Journal of Diabetes Science and Technology
Volume 7, Issue 2, March 2013

REVIEW ARTICLE

Oral Insulin Delivery: How Far Are We?

Pedro Fonte, Ms.C.,[1,2] Francisca Araújo, Ms.C.,[1] Salette Reis, Ph.D.,[2] and Bruno Sarmento, Ph.D.[1,3,4]

Abstract

Oral delivery of insulin may significantly improve the quality of life of diabetes patients who routinely receive insulin by the subcutaneous route. In fact, compared with this administration route, oral delivery of insulin in diabetes treatment offers many advantages: higher patient compliance, rapid hepatic insulinization, and avoidance of peripheral hyperinsulinemia and other adverse effects such as possible hypoglycemia and weight gain. However, the oral delivery of insulin remains a challenge because its oral absorption is limited. The main barriers faced by insulin in the gastrointestinal tract are degradation by proteolytic enzymes and lack of transport across the intestinal epithelium.

Several strategies to deliver insulin orally have been proposed, but without much clinical or commercial success. Protein encapsulation into nanoparticles is regarded as a promising alternative to administer insulin orally because they have the ability to promote insulin paracellular or transcellular transport across the intestinal mucosa. In this review, different delivery systems intended to increase the oral bioavailability of insulin will be discussed, with a special focus on nanoparticulate carrier systems, as well as the efforts that pharmaceutical companies are making to bring to the market the first oral delivery system of insulin. The toxicological and safety data of delivery systems, the clinical value and progress of oral insulin delivery, and the future prospects in this research field will be also scrutinized.

J Diabetes Sci Technol 2013;7(2):520–531

Introduction

Type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are metabolic diseases characterized by a progressive decrease of β-cell function and, when not or inappropriately treated, may lead to severe complications, which are life threatening and very costly to control. Therefore, the maintenance of blood glucose levels at near-normal levels reduces the risk of long-term complications of diabetes, such as adult blindness, cardiovascular diseases,

Author Affiliations: [1]Centro de Investigação em Ciências da Saúde (CICS), Instituto Superior de Ciências da Saúde—Norte, CESPU, Gandra, Portugal; [2]REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, Porto, Portugal; [3]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Porto, Portugal; and [4]INEB—Institute for Biomedical Engineering, University of Porto, Porto, Portugal

Abbreviations: (DPPC) dipalmitoyl phosphatidylcholine, (GIT) gastrointestinal tract, (HPMCP) hydroxypropyl methylcellulose phthalate, (MC) methyl cellulose, (MMA) methyl methacrylate, (PEG) polyethylene glycol, (PLGA) polylactic-co-glycolic acid, (SLN) solid lipid nanoparticles, (T1DM) type 1 diabetes mellitus, (T2DM) type 2 diabetes mellitus, (TPP) tripolyphosphate, (WGA) wheat germ agglutinin

Keywords: clinical trials, diabetes, hypoglycemic effect, insulin, nanoparticles, oral delivery system

Corresponding Author: Bruno Sarmento, Ph.D., Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal; email address *bruno.sarmento@ff.up.pt*

nontraumatic amputation, and diabetic nephropathy.[1] In fact, intensive insulin in T1DM patients is able to reduce the risks of nephropathy by 35% to 56%, neuropathy by 60%, and retinopathy by 50% to 70%.[2–4]

Insulin is usually administered by the subcutaneous route, which significantly reduces morbidity and mortality; however, approximately 60% of patients fail to achieve long-term glycemic control.[5] This may be due to poor patient compliance owing to the use of needles and the complexity of the insulin treatment regimen, the late stage at which insulin may be prescribed, and fear of hypoglycemia episodes and weight gain. To overcome such problems, different routes of insulin administration are being tested. The oral route remains the preferred choice for drug administration because of its noninvasive nature.[6] However, proteins such as insulin have low oral bioavailability due to their intrinsic lack of permeability through the intestinal epithelium. Therefore, to develop a delivery system intended to provide insulin orally, a proper understanding about the mucosal microenvironment and intestinal physiology is required.

During past years, different drug delivery strategies have been introduced to overcome the low oral bioavailability of insulin. In this review, the advantages and drawbacks associated with oral insulin, the delivery strategies used, and possible toxicological issues will be discussed.

Advantages and Drawbacks Associated with Oral Insulin Delivery

The high molecular weight and hydrophilicity of proteins hinder their intestinal absorption, leading to low oral bioavailability, negligible plasma levels, and high variability. Thus, therapeutic proteins are commonly administered by parenteral route, with insulin, for instance, being administered subcutaneously in the treatment of diabetes mellitus.[7] However, oral administration is regarded as a better route of administration because of its cost-effectiveness and well-established acceptability, and especially because it allows avoiding the use of needles and other injection materials. Therefore, several attempts have been made to develop an oral carrier able to deliver insulin in a continuous and efficient manner—and thus preclude contamination risks, local pain, and immune reactions—and overcome patient compliance problems. Furthermore, oral administration of insulin better mimics the normal insulin pathway in the body after endogenous secretion, providing a better glucose homeostasis.[8,9] In fact, oral delivery of insulin allows high portal vein concentrations, with no sustained peripheral hyperinsulinemia, which is associated with neuropathy and retinopathy.[10]

However, oral delivery of insulin has some limitations, which include low bioavailability due to insulin degradation in the gastrointestinal tract (GIT) by proteolytic enzymes and severe pH physiological conditions as well as poor permeability through the intestinal epithelium.[11] Notwithstanding, the oral route is back in the forefront as an alternative route of insulin administration since the pulmonary route has failed to become a real alternative.[12]

Delivery Systems for Oral Insulin Administration

To develop an adequate oral delivery system, the maintenance of insulin biological stability in the GIT and in the enterocytes cytosol must be addressed during the formulation process. Several insulin delivery systems, such as tablets, capsules, intestinal patches, hydrogels, microparticles, and nanoparticles, have been explored to deliver insulin by paracellular and/or transcellular transport throughout the ileum and colon. The delivery systems used may contain excipients, which protect insulin from aggregation and enzymatic degradation, prolong its residence time in the GIT, and enhance its intestinal uptake. **Figure 1** shows a summary of different forms of insulin and functional excipients that are used in oral delivery systems.

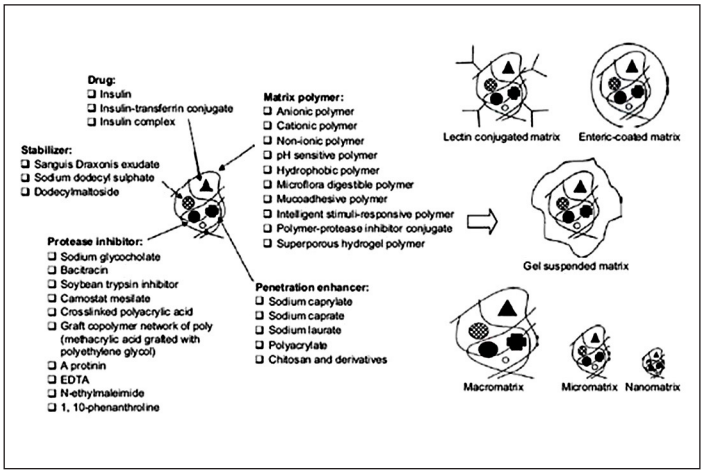

Figure 1. Different types of insulin forms, functional excipients, and delivery systems used in oral insulin dosage forms. Reproduced with permission from *Journal of Drug Targeting*.[13]

In this section, different insulin delivery systems intended for oral administration will be discussed, with a special focus on nanoparticles, which are able to permeate the intestine by different pathways (**Figure 2**).

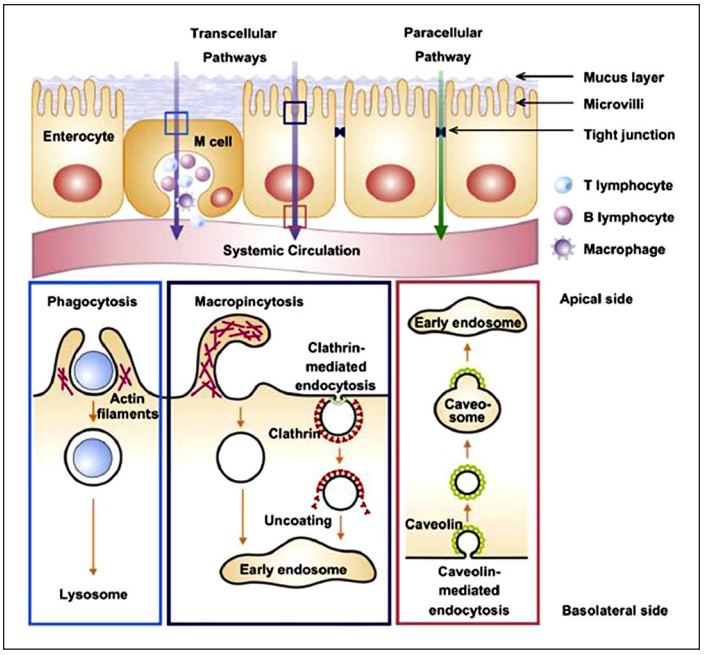

Figure 2. Pathways for insulin nanoparticle translocation through the intestinal epithelium. Schematic focus on phagocytosis, macropinocytosis, and caveolin-mediated endocytosis. Reproduced with permission from *Biomaterials*.[14]

Classical Dosage Forms

Many research groups around the world are trying to develop an oral delivery system mainly in tablet or capsule form mainly because of convenience and higher rates of patient compliance. Studies that are widely known and highly relevant to the academic and industrial fields are discussed herein.

Chitosan-4-thiobutylamidine tablets containing insulin and a Bowman–Birk inhibitor and elastatinal as enzyme inhibitors covalently linked to chitosan were developed.[15] Such covalent attachment concentrates the enzyme inhibitors in the tablets, avoiding its release in the GIT and thus minimizing local and systemic side effects. Furthermore, chitosan with mucus glycoprotein forms a mucoadhesive matrix able to deliver insulin and reduce blood glucose levels significantly in normoglycemic rats over a period of 24 h.

CODES™ is a colon-specific drug delivery system consisting of a core tablet coated with three different polymeric layers.[16] Such tablets containing insulin, lactulose, meglumine, polyethylene oxide, citric acid, and sodium glycocholate were developed.[17] Thus lactulose is used to promote the beginning of drug release in the colon, and meglumine and citric acid are used as a pH adjuster and an insulin solubilizer, respectively, whereas sodium glycocholate is used as an absorption enhancer. Their combination with polyethylene oxide in the tablet core promotes a gel barrier that allows a sustained release of insulin in the colon of dogs.

The anionic polymer Eudragit S1000 is used for intestinal delivery of drugs because of its ability to be insoluble in the stomach's acidic conditions and in an aqueous medium up to pH 6.[11] The hypoglycemic effect of Eudragit S100 enteric-coated capsules loaded with insulin and sodium salicylate as absorption enhancer formulated as a physical mixture or in polyethylene glycol (PEG) 4000 or Witepsol W35 bases was studied in hyperglycemic beagle dogs.[18] It was found that the best formulation was insulin formulated in Witepsol W35 (1 g) with sodium salicylate (50 mg) loaded in hard gelatin capsules coated with Eudragit S100. Such a system was able to reduce plasma glucose levels in approximately 25–30% and achieve a relative hypoglycemia of approximately 12.5% compared with subcutaneous insulin. Intestinal patches may also be used to deliver insulin orally, and they may be produced in a form of 1–4 mm radii discs of 400 μm in thickness and made of insulin, Carbopol 934, sodium carboxymethyl cellulose and supported by ethyl cellulose on one side of the disc. Further, the discs may be enteric-coated or filled into enteric-coated capsules to avoid the release of insulin in the stomach.[19,20]

Pharmaceutical companies are also trying to develop an adequate system to deliver insulin orally. **Table 1** summarizes such efforts, showing the technology used and the current status of development.

Diabetology, a biopharmaceutical company, compared the pharmacokinetic and pharmacodynamic properties of Actrapid®, a subcutaneous regular human insulin, and Capsulin™.[32] In a phase 2 study, it was found that the administration of Actrapid or Capsulin (150 and 300 U) was able to increase the glucose infusion rates, reaching maximum values at approximately 280–330 min. The maximum glucose infusion rate values were higher for Actrapid than either dose of Capsulin. No differences between 150 and 300 U of Capsulin were observed, and after its administration, a significant

Table 1.
Oral Insulin Delivery Systems Undergoing Clinical Trials

Product name	Company	Technology	Status	Reference
Capsulin	Diabetology (Jersey, UK)	Axcess™; enteric-coated capsule filled with a mixture of insulin, an absorption enhancer, and a solubilizer	Phase IIa in T1DM and phase II in T2DM completed; agreement with USV Limited (Mumbai India) to complete the development and commercialize for the Indian market	21
ORMD-0801	Oramed (Jerusalem, Israel)	Enteric-coated capsule containing insulin and adjuvants to protect the protein and promote its intestinal uptake	Phase IIa in T1DM and phase IIb in T2DM	22
ORA2	BOWS Pharmaceuticals AG (Zug, Switzerland)	Capsule containing insulin in dextran matrix	Phase II in T2DM; agreement with Orin Pharmaceuticals AG (Stockholm, Sweden) for the development	23
—	Emisphere Technologies (Cedar Knolls, NJ)	Eligen®; capsule containing insulin and an absorption enhancer that facilitates the passive transcellular transport	Phase II in T2DM suspended	24
NN1952	Novo Nordisk (Bagsvaerd, Denmark)	GIPET® from Merrion Pharmaceuticals (Dublin, Ireland); capsule or tablet containing absorption enhancers that activate micelle formation, facilitating transport of insulin	Cancelled after phase II	25,26
NN1953; NN1954	Novo Nordisk (Bagsvaerd, Denmark)	Tablet of long-acting insulin analog	Phase I in T1DM and T2DM	25
IN-105	Biocon (Bangalore, India)	Insulin modified with a small PEG	Phase II; searching for other company to pursue development	27
HDV-I	Diasome (Conshohocken, PA)	Liposomal insulin, which is hepatic-directed vesicles–insulin, HDV-I, in orally administered forms	Phase III	28,29
—	Biolaxy (Shanghai, China)	NOD Technology; insulin-loaded bioadhesive nanoparticles	Phase I	30
—	Access Pharmaceuticals (Dallas, TX)	CobaCyte™; nanoparticle or polymer containing insulin, coated with vitamin B_{12} for targeted delivery	Phase I	31

hypoglycemic effect over 6 h was verified. In a phase 2a study, using male volunteers with T1DM, Capsulin (150 and 300 U) appeared to be safe and well tolerated and was able to produce consistent increases in blood insulin levels within 30–120 minutes after dosing, and to control blood glucose levels for an extended time period. It was also found that both increases in insulin levels and control of glucose levels were dose dependent.[21]

Oramed performed a clinical study using 29 T2DM patients; placebo and Oramed's insulin-loaded capsules (8 mg/capsule, two capsules/day) were administered to 8 and 21 patients, respectively, who were monitored during 6 weeks.[33] A good safety and tolerability of ORMD-0801 was found, and it demonstrated a relevant clinical impact. However, such positive results should be further confirmed in a larger population. In another study, two capsules of ORMD-0801 (8 mg insulin each) were administered to fasting T1DM patients, and blood samples were collected after 6 h.[34] ORMD-0801 was demonstrated to be safe, since it was cleared within 300 min, and proved to be biologically active upon oral, preprandial administration and was also able to prevent the expected rise of glucose levels in fasting T1DM patients upon insulin withdrawal. In another study, 10 healthy male fasting subjects were administered one capsule of F130 or F130GT, which are two ORMD-0801 variants just differing in their emulsifier content.[35] It was found that, besides F130- and F130GT-induced similar plasma C-peptide patterns, which decrease during the 5 h monitoring session, the mean C_{min} registered after administration of F130 was lower than after F130GT administration (0.12 versus 0.2 ng/ml, respectively). Furthermore, F130 decreased glucose significantly compared with F130GT, with a two-fold increase in the mean area above the curve (768.3 versus 1626.4 mg/dl/min, respectively). No adverse effects during the study were verified, and the emulsifiers used and excipient ratios were shown to preserve insulin bioactivity.

Overall, despite the positive results achieved so far, further work is needed to develop an optimal formulation with proven advantages over the subcutaneous delivery route because no oral delivery system developed, thus far, has demonstrated a clear clinical advantage.

Lipid-Based Insulin Nanoparticles

Concerning lipid-based insulin nanoparticles, the focus goes to solid lipid nanoparticles (SLN) and liposomes, which are the most commonly used systems.

Solid lipid nanoparticles have been developed as an alternative carrier system to polymeric nanoparticles since the 1990s[36,37] because of their good tolerability, biodegradation, possibility of large-scale production,[37] and their possible composition of physiological lipids, which minimizes the risk of acute and chronic toxicity.[38] In fact, SLN in contact with gastrointestinal fluids can protect insulin from degradation and improve its absorption.[39] It was reported that the glucose levels of rats after oral administration of insulin-loaded SLN were lower than those obtained after administration of oral insulin solution and empty SLN.[40] In another study, both lectin-modified SLN and wheat germ agglutinin (WGA)-modified SLN were shown to protect insulin from digestive enzymes degradation *in vitro*, and the latter showed a better stabilizing effect.[41] After oral administration of insulin-loaded SLN or WGA-modified SLN to rats, the relative pharmacological bioavailabilities were 4.46% and 6.08%, and the relative bioavailabilities were 4.99% and 7.11%, respectively, compared with insulin subcutaneous injection.

The main advantage of liposomes as a carrier is that they are composed of physiological materials, e.g., phospholipids. Since Patel and Ryman in 1976,[42] many studies reported that insulin-loaded liposomes showed a significant hypoglycemic effect.[43–45] In those studies, it was found that liposome composition is crucial for both insulin hypoglycemic effect via oral route and *in vivo* liposome stability. Only a little effect was observed when insulin-loaded liposomes composed of only dicetyl phosphate, cholesterol, or egg lecithin were administered to diabetic animals.[46] Therefore, bioadhesive dosage forms may be used to overcome this low bioavailability. A chitosan-coated liposome was prepared, and its properties were evaluated *in vitro* and *in vivo*.[47,48] It was demonstrated that an improved absorption of insulin can be obtained using chitosan-coated liposomes.[49] An identical technique was successfully applied to the preparation of Carbopol-coated liposomes.[50] In another study, insulin liposomal formulations were prepared using insulin or protamine-containing insulin with cholesterol, dipalmitoyl phosphatidylcholine (DPPC; egg)–cholesterol mixture and mucoadhesive agent methyl cellulose (MC)-added DPPC–cholesterol mixture.[51] These formulations showed reduced blood glucose levels when orally administered to mice and rats. In fact, the phospholipid composition (DPPC, cholesterol, and MC mixture) was found to be quite effective in promoting insulin uptake and reducing blood glucose levels. Sometimes, the effect of liposomes on drug absorption is decreased due to its degradation in the GIT because of interaction with bile salts. To avoid this, liposomes coated with PEG 2000 or the sugar chain of mucin were useful because of their ability to became resistant to bile salts digestion.[44] Particularly, the oral administration of insulin-loaded liposomes coated with PEG 2000 showed an enhanced and sustained hypoglycemic effect in rats, compared with normal liposomes.

Polymeric-Based Insulin Nanoparticles

A previous work showed a sustained reduction of glycemia in diabetic rats treated with insulin-loaded nanoparticles for 1 to 3 weeks.[52] Until now, different studies have been carried out developing polymeric-based nanoparticles for insulin oral delivery. The polymers used may differ from their origin if they are natural or synthetic.

Chitosan has been the natural polymer most used to develop nanoparticles, because it adheres to the mucus layer and transiently opens the tight junctions between the intestinal epithelial cells.[53,54] In one study, insulin-loaded chitosan nanoparticles were obtained by ionotropic gelation of chitosan with tripolyphosphate (TPP) anions.[55] These nanoparticles were orally administered (21 IU/kg) to diabetic rats, resulting in a hypoglycemic effect prolonged over 15 h and a pharmacological bioavailability of approximately 15% compared with subcutaneous insulin. Chitosan has also been used as hydrophilic polymeric coating, increasing insulin transport through the intestinal membrane.[56,57] Nanoparticles made by ionic cross linking of chitosan with hydroxypropyl methylcellulose phthalate (HPMCP) showed better stability under simulated acidic conditions. Furthermore, the oral administration of chitosan/

HPMCP nanoparticles in rats increased the hypoglycemic effect of insulin by more than 9.8 compared with an oral insulin solution, and such effect was even 2.8-fold higher compared with insulin-loaded chitosan/TPP nanoparticles.[58]

N-trimethyl chitosan (a partially quaternized chitosan derivative), N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride, and chitosan-graft methyl methacrylate (MMA) monomers have replaced chitosan in the encapsulation of insulin because they provide greater stability through enhanced electrostatic interactions while maintaining the mucoadhesive and permeation-enhancing properties.[59–61] It was reported that insulin-loaded chitosan, N-triethyl chitosan and N,N-dimethyl N-ethyl chitosan nanoparticles used in *ex vivo* studies on excised rat colon showed that quaternized derivatives of chitosan had better permeation-enhancing properties than chitosan.[62] In one study, nanoparticles made of lauryl succinyl chitosan were developed, and it was found that the presence of succinyl carboxyl groups had inhibitory effects on *in vitro* release of insulin at pH 1.2.[63] Such nanoparticles, when administered to diabetic rats, were also able to reduce blood glucose levels for approximately 6 h.

Alginate has also been applied for oral insulin delivery.[56,64] Insulin encapsulated into alginate nanoparticles reduced the basal serum glucose levels by 40% after oral administration to diabetic rats.[56] In another study, insulin complexed with cationic β-cyclodextrin polymers in alginate/chitosan nanoparticles showed the ability of cationic β-cyclodextrin polymers to protect insulin from degradation under simulated gastrointestinal conditions and to have controlled-release properties.[65]

Nanoparticles formulated with dextran and coated with chitosan showed a sustainable-release profile and significantly improved the hypoglycemic effect of insulin after administration to diabetic animals.[66] Encapsulation of insulin in vitamin B12-coated dextran nanoparticles has been considered in complementing diabetes therapy[67,68] by taking advantage of enhanced insulin absorption through vitamin B12 intrinsic factor receptor ligand-mediated endocytosis via intestine ileocytes.[68]

The introduction of synthetic polymers in insulin delivery was based on the advantage of sustained release over a period of days to several weeks compared with natural polymers.[69] Polylactic acid, polylactic-co-glycolic acid (PLGA), and poly(ε-caprolactone) polymers have attracted significant interest[70–72] because of their biodegradability and biocompatibility; however, encapsulation of insulin into hydrophobic polyester nanoparticles should be carried out using the water-in-oil-in-water double emulsion method.[73] In addition, possible modifications of PLGA nanoparticles have been proposed, which include a blended matrix formed by the PLGA copolymer and polyoxyethylene derivatives (PLGA:poloxamer and PLGA:poloxamine compositions),[74] PLGA mannosamine,[75] and PLGA and HPMCP.[76] The latter is a pH-sensitive cellulose coating in which insulin-loaded nanoparticles have shown to significantly reduce the serum glucose level over 24 h in diabetic rats, compared with insulin-loaded PLGA nanoparticles.[76] In a previous work, sodium oleate was complexed with insulin to improve its liposolubility and encapsulated into PLGA nanoparticles. Such nanoparticles were administered to diabetic rats (20 IU/kg) and were able to reduce plasma glucose level in approximately 23.8% 12 h after oral administration, prolonging such hypoglycemic effect until 24 h.[77] In another study, PLGA and PLGA-Hp55 nanoparticles showed an initial *in vitro* insulin release of approximately 50.5% and 19.8%, respectively.[76] The nanoparticles were further administered orally to diabetic rats, and the relative bioavailability of PLGA and PLGA-Hp55 nanoparticles compared with subcutaneous administration was approximately 3.7% and 6.3%, respectively.

Nanoparticles formulated with polylactic acid, ethylene oxide, and propylene oxide triblock demonstrated potential capacity in delivering insulin orally, decreasing blood glucose concentration in diabetic animals and maintaining a significant hypoglycemic effect for 24 h.[78] Insulin encapsulated into nanoparticles prepared with poly(ε-caprolactone) and Eudragit decreased fasted glycemia in a dose-dependent manner, mainly due to the mucoadhesive properties of Eudragit.[79]

Polyacrylic acid–cysteine conjugate polyvinyl pyrrolidone nanoparticles encapsulating insulin showed stability in a gastric environment and significantly reduced blood glucose levels.[80] It seems that the mucoadhesive properties of polyacrylic acid–cysteine plays an essential role in insulin uptake.

Polyalkylcyanoacrylates have also been used in insulin nanoparticles because of their excellent nontoxical adhesive properties, resulting from the strong bonds with the mucosa.[81] It was reported that insulin-loaded polybutylcyanoacrylate nanoparticles showed better insulin protection from degradation by proteolytic enzymes compared with the same nanoparticles in aqueous medium.[82] Furthermore, the apparent bioavailability of nanoparticle oral administration in rats (50 IU/kg) in the oily medium versus subcutaneous administration of insulin (2 IU/kg) was 22.4%, which was much higher than the 15.5% obtained by the nanoparticles in aqueous medium. Methacrylic acid grafted with PEG and acrylic acid grafted with PEG nanoparticles significantly reduced serum glucose levels.[83] Polymethacrylic acid–chitosan–polyether (PEG-polypropylene glycol copolymer) nanoparticles containing a hydroxypropyl β cyclodextrin–insulin complex were found to have good mucoadhesive properties in excised rat intestinal mucosa, being suggested as a good carrier to deliver insulin orally.[84]

Functionalized Insulin Nanoparticles

The particle size and surface properties are important parameters for insulin nanoparticle uptake and translocation through the intestinal mucosa.[71,85] In addition, targeting strategies to improve the interaction of insulin nanoparticles with the intestinal absorptive cells and M cells of Peyer's patches have been designed. The modification of surface properties or coupling of a targeting moiety at the surface of nanoparticles are examples of used strategies.[86]

One strategy consists of coating nanoparticles with a hydrophilic stabilizing agent or incorporating it in the nanoparticle structure or using bioadhesive polymers such as chitosan and poly(methacrylic acid) or surfactant molecules.[86] Fonte and coauthors[87] reported that insulin-loaded chitosan-coated SLN nanoparticles were able to better improve insulin permeation through Caco-2 cell monolayer and Caco-2/HT-29 coculture monolayer models. Such nanoparticles were able to further produce a prolonged hypoglycemic effect up to 24 h in diabetic rats. Functionalized graft copolymer nanoparticles consisting of chitosan and the monomer MMA, N-dimethylaminoethyl methacrylate hydrochloride, and N-trimethylaminoethyl methacrylate chloride loaded with insulin showed an encapsulation efficiency of approximately 100% and an initial burst release followed by sustained release for more than 24 h.[60] Functionalizing nanoparticles by changing the zeta potential influences the interactions of insulin nanoparticles with the intestinal mucosa, specifically with the negatively charged mucin.[85,88] Calcium phosphate nanoparticles coated with PEG are able to protect insulin from the gastric environment and preserve insulin structure.[89] Insulin-loaded nanoparticles composed by alginate and dextran sulfate nucleating around calcium and binding to poloxamer, stabilized by chitosan, and coated with albumin were able to maintain insulin bioactivity and enhance pharmacological bioavailability by shielding insulin from degradation, thus facilitating its permeation.[90] These nanoparticles were administered to diabetic rats and reduced the plasma glucose levels to 40% of the basal values, with a sustained hypoglycemic effect over 24 h. Furthermore, a dose of 50 IU/kg showed an oral bioavailability (13%) three-fold higher than orally administered insulin. Chitosan-coated PLGA nanoparticles may also be used to take advantage of chitosan mucoadhesive properties.[91]

Another strategy is grafting a ligand at the nanoparticle surface for specifically targeting nanoparticles to receptors on enterocytes or M cells.[86] For instance, lectins are involved in many cell recognition and adhesion processes that significantly increase nanoparticle transport through the intestine.[92] Both lectin-modified SLN and WGA–N-glutaryl-phosphatidylethanolamine-modified SLN containing insulin, when administered orally to rats, were able to improve insulin bioavailability and protect insulin from *in vitro* degradation enzymes.[41] Covalent coupling of vitamin B12 to dextran nanoparticles has been used as a strategy to increase insulin oral bioavailability,[67] and the transferrin molecule is another example of a targeting molecule.[92] The potential of vitamin B12 on nanoparticle transport enhancement through the intestinal epithelium has been demonstrated.[68] Vitamin B12 bound to dextran nanoparticles administered to diabetic rats showed a blood glucose reduction of approximately 70–75%, prolonging such effect for approximately 54 h. Insulin-loaded chitosan-6-mercaptonicotinic acid nanoparticles were orally administered to rats and the area under the curve of insulin after its administration was four times higher than unmodified chitosan nanoparticles.[93] Mannosylated nanoparticles may be also a promising delivery system to consider.[94]

Toxicological Issues of Insulin Delivery Systems

Toxicological issues must be focused on the binomial insulin carrier. Toxicity is a critical factor to be considered when evaluating the potential of insulin-loaded nanoparticles. Given that nanoparticles are engineered to interact with

cells, it is important to ensure that they do not cause any adverse effects or even damage the intestinal epithelium. The important issue is that, whether naked or coated, nanoparticles will undergo biodegradation in the cellular environment and may affect cellular responses.[95] For instance, biodegraded nanoparticles can accumulate inside the cells and lead to intracellular changes, such as disruption of organelle integrity or gene alterations, which cause severe toxicity. The cytotoxicity test is a sensitive, rapid, and inexpensive method to detect the potential ability of nanoparticles to induce sublethal or lethal effects in cells. However, cytotoxicity may not be the only adverse effect because cell immunological response may also be affected.[95] Furthermore, molecules delivered to unnatural sites in unnatural quantities are likely to behave in unexpected ways, so from a toxicological perspective, oral insulin delivery may be questionable. If insulin is entrapped and not released from carrier systems until it reaches the systemic circulation, then this may not be an issue, but this approach is questionable, because insulin may cause gastroparesis.[96] The use of absorption enhancers may lead to a long-term toxicity, and surfactants can also damage intestinal epithelium. Indeed, absorption enhancers, when administered in a continuous manner, may also promote permeation of pathogens and toxins.[97] Protease inhibitors also have some safety concerns due to a possible interference with the digestion of dietary proteins. Moreover, mucoadhesive systems may affect mucus turnover and consequently alter the physiology of intestinal membrane.[98]

Toxic effects of insulin nanoparticles of alginate–dextran complexed with chitosan–PEG–albumin were evaluated for 15 days after oral administration to diabetic rats.[99] The rats showed lower levels of alanine transaminase, aspartate aminotransaminase, and alkaline phosphatase, suggesting the absence of liver damage or biliary obstruction. In a similar study, insulin-loaded nanoparticles administered to diabetic rats did not change the liver and kidney functions.[100] Furthermore, the increase in some hepatic parameters was attributed to chemical inducement of diabetes and its physiopathology and not to nanoparticles. Concerning kidney function, creatinine and urea nitrogen values were also similar to normal rats, with the exception of glycosuria, again attributed to the chemical inducement of diabetes because nondosed diabetic rats showed the same alteration. The glycosuria levels of dosed animals were lower than control diabetic rats, which indicate an effective hypoglycemic response.

In another study, the safety of self-assembled nanoparticles, obtained mixing a poly-γ-glutamic acid solution with chitosan in presence of $MgSO_4$ and sodium TPP, after oral administration in a diabetic rat model were assessed by an *in vivo* toxicity study.[101] These nanoparticles were well tolerated, even at a dose 18 times higher than used in the pharmacodynamic/pharmacokinetic study. Another biocompatibility assessment of N-trimethyl chitosan–cysteine nanoparticles when administered to rats revealed lack of toxicity.[102] Moreover, the cytotoxicity of PLGA nanoparticles was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and it was proven that no extra toxicity was introduced by nanoparticles.[103]

Conclusions and Future Prospects

Oral insulin delivery has been an interesting and promising research field that promises to revolutionize the way diabetes mellitus is treated. Several studies have achieved some positive results, and some delivery systems are in advanced stages of development. However, despite all efforts made since the 1980s, there appears to be no progress because the delivery systems developed have not shown a clear clinical advantage over the subcutaneous insulin route. Several concerns need to be properly addressed. Long-term efficacy and safety need to be demonstrated through adequately powered studies in different patient populations across the diabetes spectrum. Furthermore, a reproducible absorption of the drug and understanding of meal-related absorption are also important goals for developing a drug that needs to be administered lifelong. In addition, clinical studies need to clearly demonstrate superiority over parenteric insulin formulations and oral hypoglycemic agents, including improved hypoglycemic profile, reduced weight gain, and better disease progression outcome in long-term studies. The toxicological profile of the developed delivery systems must be also properly assessed.

Overall, the success of oral insulin depends on the ability to manufacture insulin both in sufficient quantities for oral delivery as well as efficiently in a cost-conscious pharmaceutical marketplace. Pharmaceutical companies are on the frontline in developing a system to deliver insulin orally; however, most of them abandon their delivery systems at

the first stages of development. It would be very interesting if they would clearly disclose what went wrong in those studies.

It is clear that further work needs to be done to bring the first insulin oral delivery system to the market. Addressing these issues successfully will create a new paradigm in diabetes treatment.

Funding:

Pedro Fonte and Bruno Sarmento acknowledge financial support from Fundação para a Ciência e a Tecnologia, Portugal (grants SFRH/BD/78127/2011 and SFRH/BPD/35996/2007).

References:

1. Meetoo D, McGovern P, Safadi R. An epidemiological overview of diabetes across the world. Br J Nurs. 2007;16(16):1002–7.
2. Reichard P, Nilsson BY, Rosenqvist U. The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus. N Engl J Med. 1993;329(5):304–9.
3. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977–86.
4. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. JAMA. 2002;287(19):2563–9.
5. Saaddine JB, Cadwell B, Gregg EW, Engelgau MM, Vinicor F, Imperatore G, Narayan KM. Improvements in diabetes processes of care and intermediate outcomes: United States, 1988-2002. Ann Intern Med. 2006;144(7):465–74.
6. Heinemann L, Jacques Y. Oral insulin and buccal insulin: a critical reappraisal. J Diabetes Sci Technol. 2009;3(3):568–84.
7. Beals JM, Kovach P. Crommelin DJ, Sindelar RD. Insulin. In: Pharmaceutical biotechnology. Netherlands: Harwood Academic Publishers; 1997, 229–39.
8. Hoffman A, Ziv E. Pharmacokinetic considerations of new insulin formulations and routes of administration. Clin Pharmacokinet. 1997;33(4):285–301.
9. Owens DR. New horizons--alternative routes for insulin therapy. Nat Rev Drug Discov. 2002;1(7):529–40.
10. Khafagy el-S, Morishita M, Onuki Y, Takayama K. Current challenges in non-invasive insulin delivery systems: a comparative review. Adv Drug Deliv Rev. 2007;59(15):1521–46.
11. Carino GP, Mathiowitz E. Oral insulin delivery. Adv Drug Deliv Rev. 1999;35(2-3):249–57.
12. Heinemann L. New ways of insulin delivery. Int J Clin Pract Suppl. 2011;(170):31–46.
13. Wong TW. Design of oral insulin delivery systems. J Drug Target. 2010;18(2):79–92.
14. Chen MC, Sonaje K, Chen KJ, Sung HW. A review of the prospects for polymeric nanoparticle platforms in oral insulin delivery. Biomaterials. 2011;32(36):9826–38.
15. Krauland AH, Guggi D, Bernkop-Schnurch A. Oral insulin delivery: the potential of thiolated chitosan-insulin tablets on non-diabetic rats. J Control Release. 2004;95(3):547–55.
16. Li J, Yang L, Ferguson SM, Hudson TJ, Watanabe S, Katsuma M, Fix JA. *In vitro* evaluation of dissolution behavior for a colon-specific drug delivery system (CODES) in multi-pH media using United States Pharmacopeia apparatus II and III. AAPS PharmSciTech. 2002;3(4):E33.
17. Katsuma M, Watanabe S, Kawai H, Takemura S, Sako K. Effects of absorption promoters on insulin absorption through colon-targeted delivery. Int J Pharm. 2006;307(2):156–62.
18. Hosny EA, Al-Shora HI, Elmazar MM. Oral delivery of insulin from enteric-coated capsules containing sodium salicylate: effect on relative hypoglycemia of diabetic beagle dogs. Int J Pharm. 2002;237(1-2):71–6.
19. Whitehead K, Shen Z, Mitragotri S. Oral delivery of macromolecules using intestinal patches: applications for insulin delivery. J Control Release. 2004;98(1):37–45.
20. Grabovac V, Foger F, Bernkop-Schnurch A. Design and *in vivo* evaluation of a patch delivery system for insulin based on thiolated polymers. Int J Pharm. 2008;348(1-2):169–74.
21. Diabetology. http://www.diabetology.co.uk/. Accessed January 21, 2012.
22. Oramed Pharmaceuticals. http://www.oramed.com/. Accessed January 22, 2012.
23. BOWS Pharmaceuticals AG. http://www.bowspharma.com/. Accessed January 22, 2012.
24. Emisphere Technologies. http://www.emisphere.com/. Accessed January 22, 2012.
25. Novo Nordisk. http://www.novonordisk.com/. Accessed January 22, 2012.

26. Merrion Pharmaceuticals. http://www.merrionpharma.com/. Accessed January 21, 2012.
27. Biocon Biopharmaceuticals. http://www.biocon.com/. Accessed January 21, 2012.
28. Diasome Pharmaceuticals. http://www.diasome.com/. Accessed January 21, 2012.
29. Geho WB, Geho HC, Lau JR, Gana TJ. Hepatic-directed vesicle insulin: a review of formulation development and preclinical evaluation. J Diabetes Sci Technol. 2009;3(6):1451–9.
30. Biolaxy. http://www.biolaxy.com/. Accessed January 21, 2012.
31. Access Pharmaceuticals. http://www.accesspharma.com. Accessed January 22, 2012.
32. Luzio SD, Dunseath G, Lockett A, Broke-Smith TP, New RR, Owens DR. The glucose lowering effect of an oral insulin (Capsulin) during an isoglycaemic clamp study in persons with type 2 diabetes. Diabetes Obes Metab. 2010;12(1):82–7.
33. Kidron M, Eldor R, Miteva Y, Freier R, Arbit E. Extended exposure to an oral insulin formulation yields decreased insulin secretion in type II diabetes subjects. Presented at the Diabetes Technology Meeting, Bethesda, MD, November 11–13, 2010.
34. Eldor R, Arbit E, Miteva Y, Freier R, Kidron M. Oral insulin: type I diabetes (T1DM) patient response upon preprandial administration. Presented at the 70th American Diabetes Association Scientific Sessions, Orlando, FL, June 25–29, 2010.
35. Eldor R, Arbit E, Miteva Y, Kidron M. Evaluation of the safety and efficacy of two oral insulin formulations in healthy volunteers. Presented at the Diabetes Technology Meeting, San Francisco, CA, October 2011.
36. Yang S, Zhu J, Lu Y, Liang B, Yang C. Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharm Res. 1999;16(5):751–7.
37. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. Eur J Pharm Biopharm. 2000;50(1):161–77.
38. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001;47(2-3):165–96.
39. García-Fuentes M, Torres D, Alonso MJ. Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. Coll Surf B Biointerf. 2002;27:159–68.
40. Sarmento B, Martins S, Ferreira D, Souto EB. Oral insulin delivery by means of solid lipid nanoparticles. Int J Nanomedicine. 2007;2(4):743–9.
41. Zhang N, Ping Q, Huang G, Xu W, Cheng Y, Han X. Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. Int J Pharm. 2006;327(1-2):153–9.
42. Patel HM, Ryman BE. Oral administration of insulin by encapsulation within liposomes. FEBS Lett. 1976;62(1):60–3.
43. Axt J, Sarrach D, Zipper J. [Biopharmaceutical studies on phospholipid liposomes as carriers for the oral administration of insulin]. Pharmazie. 1983;38(4):246–8.
44. Iwanaga K, Ono S, Narioka K, Morimoto K, Kakemi M, Yamashita S, Nango M, Oku N. Oral delivery of insulin by using surface coating liposomes: improvement of stability of insulin in GI tract. Int J Pharm. 1997;157(1):73–80.
45. Kisel MA, Kulik LN, Tsybovsky IS, Vlasov AP, Vorob'yov MS, Kholodova EA, Zabarovskaya ZV. Liposomes with phosphatidylethanol as a carrier for oral delivery of insulin: studies in the rat. Int J Pharm. 2001;216(1-2):105–14.
46. Das N, Basu MK, Das MK. Oral application of insulin encapsulated liposomes. Biochem Int. 1988;16(6):983–9.
47. Takeuchi H, Yamamoto H, Niwa T, Hino T, Kawashima Y. Mucoadhesion of polymer-coated liposomes to rat intestine *in vitro*. Chem Pharm Bull (Tokyo). 1994;42(9):1954–6.
48. Takeuchi H, Yamamoto H, Niwa T, Hino T, Kawashima Y. Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. Pharm Res. 1996;13(6):896–901.
49. Takeuchi H, Yamamoto H, Kawashima Y. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv Drug Deliv Rev. 2001;47(1):39–54.
50. Takeuchi H, Matsui Y, Yamamoto H, Kawashima Y. Mucoadhesive properties of carbopol or chitosan-coated liposomes and their effectiveness in the oral administration of calcitonin to rats. J Control Release. 2003;86(2-3):235–42.
51. Değim IT, Gümüşel B, Değim Z, Ozçelikay T, Tay A, Güner S. Oral administration of liposomal insulin. J Nanosci Nanotechnol. 2006;6(9-10):2945–9.
52. Damge C, Michel C, Aprahamian M, Couvreur P. New approach for oral administration of insulin with polyalkylcyanoacrylate nanocapsules as drug carrier. Diabetes. 1988;37(2):246–51.
53. Pan Y, Zheng JM, Zhao HY, Li YJ, Xu H, Wei G. Relationship between drug effects and particle size of insulin-loaded bioadhesive microspheres. Acta Pharmacol Sin. 2002;23(11):1051–6.
54. Janes KA, Calvo P, Alonso MJ. Polysaccharide colloidal particles as delivery systems for macromolecules. Adv Drug Deliv Rev. 2001;47(1):83–97.
55. Pan Y, Li YJ, Zhao HY, Zheng JM, Xu H, Wei G, Hao JS, Cui FD. Bioadhesive polysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin *in vivo*. Int J Pharm. 2002;249(1-2):139–47.
56. Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D. Alginate/chitosan nanoparticles are effective for oral insulin delivery. Pharm Res. 2007;24(12):2198–206.
57. Lin YH, Mi FL, Chen CT, Chang WC, Peng SF, Liang HF, Sung HW. Preparation and characterization of nanoparticles shelled with chitosan for oral insulin delivery. Biomacromolecules. 2007;8(1):146–52.

58. Makhlof A, Tozuka Y, Takeuchi H. Design and evaluation of novel pH-sensitive chitosan nanoparticles for oral insulin delivery. Eur J Pharm Sci. 2011;42(5):445–51.
59. Van der Merwe SM, Verhoef JC, Verheijden JH, Kotzé AF, Junginger HE. Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. Eur J Pharm Biopharm. 2004;58(2):225–35.
60. Qian F, Cui F, Ding J, Tang C, Yin C. Chitosan graft copolymer nanoparticles for oral protein drug delivery: preparation and characterization. Biomacromolecules. 2006;7(10):2722–7.
61. Kotze AF, Luessen HL, de Leeuw BJ, de Boer BG, Verhoef JC, Junginger HE. N-trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: *in vitro* evaluation in intestinal epithelial cells (Caco-2). Pharm Res. 1997;14(9):1197–202.
62. Bayat A, Dorkoosh FA, Dehpour AR, Moezi L, Larijani B, Junginger HE, Rafiee-Tehrani M. Nanoparticles of quaternized chitosan derivatives as a carrier for colon delivery of insulin: *ex vivo* and *in vivo* studies. Int J Pharm. 2008;356(1-2):259–66.
63. Rekha MR, Sharma CP. Synthesis and evaluation of lauryl succinyl chitosan particles towards oral insulin delivery and absorption. J Control Release. 2009;135(2):144–51.
64. Reis CP, Ribeiro AJ, Houng S, Veiga F, Neufeld RJ. Nanoparticulate delivery system for insulin: design, characterization and *in vitro/in vivo* bioactivity. Eur J Pharm Sci. 2007;30(5):392–7.
65. Zhang N, Li J, Jiang W, Ren C, Li J, Xin J, Li K. Effective protection and controlled release of insulin by cationic beta-cyclodextrin polymers from alginate/chitosan nanoparticles. Int J Pharm. 2010;393(1-2):212–8.
66. Sarmento B, Ribeiro A, Veiga F, Ferreira D, Neufeld R. Oral bioavailability of insulin contained in polysaccharide nanoparticles. Biomacromolecules. 2007;8(10):3054–60.
67. Chalasani KB, Russell-Jones GJ, Yandrapu SK, Diwan PV, Jain SK. A novel vitamin B12-nanosphere conjugate carrier system for peroral delivery of insulin. J Control Release. 2007;117(3):421–9.
68. Chalasani KB, Russell-Jones GJ, Jain AK, Diwan PV, Jain SK. Effective oral delivery of insulin in animal models using vitamin B12-coated dextran nanoparticles. J Control Release. 2007;122(2):141–50.
69. Sarmento B, Ferreira D, Vasconcelos T. Polymer-based delivery systems for oral delivery of peptides and proteins. In: Jorgensen L, Nielsen HM, eds. Delivery technologies for biopharmaceuticals: peptides, proteins, nucleic acids and vaccines. Chichester: John Wiley & Sons; 2009, 207–26.
70. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release. 2001;70(1-2):1–20.
71. Des Rieux A, Fievez V, Garinot M, Schneider YJ, Preat V. Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J Control Release. 2006;116(1):1–27.
72. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Deliv Rev. 2003;55(3):329–47.
73. Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM. Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. J Control Release. 2008;125(3):193–209.
74. Csaba N, Gonzalez L, Sanchez A, Alonso MJ. Design and characterisation of new nanoparticulate polymer blends for drug delivery. J Biomater Sci Polym Ed. 2004;15(9):1137–51.
75. Alonso-Sande M, Delgado A, Evora C, Zoniou M, Remuñán-López C, Alonso MJ. PLGA-mannosamine nanoparticles as new carriers for oral immunization. 2nd NanoSpain Workshop, Barcelona, Spain, March 14–17, 2005.
76. Cui FD, Tao AJ, Cun DM, Zhang LQ, Shi K. Preparation of insulin loaded PLGA-Hp55 nanoparticles for oral delivery. J Pharm Sci. 2007;96(2):421–7.
77. Sun S, Liang N, Piao H, Yamamoto H, Kawashima Y, Cui F. Insulin-S.O (sodium oleate) complex-loaded PLGA nanoparticles: formulation, characterization and *in vivo* evaluation. J Microencapsul. 2010;27(6):471–8.
78. Xiong XY, Li YP, Li ZL, Zhou CL, Tam KC, Liu ZY, Xie GX. Vesicles from Pluronic/poly(lactic acid) block copolymers as new carriers for oral insulin delivery. J Control Release. 2007;120(1-2):11–7.
79. Damge C, Maincent P, Ubrich N. Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats. J Control Release. 2007;117(2):163–70.
80. Deutel B, Greindl M, Thaurer M, Bernkop-Schnurch A. Novel insulin thiomer nanoparticles: *in vivo* evaluation of an oral drug delivery system. Biomacromolecules. 2008;9(1):278–85.
81. Vauthier C, Dubernet C, Fattal E, Pinto-Alphandary H, Couvreur P. Poly(alkylcyanoacrylates) as biodegradable materials for biomedical applications. Adv Drug Deliv Rev. 2003;55(4):519–48.
82. Hou ZQ, Zhang ZX, Xu ZH, Zhang H, Tong ZF, Leng YS. The stability of insulin-loaded polybutylcyanoacrylate nanoparticles in an oily medium and the hypoglycemic effect in diabetic rats. Yao Xue Xue Bao. 2005;40(1):57–64.
83. Foss AC, Goto T, Morishita M, Peppas NA. Development of acrylic-based copolymers for oral insulin delivery. Eur J Pharm Biopharm. 2004;57(2):163–9.
84. Sajeesh S, Sharma CP. Cyclodextrin-insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery. Int J Pharm. 2006;325(1-2):147–54.
85. Jung T, Kamm W, Breitenbach A, Kaiserling E, Xiao JX, Kissel T. Biodegradable nanoparticles for oral delivery of peptides: is there a role for polymers to affect mucosal uptake? Eur J Pharm Biopharm. 2000;50(1):147–60.
86. Damge C, Reis CP, Maincent P. Nanoparticle strategies for the oral delivery of insulin. Expert Opin Drug Deliv. 2008;5(1):45–68.

87. Fonte P, Nogueira T, Gehm C, Ferreira D, Sarmento B. Chitosan-coated solid lipid nanoparticles enhance the oral absorption of insulin. Drug Deliv Transl Res. 2011;1:299–308.

88. Attivi D, Wehrle P, Ubrich N, Damge C, Hoffman M, Maincent P. Formulation of insulin-loaded polymeric nanoparticles using response surface methodology. Drug Dev Ind Pharm. 2005;31(2):179–89.

89. Ramachandran R, Paul W, Sharma CP. Synthesis and characterization of PEGylated calcium phosphate nanoparticles for oral insulin delivery. J Biomed Mater Res B Appl Biomater. 2009;88(1):41–8.

90. Woitiski CB, Neufeld RJ, Veiga F, Carvalho RA, Figueiredo IV. Pharmacological effect of orally delivered insulin facilitated by multilayered stable nanoparticles. Eur J Pharm Sci. 2010;41(3-4):556–63.

91. Zhang X, Sun M, Zheng A, Cao D, Bi Y, Sun J. Preparation and characterization of insulin-loaded bioadhesive PLGA nanoparticles for oral administration. Eur J Pharm Sci. 2012;45(5):632–8.

92. Peppas NA, Kavimandan NJ. Nanoscale analysis of protein and peptide absorption: Insulin absorption using complexation and pH-sensitive hydrogels as delivery vehicles. Eur J Pharm Sci. 2006;29(3-4):183–97.

93. Millotti G, Perera G, Vigl C, Pickl K, Sinner FM, Bernkop-Schnurch A. The use of chitosan-6-mercaptonicotinic acid nanoparticles for oral peptide drug delivery. Drug Deliv. 2011;18(3):190–7.

94. Salman HH, Gamazo C, Campanero MA, Irache JM. Bioadhesive mannosylated nanoparticles for oral drug delivery. J Nanosci Nanotechnol. 2006;6(9-10):3203–9.

95. Hossain S, Chowdhury EH, Akaike T. Nanoparticles and toxicity in therapeutic delivery: the ongoing debate. Ther Deliv. 2011;2(2):125–32.

96. Florence AT. Issues in oral nanoparticle drug carrier uptake and targeting. J Drug Target. 2004;12(2):65–70.

97. Goldberg M, Gomez-Orellana I. Challenges for the oral delivery of macromolecules. Nat Rev Drug Discov. 2003;2(4):289–95.

98. Gowthamarajan K, Kulkarni G. Oral insulin: fact or fiction? Possibilities of achieving oral delivery for insulin. Resonance. 2003;8(5):38–46.

99. Reis CP, Veiga F, Nunes P, Soares AF, Laranjeira T, Carvalho R, Jones J, Figueiredo IV, Ribeiro AJ, Cabrita AM. Toxicological *in vivo* studies of an oral insulin nanosystem. Toxicol Lett 2007; 172 Suppl 1: S90.

100. Reis CP, Nunes P, Soares AF, Figueiredo IV, Cabrita AM, Silva CF, Ribeiro AJ, Neufeld R, Damgé C, Veiga F. Toxicological assessment of orally delivered nanoparticulate insulin. Nanotoxicology. 2008;2(4):205–17.

101. Sonaje K, Lin YH, Juang JH, Wey SP, Chen CT, Sung HW. *In vivo* evaluation of safety and efficacy of self-assembled nanoparticles for oral insulin delivery. Biomaterials. 2009;30(12):2329–39.

102. Yin L, Ding J, He C, Cui L, Tang C, Yin C. Drug permeability and mucoadhesion properties of thiolated trimethyl chitosan nanoparticles in oral insulin delivery. Biomaterials. 2009;30(29):5691–700.

103. Zheng AP, Cao DY, Bi YQ, Cui X, Zhang XY, Sun JX. Evaluation of bioadhesion and toxicity of chitosan-coated insulin-loaded poly(lactide-co-glycolide) nanoparticles. Chin Pharm J. 2010;45:1005–10.