Chinese	Externally coated nanometer multiple emulsion for promoting oral absorption of insulin	Peptide: insulin Embodiments Dispersion type: multiple emulsion Dispersion additives: Ca^{2+} alginate/chitosan coating NOTE:
US20130034602	2013	Qian Y (Nano And Advanced Materials Institute Limited)	Enteric coated capsules containing cationic nanoparticles for oral insulin delivery	Peptide: insulin Embodiments Dispersion type: cationic nanoparticles Dispersion additives: biodegradable, cationic and mucoadhesive polymer. NOTE: The polymer also has permeation enhancement action. The nanoparticles are formulated in enteric coated capsules.
US20130267462	2013	Lau JR, Geho WB, (Sdg Inc)	Lipid construct for delivery of insulin to a mammal	Peptide: insulin Embodiments Dispersion type: mucoadhesive, amphiphilic hepatocye targeted NP Dispersion additives: amphipathic lipid, “extended amphipathic lipid that targets the construct to a receptor displayed by an hepatocyte” NOTE: Widely published on, in phase I trials (HDV-1)
US20130274352	2013	Whitehead et al (The Reagents of the University of California)	Oral drug devices and drug formulations	Peptide: insulin (and other peptides and drugs) Embodiments Dispersion type: admixed format Dispersion additives: two chemical permeation enhancers that synergistically. NOTE: low enhancer cytotoxicity (high overall potential)
US8361509	2013	Lopez-Belmonte Encina I et al (Laboratorios Farmaceticos Rovl SA)	Pharmaceutical dosage forms for the release of active compounds	Peptide: insulin (and other peptides and drugs) Embodiments Dispersion type: active in a polymeric matrix Dispersion additives: cationic polymer and a biodegradable polymer
WO2013083041	2013	Jin T, Hu Z, Yuan W, (Jin T)	Microspheres for controlled or sustained release delivery of therapeutics	Peptide: peptides (< 10 kDa, insulin listed) Embodiments Dispersion type: pH dependent microparticle (matrix) NOTE: drug and “Helping agent” in the form of fine particles are encapsulated in the polymeric matrix (EE 95%)
WO2013188979	2013	Gu F, Jones LWJ, Sandy S (FG, LWJJ, SS)	Mucoadhesive nanoparticle delivery system	Peptide: antidiabetic peptides Embodiments Dispersion type: mucoadhesive, amphiphilic targetted nanoparticles Dispersion additives: fatty acid acetylated amino acids

CN102144968	2012	In Chinese	Oral suspension of liposomes-encapsulated insulin lyophilized preparation and preparation process thereof	Dosage: — Peptide: insulin Embodiments Dispersion type: liposome suspension (O-SCULI) Dispersion additives: lecithin, cholesterol, polyglycol aliphatic acid ester, vitamin E, insulin, water NaCl, phosphate buffer NOTE: two-step process outlined. Oral suspension is absorbed into the hepatic portal vein
CN102319216	2012	In Chinese	Insulin liposome lyophilised powder, oral insulin compound preparation and preparation methods and applications thereof	Peptide: insulin Embodiments Dispersion type: nanoparticles (dry liposome preparation) Dispersion additives: acidified insulin is added to phosphate buffer and sodium cholate. Cholesterol and lecithin are dissolved in EtOH to which an aqueous mannitol and sodium cholate solution are added and homogenised for 10-30 minutes to generate an oil phase. Oil and aqueous phase are then mixed (2-6C) and distilled and lyophilised NOTE: —
EP2254590	2012	Vol A, Gribova O, (Oshadi Drug Admin Ltd)	Methods and compositions for oral administration of insulin	Peptide: insulin Embodiments Dispersion type: particulate in oil Dispersion additives: inert silica nanoparticles consisting of a hydrophobic surface, a polysaccharide and insulin suspended in oil
EP2523655 US 20130058999	2012	Foger FA (Novo Nordisk)	Pharmaceutical compositions for oral administration of insulin peptides	Peptide: insulin Embodiments Dispersion type: Dispersion additives: NOTE: —
US8257735	2012	Lau JR, Geho WB (SDG Inc)	Method of increasing the bioavailability of recombinant human insulin isophane	— Peptide: insulin isophane Embodiments Dispersion type: water insoluble target insoluble complex, Dispersion additives: NOTE: the complex consists of multiple linked individual units and a supra-molecular lipid construct matrix. The cationic insulin interacts with the anionic targeting complex
US8283317	2012	Sung HW, et al (Gp Medical Inc, National Tsing Hua University)	Nanoparticles for protein drug delivery	Dosage: — Peptide: insulin Embodiments Dispersion type: polyelectrolyte complexes Dispersion additives: chitosan, PGA
US8309123	2012	Bennis F, Serrano JJ, (FB)	Pharmaceutical compositions and methods for the oral delivery of insulin	Peptide: insulin Embodiments Dispersion type: admixed format Dispersion additives: buffer system (pH 4-8)
WO2012170828	2012	Williams P et al (Monosol Rx, LIc, Midatech Ltd)	Combination peptide-nanoparticles and delivery systems incorporating same	Peptide: insulin Embodiments Dispersion type: targeted NP Dispersion additives: NOTE: NP have a peptide encapsulated core and corona-Ligand
CN102144976	2011	In Chinese	Method for preparing insulin dry powder for oral administration by using micro capsulation technology	Dosage form: dry powder for oral administration) Peptide: insulin (and derivatives) Embodiments Dispersion type: admixed format (particulate capsulation) Dispersion additives: propolis antioxidant or colloidal matter, vit E, vit C, fatty acid, emulsifier, lyophilised NOTE: solving the problem of insulin oxidation, insulin is administered with water 30 min prior to ingestion of a meal, spray embedment of insulin (encapsulation)
CN102293748	2011	In Chinese	An oral PEGylated insulin and its preparation method pH-sensitive nanoparticles	Peptide: insulin Embodiments Dispersion type: covalent conjugate (PEGylation), NP carrier Dispersion additives: pH sensitive polymer, carrier additives, stabiliser NOTE: PEGylated insulin in NP (formed by multiple emulsion approach)
WO2011084618	2011	Lee WW et al (Nod Pharma)	Compositions and methods for oral drug delivery	Peptide: insulin, exenatide Embodiments Dispersion type: admixed format (TBC) Dispersion additives: permeation enhancer, pharmaceutically acceptable excipient, bioadhesive polymer. NOTE: consists of an opening for the unidirectional release of peptide and permeation enhancer
WO2011130716	2011	Zarzycki et al (Access Pharma Inc)	A nanostructure containing vitamin B_{12} for facilitated delivery of drugs across biological barriers	Peptide: insulin Embodiments Dispersion type: ligand coated nanoparticle (B_{12}) Dispersion additives: dispersion additives: dextran, cobalamin
CN 100588422	2010	In Chinese	Oral insulin composition and methods of making and using thereof	Peptide: insulin Embodiments Dispersion type: dextran microparticles (mono or multiphase) Dispersion additives:
CN100594929	2010	In Chinese	Oral insulin medicine and preparation method thereof	Peptide: insulin Embodiments Dispersion type: admixed format Dispersion additives: insulin, bile acid, bilirubin, cholesterol, lecithin NOTE: clinical data

CN101862445	2010	In Chinese	Oral insulin containing protease inhibitor	Peptide: insulin Embodiments Dispersion type: “polymersomes gel” Dispersion additives: polymerised ovomucoid from duck eggs with acrylamide coadministered with insulin NOTE: F of 20%
EP 2248531	2010	Arbit et al (Emisphere)	Antidiabetic oral insulin-biguanide combination	Peptide: insulin Embodiments Dispersion type:
WO2010060667	2010	Foger FA (Novo Nordisk)	Pharmaceutical compositions suitable for oral administration of derivatised insulin peptides	Peptide: insulin Embodiments Dispersion type: water free liquid dispersion/semi-solid dispersion Dispersion additives: one polar organic solvent, one hydrophobic component
WO2010113177	2010	Vidhya R et al (Reliance Life Sciences Pvt Ltd)	Oral insulin delivery systems for controlling diabetes	Peptide: insulin Embodiments Dispersion type: peptide encapsulated in pH sensitive polymeric microspheres Dispersion additives: Eudragit®
EP 2042166	2009	Adel G et al (The Jordanian Pharma Manu Co)	Nanocapsules for oral delivery of proteins	Peptide: insulin Embodiments Dispersion type: nanoparticle Dispersion additives:
EP2017288	2009	Not yet filed (Novo Nordisk)	Protease stabilized, pegylated insulin analogues	Peptide: insulin Embodiments Dispersion type: PEGylated insulin analogues (conjugate) Dispersion additives: NOTE: insulin analogues contain B25H A14E or A14H. PEGylation carried out at position B29K
WO2009020577	2009	Chang LC et al (LCC et al)	Innovative formulation for oral insulin delivery	Peptide: insulin Embodiments Dispersion type: particulate encapsulation with mucoadhesion
WO2009050738	2009	Khedkar et al (Biocon Ltd et al)	An orally administerable solid pharmaceutical composition and a process thereof	Peptide: insulin (and other peptides and drugs) Embodiments Dispersion type: peptide conjugates in particulate dispersion Dispersion additives: 10-60% fatty acid or fatty acid sodium salt, other polymer excipients that improve solubility, dissolution rate and effective F of peptide
CN101167699	2008	In Chinese	Insulin sustained-release oral preparation and preparation method thereof	Peptide: insulin Embodiments Dispersion type: dragon’s blood nanoparticle Dispersion additives: dragon’s blood, dextran-70, EtOH, tween 20 or tween 80, NaOH
US20080311214	2008	Rao KK (Transgene Biotek Ltd)	Polymerized solid lipid nanoparticles for oral or mucosal delivery of therapeutic proteins and peptides	Peptide: insulin Embodiments Dispersion type: ligand coated solid lipid nanoparticles Dispersion additives:
WO 2008051101	2008	Beco PRAC (Univ De Coimbra)	Oral submicron particle delivery system o r proteins and process for its production	Peptide: insulin Embodiments Dispersion type: microparticles (submicron) Dispersion additives: natural polymers NOTE: polymeric matrix that is pH and enzyme resistant and these swell in the intestine. The particles contain two coating layers that can enhance permeation of peptide (F_{REL} 34%)
WO 2008109068	2008	Doyle RP (Univ Syracuse, RPD)	A conjugate of insulin and vitamin B_{12} for oral delivery	Peptide: insulin Embodiments Dispersion type: peptide conjugate (B_{12}) Dispersion additives:
WO2008033058 EP2067484	2008	Artamonov AV, Rodionov PI (AVA, PIR, Concern O3 Company Ltd)	Method for producing insulin in the form of an oral preparation	Peptide: insulin Embodiments Dispersion type: admixed format Dispersion additives: peptide mixed with 1-50% polymer (0.4-40 kDa) with an irradiation that gives a final conc of 1-10 mg/ml and a POE:insulin ratio of 500:1
WO2008132727	2008	Shimoni E et al (Technion Res & Dev Foundation)	Oral Delivery of proteins and peptides	Dosage form: enteric coated tablets or capsules Peptide: insulin Embodiments Dispersion type: microparticles of peptide, permeation enhancer, protease inhibitor embedded in a solid matrix. NOTE: fast release.
CN1296098	2007	In Chinese	Oral insulin protecting agent	Peptide: insulin Embodiments Dispersion type: admixed format

				Dispersion additives: protease inhibitor, permeation enhancer
EP1140024	2007	Grove CF et al (The Reagents of the University of California, iMEDD)	Particles for oral delivery of peptides and proteins	Dosage: enteric coated tablets or capsules Peptide: insulin (and other peptides and drugs) Embodiments Dispersion type: asymmetrical, reservoir containing particulates within enteric coated solid dosage form Dispersion additives: selected excipients within the core to delay dissolution and release from the particle reservoir for 5-60 min NOTE: particles are enteric coated
EP17781257	2007	Shingai Emisphere Technol Inc, et al (Emisphere)	Pharmaceutical formulations containing microparticles or nanoparticles of a delivery agent	Dosage form: Solid dosage form Peptide: insulin Embodiments Dispersion type: Microparticles or nanoparticles with a delivery agent Dispersion additives:
EP1797870	2007	Badwan AA, et al (The Jordanian Pharma Manu Co)	Oral delivery of protein drugs using microemulsions	Peptide: insulin Embodiments Dispersion type: w/o microemulsion (possible SLN) Dispersion additives: biodegradable polymer such as chitosan oligonucleotides, oleic acid, plurol® (glyceryl 6-dioleate)
WO 2007032018	2007	2007 Sharma CP, Mannemcherril RR, (CPS, RRM, Council Scientific and Industrial Research)	pH sensitive nanoparticle formulation for oral delivery of proteins/peptides	Peptide: insulin Embodiments Dispersion type: pH sensitive hydrophobic nanoparticles Dispersion additives: pH sensitive fatty acid based NP stabilised with a hydrophilic polymer (30-60mg/g)
WO2007006320	2007	Abbas HSH (HSHA)	Drinkable oral insulin liquid and capsules	Peptide: insulin Embodiments Dispersion type: (i) aqueous dispersion and (ii) soft gelatin capsule Dispersion additives:
WO2007036946	2007	Devarajan PV et al (PVD)	Compositions for enhanced absorption of biologically active agents	Peptide: insulin (and other peptides and drugs) Embodiments Dispersion type: polymeric NP Dispersion additives: permeation enhancer in NPs NOTE: NP have a peptide encapsulated core and corona-Ligand
WO2007068311	2007	Mayyas AR et al (ARM et al)	Oral delivery of protein drugs using a microemulsion	Peptide: insulin Embodiments Dispersion type: w/o microemulsion (possible SLN) Dispersion additives: biodegradable polymer such as chitosan oligonucleotides, oleic acid, plurol® (glyceryl 6-dioleate)
CN1753688	2006	In Chinese	Night-time insulin therapy	Peptide: insulin Embodiments Dispersion type:
CN2792500	2006	In Chinese	Oral insulin corpusle	Peptide: insulin Embodiments Dispersion type: microparticle Dispersion additives: NOTE: “traditional Chinese medicine dragon’s blood as a carrier to form a nanometer insulin microsphere” of <80nm, direct particle absorption
WO 2006088473	2006	Kontala PR, Kontala S (PRK, SK)	Microcapsules and nanocapsules for the transmucosal delivery of therapeutic and diagnostic agents	Peptide: insulin Embodiments Dispersion type: MP and NP delivery vehicle Dispersion additives: polymeric particulates
WO2006103657	2006	Pinhasi A, Gomberg M, (Dexcel Pharma Technologies Ltd AP, MG)	A solid composition for intra-oral delivery of insulin	Peptide: insulin Embodiments Dispersion type: hydrophilic polymer matrix, phospholipid, NOTE: F of 5%
CN 1676164	2005	In Chinese	Colon positioned-release oral insulin self microemulsion formulation and capsules containing it	Peptide: insulin Embodiments Dispersion type: self microemulsified delivery system Dispersion additives: lyophilised support, stabilisers, permeation enhancer (oil), emulsifier
CN1221283	2005	In Chinese	Oral insulin granule and its preparation	Peptide: insulin Embodiments Dispersion type: particulate format (insulin precursor liposomes: gelatin complex) Dispersion additives: gelatin, plastids, silica powder NOTE: “fine granules enteric capsules of insulin”, dispersed in “plastids”
EP1072255	2005	Barantsevitch EN, Milstein SJ (Emisphere)	Oral delivery system for desferrioxamine, insulin and cromolyn sodium	Peptide: insulin Embodiments Dispersion type: non-covalent complexation (carrier) Dispersion additives: acetylated amino acids

US 20050136121	2005	Kershman A, Shear JL (KA, SJL, Shear Kershman Lab Inc)	Oral peptide delivery system with improved bioavailability	Peptide: insulin Embodiments Dispersion type: solid lipid microparticles Dispersion additives: NOTE: when melted the additives exhibit thixotropy
US 6949258	2005	Zhang J (Zhang H)	Biologically active oral preparation that can be site specific released in colon	Peptide: insulin Embodiments Dispersion type: colon specific delivery
CN1181889	2004	In Chinese	Process for preparing oral insulin nanomaterial	Peptide: insulin Embodiments Dispersion type: nanoscale insulin microspheres dispersed in oil Dispersion additives: biodegradable, non-toxic carrier (polybutylcyanoacrylate) NOTE: insulin is partially bound to the surface and partially embedded in the NP. The average diameter of microspheres is 30nm. Improved permeation in this NP-in oil dispersion
EP1469812	2004	Abbas R (Emisphere)	Oral insulin therapy	Peptide: insulin Embodiments Dispersion type: non-covalent complexation
WO2004078197	2004	Sabetsky V (VS and Technology Development Company Ltd)	Delivery system for drug and cell therapy	Peptide: insulin Embodiments Dispersion type: dextran microparticles (mono or multiphase)
WO2004080401 US8324156	2004	Arbit E, et al (EA, et al and Emisphere)	Oral insulin therapies and protocol	Peptide: insulin Embodiments Dispersion type: non-covalent complexation to a carrier NOTE: administered 10 minutes preprandially
US20030198666	2003	Abbas R et al (RA et al)	Oral insulin therapy	Dosage form: Oral Peptide: insulin Embodiments Dispersion type: non-ionic complexation Dispersion additives: delivery agent NOTE: “Attenuating diseases associated with diabetes”
US20030229010	2003	Ekwuribe N (NE)	Oral insulin-oligomer conjugates	Peptide: insulin (and other peptides and drugs) Embodiments Dispersion type: w/o microemulsion of a peptide conjugate Dispersion additives: dispersion HLB between 3-7 NOTE: conjugate moiety selected froma polyalkylene glycol moiety and a lipophilic moiety
WO2003057170	2003	Arbit E et al (EA, et al (and Emisphere))	Oral insulin therapy	Peptide: insulin Embodiments Dispersion type: non-covalent complexation NOTE: Eligen Technology (Emisphere)
EP1242013	2002	Croft J, Zhang H (Anesta Corp)	Oral transmucosal drug dosage using solid solution	Peptide: not specified Dispersion type: solid solution Dispersion additives: drug in a solid solution with a dissolution agent NOTE: Oral mucosal delivery
US 20020151467	2002	Leung F (LF)	Methods and compositions for oral insulin delivery	Peptide: insulin Embodiments Dispersion type:
WO2002085408 US20040097410	2002	Zheng C et al (University Tsinghua et al)	Method of production of insulin containing oil based preparation for oral	Peptide: insulin Embodiments Dispersion type: emulsion (oily) Dispersion additives: NOTE: dissolution of insulin in acidic medium with non-ionic surfactant (HLB 10-20) after which this is added to a continuous oil phase or emulsifier with a HLB of 0-10 to for an emulsion
WO2001027154	2001	O’Mahoney DJ, Lambkin IJ (DJO’M, IJM)	Membrane translocating peptide drug delivery system	Peptide: insulin Embodiments Dispersion type: peptide coated particulate (liposome) Dispersion additives: inclusion of a membrane translocating peptide (MTLP) coating on liposomes to facilitate insulin translocation
US5858968	1999	Eisenberth G et al (Autoimmune Inc)	Method of treating or preventing type 1 diabetes by oral administration of insulin	Peptide: insulin Embodiments Dispersion type: oral administration of insulin fragments to prevent T1DM. A formulation for oral delivery. Dispersion additives:
US5824638	1998	Belendiuk GW et al (Shire Lab Inc)	Oral insulin delivery	Peptide: insulin Embodiments Dispersion type: o/w emulsion or w/o emulsion Dispersion additives: hydrophobic phase (long chain fatty acid, ester or alcohol) and hydrophilic phase (water)
US5698515 DE19510551	1997	Ametov AS et al (Institut Neftekhimicheskogo Sinteza Imeni AV	Insulin containing polymer composition for oral administration	Peptide: insulin Embodiments Dispersion type: admixed format Dispersion additives: hydrophilic polymer modified with an enzyme inhibitor (ovomucoid from duck or turkey)

		Topchieva Rossiiskoi Akademii Nauk		
WO1996037215	1996	Belendiuk GW, et al (Pharmavene Inc)	Oral insulin delivery	Peptide: insulin Embodiments Dispersion type: o/w emulsion or w/o emulsion Dispersion additives: hydrophobic phase (long chain fatty acid, ester or alcohol) and hydrophilic phase (water)
DE4140186	1993	De Wunderlich JCH, et al (Alfatec-Pharma GmbH)	Oral dosage forms for peptide drugs – esp. insulin, contg. drug in gelatin matrix	Peptide: insulin, corticotrophin, Embodiments Dispersion type: microparticle Dispersion additives: gelatin, fractionated gelatin, collagen hydrolysate, derivatised gelatin NOTE: multilayer or core shell structure, slow release first layer fast release core, small or large intestine targeting
WO 1988001213	1988	Rosen R, Steiner SS (Clinical Technologies Ass)	Delivery systems for pharmacological agents	Peptide: insulin, heparin, physostigmine Embodiments Dispersion type: protein microspheres NOTE: acid and pepsin stable protein microspheres (average size of 10 microns) that protect cargo that pass through the stomach wall which releases cargo in the neutral pH environment.
WO1985005029	1985	Ecanow B, Ecanow CS, (Medaphore Inc)	Oral insulin and a method of making the same	Peptide: insulin Embodiments Dispersion type: two phase liquid aqueous system Dispersion additives: NOTE: to include sustained release dosage form

Dosage form stated only if information available

Table III. Classification for intestinal PEs based on criteria in preclinical models. White boxes: pass; Grey

	CLASSIFICATION	CLASS 1 FAST ENHANCEMENT STRONG ENHANCEMENT GOOD RECOVERY	CLASS 2 FAST ENHANCEMENT STRONG ENHANCEMENT POOR RECOVERY	CLASS 3 FAST ENHANCEMENT POOR ENHANCEMENT GOOD RECOVERY	CLASS 4 SLOW ENHANCEMENT STRONG ENHANCEMENT GOOD RECOVERY	CLASS 5 SLOW ENHANCEMENT STRONG ENHANCEMENT POOR RECOVERY	CLASS 6 SLOW ENHANCEMENT POOR ENHANCEMENT GOOD RECOVERY
RATE	**RATE OF ENHANCEMENT I (IN VITRO)** TIME REQUIRED FOR 80% DROP IN TEER IN CACO-2	**<15 min**	**<15 min**	**<15 min**	**>15 min**	**>15 min**	**>15 min**
	RATE OF ENHANCEMENT II (EX VIVO) TIME REQUIRED FOR AN 80% DROP IN TEER IN ISOLATED INTESTINAL MUCOSAE IN USSING CHAMBERS	**<30 min**	**<30 min**	**<30 min**	**>30 min**	**>30 min**	**>30 min**
	RATE OF ENHANCEMENT III (IN SITU) T_{MAX} FD4 IN INTESTINAL INSTILLATION	**<30 min**	**<30 min**	**<30 min**	**>30 min**	**>30 min**	**>30 min**
EXTENT	**EXTENT OF ENHANCEMENT I (IN VITRO)** P_{APP} OF MANNITOL IN CACO-2 MONOLAYERS OVER 2 HOURS	**>2 x 10^{-5} cm/s**	**>2 x 10^{-5} cm/s**	**< 2 x 10^{-5} cm/s**	**>2 x 10^{-5} cm/s**	**>2 x 10^{-5} cm/s**	**<2 x 10^{-5}cm/s**
	EXTENT OF ENHANCEMENT II (EX VIVO) P_{APP} OF MARKER IN ISOLATED INTESTINAL MUCOSAE IN USSING CHAMBERS AFTER TWO HOURS	**>1 x 10^{-5} cm/s**	**>1 x 10^{-5} cm/s**	**< 1 x 10^{-5} cm/s**	**>1 x 10^{-5} cm/s**	**>1 x 10^{-5} cm/s**	**<1 x 10^{-5}cm/s**
	EXTENT OF ENHANCEMENT III (IN SITU) F_{ABS} OF FD4 IN INTESTINAL INSTILLATION OR VIA ORAL DELIVERY	**>20% F**	**>20% F**	**<20% F**	**>20% F**	**>20% F**	**<20% F**
RECOVERY	**RECOVERY I (IN VITRO)** HISTOLOGY SCORE IN ISOLATED INTESTINAL MUCOSAE IN USSING CHAMBERS IN TWO HOURS	**1-to-3**	**4**	**1-to-3**	**1-to-3**	**4**	**1-to-3**
	RECOVERY II (IN SITU) HISTOLOGY SCORE AFTER 2 HOURS IN INTESTINAL INSTILLATION	**1-to-2**	**3-4**	**1-to-3**	**1-to-3**	**4**	**1-to-3**
	RECOVERY III (IN SITU) FABS OF FD4 BETWEEN 2-4 HOURS POST ADDITION OF ENHANCER IS <25% OF COADMINISTRATION (1-2 HOURS)	**<25% of F_{0-2}**	**>25% of F_{0-2}**	**<25% of F_{0-2}**	**<25% of F_{0-2}**	**>25% of F_{0-2}**	**<25% of F_{0-2}**

	SAFETY (GLOBAL RATING OF FIVE) ALLOWED EXCIPIENT, FOOD ADDITIVE, GRAS, NO SYSTEMIC TOXICITY, NO ACTIVATION OF CELLULAR SIGNALLING	+ + + + +	– – – – –	+ + + + +	+ + + + +	– – – – –	+ + + + +
	COMMENT	Ideal enhancer with strong development potential in oral peptide delivery	Unacceptable safety concerns, but other routes might be possible (e.g. topical)	Modest enhancer with good safety, possible delivery of potent low MW solutes	Strong enhancer that is more ideally suited to topical, rectal due to slow onset	Unacceptable safety concerns, but other routes might be possible (e.g. topical)	Weak enhancer not suited to development