Polymer-based oral peptide nanomedicines

Oral peptide delivery has been one of the major challenges of pharmaceutical sciences as it could lead to a great improvement of classical therapies, such as insulin, alongside making an important number of new therapies feasible. Successful oral delivery needs to fulfill two key tasks: to protect the macromolecules from degradation in the GI tract and to shuttle them across the intestinal epithelium in a safe and efficient fashion. Over the last decade, there have been numerous approaches based on the chemical modification of peptides and on the use of permeation enhancers, enzyme inhibitors and drug-delivery systems. Among the approaches developed to overcome these restrictions, the design of nanocarriers seems to be a particularly promising approach. This article is an overview on the state of the art of oral-peptide formulation strategies, with special attention to insulin delivery and the use of polymeric nanocarriers as delivery systems.

Peptides and proteins have been used as biopharmaceuticals since the discovery of insulin in 1922, mainly due to their high activity, specificity and effectiveness compared with more conventional drugs [1,2]. The increasing evidence of the therapeutic potential of these molecules has led not only to the production of these compounds in large quantities, but has also attracted the interest of the scientific community [3]. In fact, the development of peptide therapeutics represent a steadily growing market strategy, with 51 compounds approved by 2010, while 132 candidates were in clinical development, of which 15 were in Phase III [4]. As a general tendency, peptide candidates for metabolic disorders such as diabetes, obesity and osteoporosis that are prevalent in a sedentary, aging population are likely to enter the development pipeline in increasing numbers [5].

Unfortunately, due to their instability and restricted permeability, most of these marketed peptides have to be administered parenterally in current medical practice. This modality of administration has several important drawbacks such as pain upon injection, short half-lives in bloodstream, which makes repeated administration and high doses necessary, low patient compliance, sterility requirements and high cost of operation. Therefore, alternative ways of administration are currently being explored such as pulmonary, nasal, buccal, rectal, ocular, vaginal, transdermal and, in particular, the oral route, which is generally recognized to be the most convenient modality of administration [3]. Indeed, oral delivery is potentially cost effective, has the highest patient compliance and could overall contribute to great advances in classical therapies, leading to new methods of treatment and diagnosis of important diseases [6]. As an additional advantage, the oral route is also the ideal way for delivering certain peptides [7] as it mimics physiological pathways by which they may reach their site of action. For example, oral insulin absorption by the portal vein would directly lead insulin to the target organs, thus reducing adverse systemic effects (see ‘Nanotechnology approaches for oral insulin delivery: a case study’, below).

Despite all the efforts dedicated so far, the development of a convenient oral formulation for peptides still remains a challenge. As discussed above in further detail, the major obstacle to achieve oral peptide and protein delivery is the highly restrictive nature of the GI tract, which is specialized in degrading such compounds and does not allow their transport across the intestinal mucosa [8,9]. In particular, the extreme pH conditions and high enzymatic activity of the GI tract are critical impediments to oral peptide delivery, leading to conformational changes, inactivation and degradation [10,11]. These processes comprise hydrolytic peptide degradation by peptidases and chemical modifications such as oxidation, phosphorylation or deamidation [10].

Regarding physical and biological barriers, the first and probably most relevant barrier is the mucus overlying the intestinal mucosa. Foreign particles are efficiently retained in the mucus layer by adhesion and/or steric impedance, and then removed by means of physiological clearance mechanisms.

As a next step, the drug molecules also need to cross the epithelial barrier by means of transepithelial or paracellular pathways [12,13]. The
intestinal epithelium also comprises a small proportion of mucosa-associated lymphoid tissue (i.e., Peyer’s patches), which allows the uptake of material via the M cells and thereby the transport to the lymphatics and into the systemic circulation. M-cell mediated transport has been widely investigated for drug delivery due to the high transcytotic capacity of these cells for particulated materials [14–16]. Since this uptake mechanism is mediated by lymphatic tissues, it has been considered particularly interesting for the oral delivery of antigenic proteins or peptides for vaccination. Several clinical trials have already been conducted on this approach; however, to our knowledge; there is still no clear evidence on the effectiveness of this route for immunization [201].

Strategies & alternatives to improve oral peptide delivery

Currently, only a couple of peptides such as cyclosporin A (a hydrophobic peptide) and desmopressin (for which a very low bioavailability is required and has a relatively low production cost) can be administered orally. There have been a great number of pharmaceutical strategies to develop effective oral peptide formulations, including co-administration of enzyme inhibitors, permeation enhancers and/or chemical modification of peptides.

Permeation enhancers & enzyme inhibitors

Permeation enhancers make mucosal surfaces more permeable by temporally disrupting the physical barriers by means of increasing membrane fluidity, decreasing mucus viscosity and/or opening tight junctions [17]. However, they may not only produce the transport of the desired drug, but also the absorption of potentially toxic molecules. As a consequence, they can induce undesirable side effects and epithelial damage, which is a general concern to their implementation. Nevertheless, there are several reports in the literature indicating the possibility of increasing permeability in a reversible fashion without permanent damage to the epithelia. For instance, macromolecular enhancers, such as chitosan, increase drug transport without important toxic side effects. Recently, melittin and, in particular, its derivatives with modified amino acid sequence have also shown promising and reversible permeation enhancer characteristics in both paracellular and transcellular pathways with limited cytotoxicity [18,19]. Another current potential strategy for increasing epithelial uptake and permeation throughout the intestinal mucosa is the co-administration of the cell-penetrating peptides (CPPs) [20,21].

On the other hand, enzyme inhibitors attempt to avoid the degradation of the peptide in the GI tract. The co-administration of enzyme inhibitors with peptides has shown limited success; however, the combination of enzyme inhibitors and permeation enhancers improve the intestinal absorption of peptides [8]. Nevertheless, the use of enzyme inhibitors in long-term therapy has side effects such as the interruption of the digestion of some nutritive proteins, intestinal mucosal damage and the stimulation of protease secretion due to feedback regulation.

Chemical modifications

Peptides can be chemically modified by the addition of functional groups or by the conjugation to macromolecular entities for improving their absorption across the GI tract without producing serious side effects [22]. The use of the salt form of peptides or the covalent attachment of hydrophilic polymers to peptides can increase solubility and paracellular transport. Polyethylene glycol (PEG) and N-(2-hydroxypropyl) methacrylamide are attractive hydrophilic polymers to formulate polymer–drug conjugates. Similarly, hydrophobic lipids can be attached to peptides to enhance their transcellular uptake. Several fatty acids, such as palmitic, butyric, lauric or caprylic acid, have been used to modify peptides, such as desmopressin [23], leucine enkephalin analogue [24] and insulin [25], showing longer plasma half-life, enhanced permeability and better resistance to enzymatic degradation. Other possibility of covalent attachment is to conjugate the peptide to small molecules (cell-membrane transporters or receptors) that trigger endocytosis [17,26,27]. The major drawback of these methodologies is the risk of diminishing the biological activity of the peptide as a consequence of the chemical modification [28].

Nanoparticulate drug-delivery carriers

A promising tool to achieve oral delivery of peptides, protecting them from degradation and enhancing their transport across epithelium, is the immobilization of these macromolecules into drug-delivery carriers. Among the great number of different strategies, the design of particulated nanocarriers has been positioned recently as an especially promising approach. In fact, scientific articles describing nanosystems represent over
Polymer-based oral peptide nanomedicines

35% of all publications related to oral peptide delivery over the last 5 years [202], indicating the growing interest generated in this field. The reason behind this interest is that the submicron size of the nanocarriers and their large specific surface area significantly improves their interaction with the intestinal mucosa, as compared with larger carriers [29,30]. The possible ways of these carrier interactions are illustrated on Figure 1.

Nanometric carriers include polymeric nanosystems (mostly nanoparticles and nanocapsules) and lipidic systems (nano/microemulsions and self-nanoemulsifying systems). The present review is primarily focused on the application of polymeric biomaterials in the design of nanosystems for oral peptide delivery; however, compositions based on polymer-coated lipid nanostructures are also disclosed where relevant (i.e., nanocapsules and surface-modified solid lipid nanoparticles).

A long list of polymers has been used to generate the nanocarrier structures mentioned above (Table 1). Examples of synthetic polymers relevant for oral delivery include: polyesters (e.g., poly[lactic acid] [PLA]) [31–33], poly[lactic-coglycolic acid] [PLGA] [31,34–39], and poly[ε-caprolactone] [40,41], poly(methyl methacrylates) [42–44], poly(alkyl cyanoacrylates) [45–50] and polyanhydrides [34], which provide adjustable controlled drug-release profiles from days to weeks. Nanocarriers based on synthetic polymers can be generated by diverse techniques such as nanoprecipitation, solvent evaporation, freeze–drying or spray drying of emulsions and supercritical fluid technology [51–58].

Natural polymers are abundant in nature and relatively inexpensive compared with synthetic polymers and normally offer quicker drug-release profile [59]. In addition, the generation of nanocarriers with these natural polymers can be usually performed by using mild methods such as ionic gelation, complexation or coacervation with an excellent capacity of peptide association [31,60–62]. Albumin, agar, pectins, cyclodextrins, gelatine, alginate, collagen, dextran and chitosan, and its derivatives, have been extensively investigated in scientific literature for oral peptide delivery [60,61,63–96] (Table 1). Nevertheless, besides these positive characteristics, it is also important to keep in mind that polymers from natural sources may present homogeneity and purity problems, which in general require careful quality assessment.

The appropriate selection and combination of the biomaterials used for the preparation of these systems permit the modulation of their physicochemical properties, such as hydrophobicity, surface charge and drug-release profiles. Depending on their composition, these subcellular size carriers can also provide biodegradable and biocompatible properties, as well as controlled release. Moreover, the use of bioadhesive/mucoadhesive materials can significantly improve the performance of the carriers, allowing prolonged interaction of the delivery system with the mucosal barrier and, hence, increasing the time available for penetration across the mucosal epithelia (see Figure 1 for possible carrier interactions) [77,80]. Synthetic polymers such as polyacrylates, cellulose derivatives and natural polymers such as alginate or chitosan derivatives are relevant examples of polymers that have been applied as components for mucoadhesive particles.

An alternative approach for enhancing the interaction of the nanocarriers with the intestinal barrier—carrier penetration is their surface modification with selective ligands that can be recognized by M cells or epithelial cells [51]. Bacterial adhesins, lectins, monoclonal antibodies and specific amino acid sequences are some examples of such ‘cytoadhesive’ ligands [97].

![Figure 1. Possible interactions of nanocarriers with the intestinal barrier.](image-url)
Finally, the performance of nanocarriers for oral delivery can also be enhanced by their surface modification with hydrophilic polymers (e.g., PEG and hyaluronic acid). Several studies have shown that the presence of such hydrophilic chains on the surface of the nanocarriers can improve their efficacy by rendering them with enhanced physical and biological stability, and by prolonging the interaction of nanoparticles with the mucosal barrier [32,98].

### Nanotechnology approaches for oral insulin delivery: a case study

Diabetes mellitus is a metabolic disease caused by failure of insulin secretion (Type I diabetes) or by insulin resistance combined with reduced insulin secretion (Type II diabetes), leading to the failure of blood glucose homeostasis and hyperglycemia [99,100]. This disease is the third cause of death in developing countries [101]. It is well known that patients suffering Type I diabetes control their blood glucose levels by means of multiple insulin injections. There are several drawbacks associated to this method (e.g., physiological stress, allergy, pain and lipodystrophy, and risk of hypoglycemia [102]) and, therefore, alternative routes have been widely investigated for improved delivery of insulin [103,104].

As mentioned above, one of the main drawbacks of the current parenteral administration of insulin is that it does not follow the natural dynamics of the insulin-release profile from the

### Table 1. Overview of polymeric nanocarrier compositions for oral peptide delivery.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>System</th>
<th>Peptide</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA–PEG</td>
<td>Nanoparticle</td>
<td>Tetanus toxoid</td>
<td>[31,32]</td>
</tr>
<tr>
<td>PLGA–polyanhydrides</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[34]</td>
</tr>
<tr>
<td>PLA–cyclodextrin</td>
<td>Nanoparticle</td>
<td>BSA</td>
<td>[33]</td>
</tr>
<tr>
<td>PLGA–HP5S</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[35]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Nanoparticle</td>
<td>Albumin (BSA), insulin and calcitonin</td>
<td>[36–38]</td>
</tr>
<tr>
<td>PLGA–chitosan</td>
<td>Nanoparticle</td>
<td>Tetanus toxoid and calcitonin</td>
<td>[31,39]</td>
</tr>
<tr>
<td>PCL–eudragit RS</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[40,41]</td>
</tr>
<tr>
<td>PCL</td>
<td>Nanoparticle</td>
<td>Cyclosporine-A</td>
<td>[135]</td>
</tr>
<tr>
<td>PAA–chitosan–cyclodextrin</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[43]</td>
</tr>
<tr>
<td>PMMA–chitosan</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[42,44]</td>
</tr>
<tr>
<td>PACA</td>
<td>Nanoparticle and nanocapsule</td>
<td>Insulin, calcitonin and octreotide</td>
<td>[45–50,136]</td>
</tr>
<tr>
<td>Dextran–alginate</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[64,65,72]</td>
</tr>
<tr>
<td>Dextran–vitamin B12</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[66,73]</td>
</tr>
<tr>
<td>Dextran–alginate–chitosan</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[74–77]</td>
</tr>
<tr>
<td>Dextran–chitosan</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[78,79]</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Nanoparticle, nanocapsule and SLN</td>
<td>Insulin, calcitonin, BSA, tetanus and diphtheria toxoids and cyclosporine-A</td>
<td>[61,90,91,93–96,137]</td>
</tr>
<tr>
<td>Chitosan–alginate</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[80,87]</td>
</tr>
<tr>
<td>Chitosan–glucomannan</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[60]</td>
</tr>
<tr>
<td>Chitosan–arabic gum</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[92]</td>
</tr>
<tr>
<td>Chitosan–cyclodextrin</td>
<td>Nanoparticle</td>
<td>Glutathione</td>
<td>[68,88]</td>
</tr>
<tr>
<td>Chitosan–alginate–cyclodextrin</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[89]</td>
</tr>
<tr>
<td>Chitosan–α–PGA</td>
<td>Nanoparticle and nanocapsule</td>
<td>Insulin</td>
<td>[81–84]</td>
</tr>
<tr>
<td>Chitosan–hyaluronic acid</td>
<td>Nanoparticle</td>
<td>VEG, PDGF-BB</td>
<td>[67]</td>
</tr>
<tr>
<td>Chitosan–PASP–PEG</td>
<td>Nanoparticle</td>
<td>BSA</td>
<td>[85]</td>
</tr>
<tr>
<td>Chitosan–HPMCP</td>
<td>Nanoparticle</td>
<td>Insulin</td>
<td>[86]</td>
</tr>
</tbody>
</table>

BSA: Bovine serum albumin; HP55: Hypromellose phthalate; HPMCP: Hydroxypropyl methylcellulose phthalate; PAA: Polymethacrylic acid; PACA: Poly(alkylcyanoacrylate); PASP: Poly(l-aspartic acid); PCL: Poly(caprolactone); PEG: Polyethylene glycol; PGA: Polglycolide; PLGA: Poly(lactic acid); PLGA: Poly(lactic-co-glycolic acid); PMMA: Poly(methyl methacrylate); SLN: Solid lipid nanoparticle.

**Key Term**

Acrylic polymers: Synthetic mucoadhesive polymers, principally used for oral drug delivery.
pancreas, leading to inappropriate tissue distribution that provides the liver only with a small fraction of the total injected dose [105]. On the contrary, upon oral delivery, absorption by the enterocytes would directly lead insulin to the target organ (liver followed by the pancreas) through the portal vein, which is the natural exposure route for the hormone. This shortened pathway would reduce adverse systemic effects. In addition, early use of an oral insulin formulation in therapy would also be of great benefit for avoiding further diabetic complications and concomitant diseases (e.g., cardiovascular diseases) [102]. Based on this, it is easy to understand that a great portion of the articles and patents related to oral peptide administration refer to insulin (over 90% according to ISI Web of Knowledge) [202].

Enzyme inhibitors, such as sodium cholate, leupeptin or chicken and duck ovomucoid, decrease the degradation of insulin, increasing the available amount of the peptide for absorption [106,107]. However, the use of these substances for long periods of time produces the intestinal absorption of undesired proteins, perturbation of digestion processes and increased protease secretion [108].

Absorption enhancers, such as bile salts, surfactants, trisodium citrates, ethylenediaminetetraacetic acid [109], zonula occludens toxin [110,111], labrasol [112] and 4-CNAB [113], and cell-permeating peptides, such as octaarginine R8 and 1-penetratin [20,21,114], have been used to permeate insulin across the epithelium via the paracellular route by modulating tight junctions. Most of these compounds have shown lack of specificity and some may even produce damage of mcosa and cellular membranes [102]; however, the possibility of opening the tight junctions in a reversible fashion without damaging the epithilia is reported in literature. In fact, macromolecular permeation enhancers, such as chitosan, may increase epithelial transport without toxic side effects.

Modification of the chemical structure of insulin, typically by covalent modifications for increasing the permeation across the epithelium and decreasing the enzymatic degradation, is an opportunity to enhance bioavailability without modifying the mucosal membrane [115]. PEGylation of insulin (Biocon Limited) [116] in a Phase III clinical trial, the development of a hexyl-insulin-monoconjugate or HIM 2 (Biocon Limited and Nobex Corporation) [115] that reached Phase III clinical trials, and the conjugation of insulin with B12 vitamin [73,117], are examples of this interesting approach to improve the oral delivery of insulin by chemical conjugation (Table 2).

Regarding the use of nanocarriers, the efforts to develop an oral insulin nanotech delivery system started in the late 1980s with poly(alklycyano acrylate) nanocapsules, for which a remarkable hypoglycemic effect has been reported [46]. This pioneering work has opened a new field of research and, since then, there have been over 200 articles describing a variety of nanocarriers for developing oral forms of insulin and to a minor extent, other antidiabetic peptides. Besides acrylic polymers, other relevant polymeric biomaterials used for the development of the most promising compositions have been polyesters, such as PLGA or poly(caprolactone), and polyanhydrides, polysaccharides (chitosan, dextran and alginate), polyaminoacids (polyglutamic acid and polyaspartic acid), and their combinations and/or derivatives [202]. Some of these carriers also comprise bioadhesive-targeting entities, such as lectins [118,119], heparin-targeting ligands (biotin-phosphatidylethanolamine) [120], cell-penetrating peptides [114] or vitamin B12 [66,73]. In addition, PEG, poloxamers or protease inhibitors have also been included in the nanocompositions as protective agents against insulin degradation [121]. In the following section, the most promising nanocarrier compositions will be presented in more detail. In addition to this information, Table 2 also describes a summary of oral insulin formulations currently in clinical trials, highlighting the progress in the way of translation of current oral technologies into clinical development.

Since the first reports of Damgé and Couvreur [46], the use of acrylic polymers for drug nanocarrier preparation has widely extended, evolving towards a series of other promising systems such as poly-acrylate nanoparticles (e.g., poly[isobutyl-acrylate], poly[isobutyl-cyanoacrylate] and poly[ethyl-cyanoacrylate]; Table 1) and ‘smart’ polymethacrylate–PEG complexation hydrogels/nanosystems. In the latter case, the carboxylic groups of the methacrylate component enhance the penetration capacity of the system by promoting the opening of tight junctions, while the presence of the PEG chains promote mucosal adhesion and improves protection against protein degradation. Similar systems have also been developed by combining methacrylic copolymers with chitosan [122]. The pH-sensitive character of these systems allows
enhanced protection of insulin at the acidic pH of the stomach, while at higher pH it promotes interaction and transport through the intestinal mucosa, thereby increasing the bioavailability of the associated peptide.

Acrylic polymers have also been used in combination with other biomaterials for insulin delivery. For example, Damgé et al. reported a blend nanoparticle composition of the non-biodegradable polycationic acrylic polymer Eudragit® (RS) with the biodegradable poly(e-caprolactone), which achieved over 13% relative bioavailability [40,41]. These particles also showed improved glycemic response to an oral glucose challenge for prolonged periods. The bio adhesive character of the cationic acrylic component was identified as main contributor to the success of the carriers. The highly efficient interaction of these insulin-loaded carriers with the intestinal mucosa is shown in Figure 2, in comparison to insulin administered alone. The same authors have also shown, in another interesting study, that it is possible to modulate and improve the efficacy of nanocarriers by modifying the configuration of the encapsulated insulin molecule [40,41].

In addition to polycaprolactone, other polyesters such as PLA and PLGA have also been widely explored for oral insulin delivery (Table 1). In general, the major drawback of these materials is their hydrophobic nature, which makes the efficient entrapment of such a hydrophilic macromolecule difficult. In order to overcome these difficulties, several blend and copolymer combinations have been proposed such as the co-encapsulation of phospholipids or the use of chemically conjugated cyclodextrin–PLGA, diblock and triblock PLGA–polyoxyethylenes [35,123,124].

Within this regard, it is also very important to mention the early work of Carino et al. [34] describing nanoparticles made of PLGA and poly(fumaric-co-sebacic anhydride) (poly[FA:SA]), which has had a great impact in the transmucosal drug-delivery field. These nanosystems have achieved 11% pharmacological bioavailability (calculated from the serum glucose levels following the oral administration of the carriers). The authors of this work identified the polyanhydride component as a critical factor providing bioadhesive properties to the delivery system. However, the carriers elaborated solely of poly[FA:SA] resulted much less efficient than the blend system which combined poly[FA:SA] with PLGA. In fact, despite these very promising early results, polyanhydride-based nanocarriers have not fulfilled further expectations regarding their application for oral peptide delivery.

Table 2. Summary of oral insulin formulations currently in clinical trials.

<table>
<thead>
<tr>
<th>Company</th>
<th>Name</th>
<th>Composition</th>
<th>Clinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biocon</td>
<td>IN-105</td>
<td>Insulin–PEG (tablet)</td>
<td>Phase III</td>
</tr>
<tr>
<td>Oramed Pharm.</td>
<td>ORMD-0801</td>
<td>Enteric coating and absorption enhancers</td>
<td>Phase IIb – completed (2008)</td>
</tr>
<tr>
<td>Diabetology</td>
<td>Capsulin</td>
<td>Enteric coating and absorption enhancers</td>
<td>Phase II</td>
</tr>
<tr>
<td>Diasome Pharmaceuticals</td>
<td>HDV-I</td>
<td>Liposomes with hepatic targeting</td>
<td>Phase II–III</td>
</tr>
<tr>
<td>Apollo Pharmaceuticals</td>
<td>Oradel</td>
<td>Carbohydrate – B12 nanoparticles</td>
<td>Phase I – competed (2007)</td>
</tr>
<tr>
<td>NodPharmaceuticals</td>
<td>Nodlin</td>
<td>Bioadhesive – enteric coated nanoparticles</td>
<td>Phase I</td>
</tr>
<tr>
<td>Oshadi drug administration</td>
<td>Oshadi oral insulin</td>
<td>Silica nanoparticles in enteric capsules</td>
<td>Phase I – completed (2011)</td>
</tr>
<tr>
<td>National Institute of Diabetes and Digestive and Kidney Diseases</td>
<td>4 CNAB – insulin</td>
<td>N-(4-chlorosalicyloyl)-4-aminobutyrate</td>
<td>Phase II–III</td>
</tr>
</tbody>
</table>

PEG: Polyethylene glycol.

Figure 2. Fluorescence microscopy images of rat intestinal epithelium following intra-ileal administration. (A) Fluorescein isothiocyanate-labeled insulin alone and (B) encapsulated into polymeric nanoparticles. Reprinted with permission from [41] © Elsevier (2007).
The use of polysaccharides represents another important strategy for oral insulin delivery. In fact, chitosan and its derivatives have been one of the most investigated biomaterials in oral delivery. This cationic polysaccharide is mucoadhesive, has pH-sensitive properties and penetration enhancer capacity by opening epithelial tight junctions. Nevertheless, as indicated by comparing the in vivo performance of chitosan in solution and as nanoparticles, these attractive properties are not resulting in efficient peptide delivery unless formulated into a nanostructured delivery system [125]. Most of the chitosan-based nanocarriers are prepared by the ionic gelation technique, which allows highly efficient entrapment of hydrophilic macromolecules. This mild and easy technology, first described by Calvo et al. in 1997, has been attracting great interest over the last few years [61]. Currently, there are more than 100 reports related to the development of such ionically crosslinked nanosystems composed of chitosan and/or other polysaccharides for oral delivery of peptides [126–128]. Most promising recent developments include: composite chitosan–glucomannan, chitosan–dextran and chitosan–alginate nanoparticles [60,80,87] and those based on novel chitosan derivatives such as thiolated chitosans [69–71,129–131] or lauryl succinyl chitosan [132].

The combination of chitosan with polyaminoacids has also been explored recently for oral insulin delivery [81,85]. Within this regard, the recent work of Sonaje et al. [81–83,133] is particularly interesting. Using the ionic gelation technique, these authors have prepared a nanosystem based on chitosan-polyglutamic acid, for which a 16% relative oral bioavailability was obtained. This percentage could be further enhanced by the incorporation of freeze–dried nanocarriers in Eudragit L enteric capsules, thereby avoiding premature insulin release and degradation in the stomach. The use of such capsules did not alter the original characteristics of the incorporated carriers and resulted in relative bioavailabilities over 20% (Figure 3).

Another different nanocarrier type is based on a complex multilayer composition of different polysaccharides (chitosan, alginate and dextrane sulfate) with an additional albumin coating as a ‘sacrificial target’ for preventing insulin degradation. This composition has achieved pharmacological bioavailabilities in the range of 11–13% [76]. The mechanism of action of this formulation has been studied by detailed in vitro and ex vivo studies in the presence/absence of mucus. The increase of uptake for encapsulated insulin was significantly higher for mucus secreting co-cultures and excised mucosa than for single Caco-2 cell monolayers (3.7-fold and 3.9-fold increase, respectively, vs 2.1-fold for single Caco-2 cell monolayers), highlighting the importance of interactions between the carrier and the mucosa [134].
Future perspective & conclusion

Overall, there is no doubt that the advances in this field have been significant; however, there remains skepticism of whether these approaches will finally result in a marketed oral insulin product. In fact, despite the number of formulations in clinical trials (Table 2), there has also been a significant number of drop-outs. Failure has been associated with high intra-individual variation in insulin absorption at the moderate oral bioavailability levels detected, as well as perceived toxicology issues. This evidence, along with the discovery of new anti-obesity, antidiabetes peptides, is currently moving research towards the development of new nanotherapeutic products with a higher chance of success than those attempted to date.

As a consequence of the significant efforts oriented to the design of nanostructures for overcoming critical barriers associated to the oral route of administration, it has become clear that there are a number of interconnected features that need to be taken into account in the design of new nanocarriers:

- Particle size distribution and, thereby, the specific surface area, is a critical parameter in terms of the capacity of nanocarriers to interact with the intestinal mucosa. It is suggested that the upper size limit for this functional ability depends on the nanocarrier composition and shape;

- The stability of the nanostructures in the biological fluids is another determinant factor as it affects size distribution after in vivo administration as well as the premature delivery and/or degradation of the associated active compound;

- Surface chemical composition and, thus, the lipophilicity, fluidity and surface charge may influence the stability of the nanocarrier in the biological environment and also its ability to interact with and be transported across the barrier;

- The internal chemical composition, together with hydrophilicity, rigidity and porosity may influence the stability and delivery rate of the associated peptides;

- The targeting or affinity of the nanocarriers for specific apical membrane receptors may be critical for overcoming biological barriers;

It should be kept in mind that these five parameters together may have an impact on the biocompatibility, biodegradability and potential immune responses of the nanostructures.

It has also become clear that there is still a need to generate and integrate more knowledge for the proper design of oral nanocarriers. In particular, it would be critical to understand in detail the interaction of nanomaterials with the intestinal mucosa, at the cell and tissue levels, as well as their biodistribution, kinetics and toxicological–immunological responses in order to advance towards making oral peptide delivery a reality.

Taken as a whole, research and development of peptide-based therapeutics is a dynamic field of research, with increasing numbers of candidates entering clinical studies in a wide variety of therapeutic categories. There is no doubt that pharmaceutical and biotechnology industries will continue to focus on these versatile molecules in association with the development of new formulation and delivery technologies.

Financial & competing interests disclosure

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