Expert Opinion

Developments in polymeric devices for oral insulin delivery

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Background: Development of improved oral insulin administration is necessary for the treatment of diabetes mellitus, to overcome the problem of daily subcutaneous injections. The vast amount of literature data on oral insulin delivery prompted us to cover this area in a review. *Objective*: Insulin delivery using polymeric devices is discussed, with an ultimate aim of addressing the technological development in this area. *Methods*: The development of oral delivery devices for insulin using hydrogels and micro/nanoparticles is discussed with reference to polymers. These efforts must be directed to increase the residence time of insulin near the intestinal absorptive cells. *Results/conclusion*: The published results on oral insulin delivery devices, particularly on inter-polymer complexes of the grafted copolymers, are discussed in greater depth. The use of absorption enhancers like cyclodextrins, bile salts and surfactants is covered. The state-of-the-art technology and challenges in this area are discussed, with typical examples.

Keywords: absorption, enzymatic degradation, hydrogels, microparticles, nanoparticles, oral insulin delivery

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1. Introduction

Insulin is necessary for normal carbohydrate, protein and fat metabolism. Pancreatic beta cells secrete insulin, which allows the glucose to be absorbed by cells all over the body, primarily in muscle and fat tissue [1,2]. The principal regulator of blood glucose concentration is insulin, a hormone synthesized by the β islet cells of the pancreas and secreted in response to elevated blood glucose levels. People with type 1 diabetes mellitus do not produce enough insulin to sustain life and become dependent on exogenous insulin for survival. In contrast, people with type 2 diabetes are not dependent on exogenous insulin for survival. Over time, many of these individuals will show decreased insulin production, thereby requiring supplemental insulin for adequate blood glucose control, especially during times of stress or illness. Insulin is normally obtained either from pork pancreas, or prepared by recombinant DNA technology identical to human insulin, or by chemical modification of pork insulin. Insulin analogs are developed by modifying the amino acid sequence of insulin. Insulin in rapid-, short-, intermediate- and long-acting types are injected separately or in mixture. Rapid-acting insulin analogs (insulin lispro and insulin aspart) are available, while other analogs are under development. Regular insulin is short-acting. Intermediate-acting insulins include lente and NPH. Ultralente and insulin glargine are long-acting insulins.

According to the International Diabetes Federation, the global prevalence of diabetes is estimated at 200 million, which is about 5% of the world's adult population. According to the World Health Organization (WHO), this number

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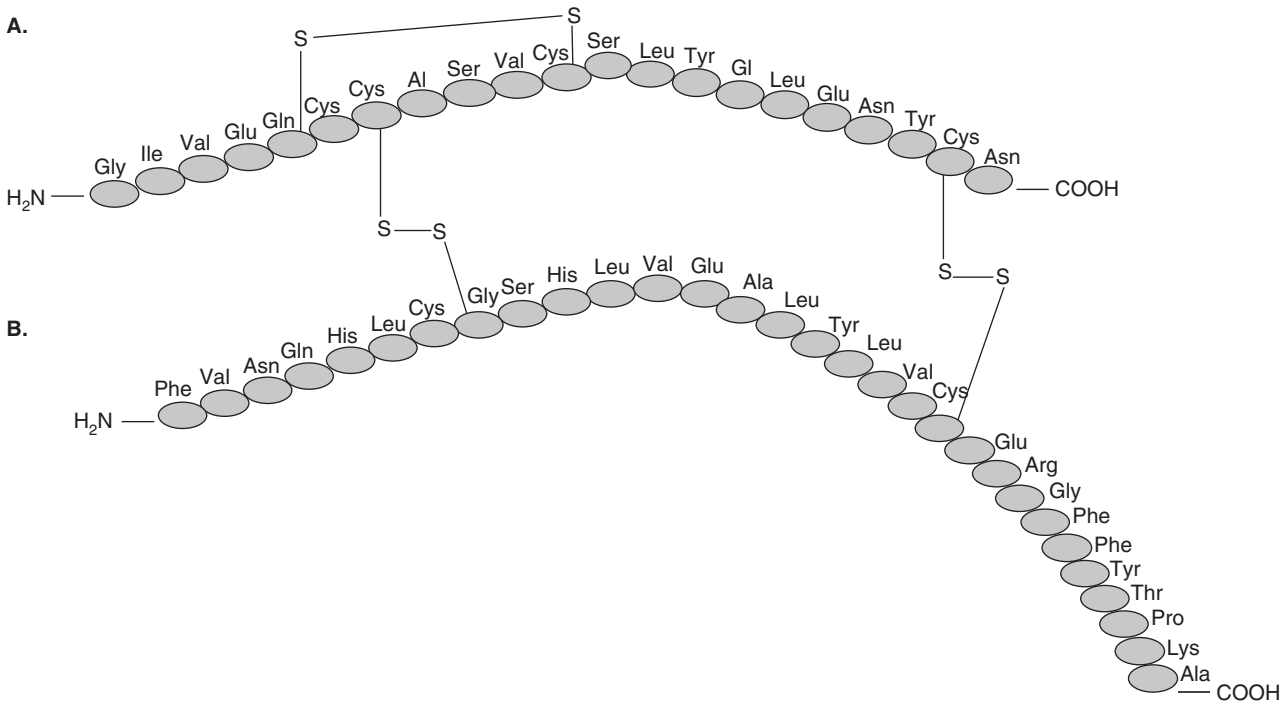

Figure 1. The structure of insulin: chains A and B are linked by two disulfide bonds.

may exceed 370 million by 2030. Approximately 20 – 30% of all diabetic patients take daily insulin injections to maintain their glucose levels. For all type 1 diabetic patients and many type 2 diabetic patients, the time course of insulin action requires three or more injections per day to meet glycemic levels. Thus type 1 patients require regular insulin to survive, while type 2 patients can manage with diet and exercise along with oral medication to control blood glucose levels. Only when diet and oral hyperglycemic agents fail to provide satisfactory control must type 2 patients take insulin injections. Recently, the trend is increasing to treat type 2 patients with oral insulin to prevent pancreatic failure and to avoid potential complications resulting from hyperglycemia.

Therapeutic strategies for treating diabetes have been under active investigation for over a decade and investigations into oral delivery devices are being explored intensively. Novel drug delivery strategies have increased the bioavailability of orally administered insulin. Recent trends for optimizing oral insulin delivery devices include pH-sensitive hydrogels, micro and nanoparticles prepared from biodegradable polymers, where the insulin payload is encapsulated within the cargo system. Specifically engineered delivery polymers that transport across biological barriers, including the gastrointestinal tract (GIT), are required. This review will address the challenges and significant advances made over the past decade on the carrier systems used in oral insulin delivery.

2. The chemistry of insulin

Insulin, a polypeptide hormone (see **Figure 1**), is formed after the elimination of C peptide by hydrolysis. The two chains A (21 amino acids) and B (30 amino acids) are connected by two disulfide bridges; an additional disulfide is formed within A chain. It is secreted by β cells of the islets of Langerhans of the pancreas, exerts hypoglycemic action and belongs to the group of peptides called IGF (insulin-like growth factors) or somatomedins.

Based on the onset and duration of action, many companies have adopted different names for insulin: short-, intermediate-, or long-acting types of insulin or combinations thereof. Human insulins have a more rapid onset and shorter duration of activity than pork insulins. The common insulin formulations available in the market today are given in **Table 1**.

Insulin glargine, sold under the name Lantus, is a long-acting basal insulin analogue, usually given once or twice daily to help control the blood sugar level of diabetic patients. Its advantage is that it has a 24 h duration of action, with a 'peakless' profile. Insulins, glargine and detemir, have several advantages over the NPH insulin [3]. After careful consideration, Eli Lilly has decided to stop producing Humulin N and hence the use of these intermediate-acting insulins has declined by more than 70% over the past years due to the development of newer insulin therapies for diabetic patients. It may be noted that

Table 1. The common insulin formulations available in the market.

Type	Brand name	Company	Onset of action (min)	Peak of action (min)	Duration of action (h)
Rapid-acting	Humalog	Eli Lilly	15	30 – 90	3 – 5
	Novolog	Novo Nordisk	15	40 – 50	
Short-acting (regular)	Humulin R	Eli Lilly	30 – 60	50 – 120	5 – 8
	Novolin R	Novo Nordisk	30 – 60	50 – 120	5 – 8
Intermediate-acting (NPH)	Humulin N	Eli Lilly	60 – 180	480	20
	Novolin N	Novo Nordisk	60 – 180	480	20
	Humulin L	Eli Lilly	60 – 150	420 – 900	18 – 24
	Novolin L	Novo Nordisk	60 – 150	420 – 900	18 – 24
Long-acting	UltraLente	Eli Lilly	240 – 480	480 – 720	36
	Lantus	Aventis	60	–	24

ULTRALENTE® and LENTE® human insulin (rDNA origin) zinc suspensions have also been discontinued by Lilly.

3. Development of oral formulation for insulin therapy

Oral insulin delivery has many potential advantages over other forms of insulin delivery, since it most closely mimics the natural insulin secretion pathway from pancreas to liver. By delivering insulin to the liver as the oral route does, the main organ of glucose regulation is solicited, and insulin side effects in the peripheral circulation (from other routes of administration) are avoided.

Recently, with advances in recombinant biotechnology, a large number of bioengineered products, including peptides and proteins, have become available. Insulin is normally administered parenterally due to its inherent instability in the GIT and its low permeability across biological membranes, due to their high molecular weight and hydrophilic nature. Several strategies have been adopted to achieve oral delivery of insulin, including co-administration with absorption enhancers, enzyme inhibitors, chemical modification, polymeric carriers and lipid-based carriers such as liposomes. These approaches are hampered with drawbacks like low bioavailability, irritation of the intestinal mucosal membrane and impairment of the membrane barrier. Therefore, the development of effective and reliable oral delivery systems for insulin that would circumvent all the above-mentioned hurdles is required.

There are other reasons why oral delivery of insulin is of prime importance. For example, oral insulin mimics the natural insulin release from the pancreas (first to the portal vein, then to the liver, followed by the peripheral circulation). Other routes of administration result in peripheral hyperinsulinemia, which predisposes to hypoglycemia and is thought to be linked to weight gain or other metabolic abnormalities, which in turn will lead to micro- and macro-vascular diseases. The liver has been shown to be more clinically significant than muscle and fat to control the postprandial glucose and maintain normal glycemic index. However, the critical issue is that insulin requirements vary from minute to minute in most patients, causing changes in the rates of absorption and leading to hypo- and hyperglycemia. Insulin is quite different from the average orally administered drug and hence it is not sufficient simply to achieve bioavailability over the course of hours, but other issues like the effects of food on absorption and gastric emptying are equally important.

The conventional treatment of advanced diabetes typically involves taking regular injections of insulin, but the inconvenience of this has led to the development of alternative routes of insulin delivery [4]. For such developments to succeed, oral insulin delivery systems must meet at least two requirements. First, the delivery system should protect insulin from enzymatic degradation; and second, the system should increase insulin permeability within the intestinal membrane. The fragile nature and short biological half-life of insulin poses additional obstacles in developing successful oral dosage formulations. One formulation that satisfies these requirements is the encapsulation of insulin in a polymeric carrier. Although various polymeric carriers have been developed for insulin release, such systems have not shown sufficient bioavailability when administered orally. Among the class of polymers used in developing successful oral insulin delivery devices, hydrogel networks of acrylic acid-based polymers and natural polymers like chitosan (CS), pectins, sodium alginate (NaAlg), cyclodextrins (CD), etc, are the most widely explored ones. Other systems involving biodegradable lactide-based polymers, liposomes and polymeric micelles are also used. The present review will concentrate primarily on the grafted hydrogel copolymers of poly(methacrylic acid)/poly(ethylene glycol) and the inter-polymer complexes formed in these and other similar systems. We will also briefly address issues concerning the use of microparticles (MPs) and nanoparticles (NPs) of other biopolymers. From a perusal of literature, it is realized that complexation hydrogels are a suitable carrier system for oral insulin delivery, because with insulin and the carrier formulation, they do not possess the unexpected toxicity or safety issues and have a predictable onset of activity.

Table 2. Polymers used in oral insulin delivery.

Polymer type	Source
Polymethacrylic acid-*g*-PEG	[35]
Pluronic/poly(lactic acid)	[37]
Chitosan/poly(methacrylic acid)	[49]
Low mol. wt. PLGA	[52]
Poly (lactic acid)/pluronics	[53]
Cyclodextrins	[53]
Eudragits	[58]
Poly(ε-caprolactone)	[58]

3.1 Oral insulin delivery devices

Conventional methods of insulin encapsulation have met with several limitations such as high shear stress and exposure to organic solvents, in addition to extreme temperatures that degrade the insulin [5]. However, recent research efforts have circumvented some of these problems associated with developing successful formulations. Although conventional biodegradable polymers in the form of MPs and NPs have been used as devices in delivering insulin [6-10], the present research trends are moving more in the direction of utilizing pH-sensitive hydrogels. Several biopolymers have been used for oral insulin delivery [11-14] along with permeation enhancers and protein–ligand conjugates. In addition, liposome-based systems have also been employed [15-19].

Table 2 summarizes some typical polymers used in oral insulin delivery. The low surface area of the hydrogels posed problems of limited permeability and bioavailability of insulin, while NPs do not easily degrade. Liposomes and surface-coated liposomes have good permeation properties, since their bilayer structures are similar to a cell membrane, but their stability in the biological environment is of great concern. Polymeric vesicles with similar basic architectures, that are more stable than liposomes due to their low critical micellar concentration, have also been suggested for oral insulin delivery [20-24]. While the literature on micro or nanoparticles is exhaustive, the importance of complexation hydrogels in oral insulin therapy is discussed here in greater detail.

3.2 pH-sensitive methacrylic/acrylic acid-based polymeric hydrogels

Hydrogels are the cross-linked polymer networks that can be swollen in water, buffer or physiological media that are classified into three general categories based on their physical forms, as depicted in **Figure 2**. These are: i) linear free chains in solution, where the polymer undergoes a reversible collapse after application of external stimulus; ii) covalently cross-linked polymers that are reversible and are either microscopic or macroscopic networks, whose swelling can be externally triggered; and iii) chain adsorbed or surface-grafted, where the polymer reversibly swells or collapses on the surface, converting the interface from hydrophilic to hydrophobic and vice versa when the specific external parameter is modified. Hydrogels in solution can also be conjugated with biomolecules, thus widening their potential applications. Biomacromolecules like proteins and oligopeptides, sugars and polysaccharides, single- and double-stranded oligonucleotides and DNA plasmids, simple lipids and phospholipids, and other recognition ligands as well as synthetic drugs can be conjugated with hydrogels. Earlier reports have described various forms of stimuli-responsive polymers and their potential applications in drug delivery [25,26].

In recent years, there has been considerable research activity into developing hydrogels that are sensitive to the surrounding physiological pH, and these are found to be the ideal systems for site-specific delivery [27-31]. A particularly interesting application is the mucosal delivery of insulin. Bioadhesive poly(acrylates) like carbopol and polycarbophil have the potential to protect insulin from proteolytic degradation by inhibiting proteases from the GIT [32,33]. A major contribution to the development of novel types of hydrogels for use in oral insulin delivery comes from the intense efforts of Peppas *et al.* [27,34]. Their oral insulin delivery devices consist of: i) biodegradable poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactic acid-*co*-glycolic acid) (PLGA), poly[lactic acid-*co*-poly(ethylene glycol)] (PLA-PEG), dextran-PEG; ii) pH-sensitive polymers like poly(acrylic acid) (PAA) and poly(methacrylic acid) (PMAA); and iii) complexing hydrogel graft polymers like P(MAA-*g*-EG), P(PAA-*g*-EG) as well as other biopolymers such as chitosan, cyclodextrin, etc., in various combinations with methacrylic or acrylic-based polymers. Temperature-sensitive polymers like poly(*N*-isopropylacrylamide) (PNIPAAm) are rarely used in these applications.

The pH-sensitive graft copolymers of poly(methacrylic acid) and poly(ethylene glycol), designated as P(MAA-*g*-EG), are the most widely explored systems for oral insulin delivery. These polymers exhibit pH-sensitive swelling due to the reversible formation of inter-polymer complexes stabilized by hydrogen-bonding between ethereal groups of the grafted PEG chains and carboxylic acidic protons of PMAA network [35]. This type of complex formation in insoluble copolymers is very sensitive to the pH of the media as well as copolymer composition and graft chain length. In the acidic environment of the stomach, such hydrogels are in a complexed state, such that insulin cannot readily diffuse through the membrane barrier, due to its small mesh size [28,29]. As the polymer passes through the stomach into the intestine, the pH increases above the transition pH of the hydrogel and the complex immediately dissociates; the network pore size rapidly increase, leading to the release of the insulin.

Figure 3 displays the unique pH-responsive property of P(MAA-*g*-EG) hydrogels in which inter-polymer complexes

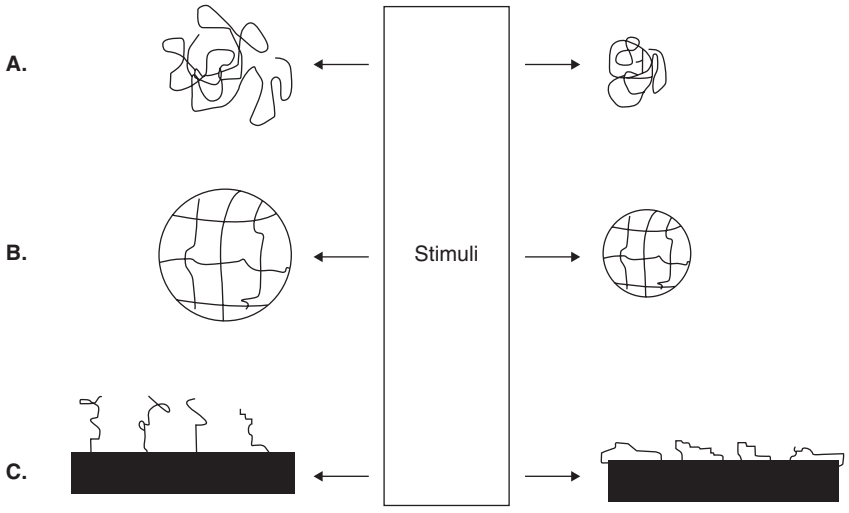

Figure 2. Different physical forms of hydrogels upon application of external stimuli. (**A**) Linear chains in solution where the polymer undergoes reversible collapse; (**B**) covalently cross-linked reversible gels in which swelling or shrinkage occurs; and (**C**) chain adsorbed or surface-grafted forms in which polymer reversibly swells/collapses on the surface.

are formed in acidic media and dissociated in a neutral environment. Such systems containing equimolar amounts of MAA and PEG are reported [27] to be capable of efficient insulin encapsulation up to 90% or more using the equilibrium partitioning (90%). Insulin release can be significantly retarded in acidic media, while rapidly releasing insulin in neutral/basic conditions. In contrast, at a higher amount of MAA of the polymer, the encapsulation efficiency of insulin within the hydrogel would greatly reduce, resulting in the release of insulin from the hydrogel network in acidic and neutral media.

These characteristics mean that hydrogels are promising delivery devices for oral insulin delivery. It has been realized that P(MAA-*g*-EG) hydrogels have the potential to bind calcium [30], thereby affecting the proteolytic activity of calcium-dependent enzymes, such as trypsin. Thus, when insulin is incorporated into P(MAA-*g*-EG) hydrogels of 100 – 150 µm size, they successfully enhanced insulin absorption in both streptozotocin-induced diabetic and non-diabetic rats, achieving 4.2% bioavailability (relative to subcutaneous (s.c.) administration) and giving significant hypoglycemic effects. Thus, one can envisage their carrier ability for insulin via the oral route; additional advantages of these systems are that they would protect insulin from the harsh acidic environment of the stomach before releasing it in small intestine. In the acidic environment of the stomach, these hydrogels do not become swollen due to the formation of intermolecular polymer complexes. The insulin remains in the hydrogel matrix and is well protected from proteolytic degradation. In addition, these systems possess mucoadhesive properties due to the presence of graft PEG chains, which serve as adhesion promoters. The pK_a of PMAA is 4.8; at neutral pH, the MAA groups of the network are almost entirely deprotonated and hydrogen bonds present at low pH dissociate in neutral pH, resulting in the swelling of hydrogel network as indicated in **Figure 3**.

Fluorescein-isothiocyanate labelled dextrans (FITC-Ds) of different molecular weights have been evaluated to establish the relationship between molecular weight and encapsulation efficiency [36]. However, hydrogels are quite effective in encapsulating insulin to protect it from the acidic stomach. The bioavailability data of hydrogels ranged from 4.6 – 7.2% to release insulin rapidly in 1 h of swelling in phosphate buffer media (pH = 7.4) [37], giving a burst effect at the upper small intestine and degradation of insulin before its absorption. To circumvent these problems, three insulin-loaded P(MAA-*g*-EG) systems were developed by Morishita *et al.* [38] in the size ranges of 180 – 230 µm, 43 – 89 µm and < 43 µm. Intra- and inter-cellular integrity and/or damage were examined by lactate dehydrogenase leakage and membrane electrical resistance changes to ensure their safety for the oral route. The smaller size particles (< 43 µm) showed rapid burst release with higher insulin absorption as compared to large size particles (180 – 230 µm), resulting in greater hypoglycemic effects without any detectable mucosal damage. The smaller size particles demonstrated higher mucoadhesive properties to jejunum and ileum than those of bigger size particles. Moreover, smaller size systems enhanced the insulin's mucosal absorption that showed site-specificity, demonstrating the maximum effect at the ileal segment. These results provide clear proof that particle size and delivery site are important in increasing the bioavailability of insulin following oral administration.

Foss *et al.* [39] developed the cross-linked networks of MAA grafted onto PEG (size = 200 nm) by the free radical

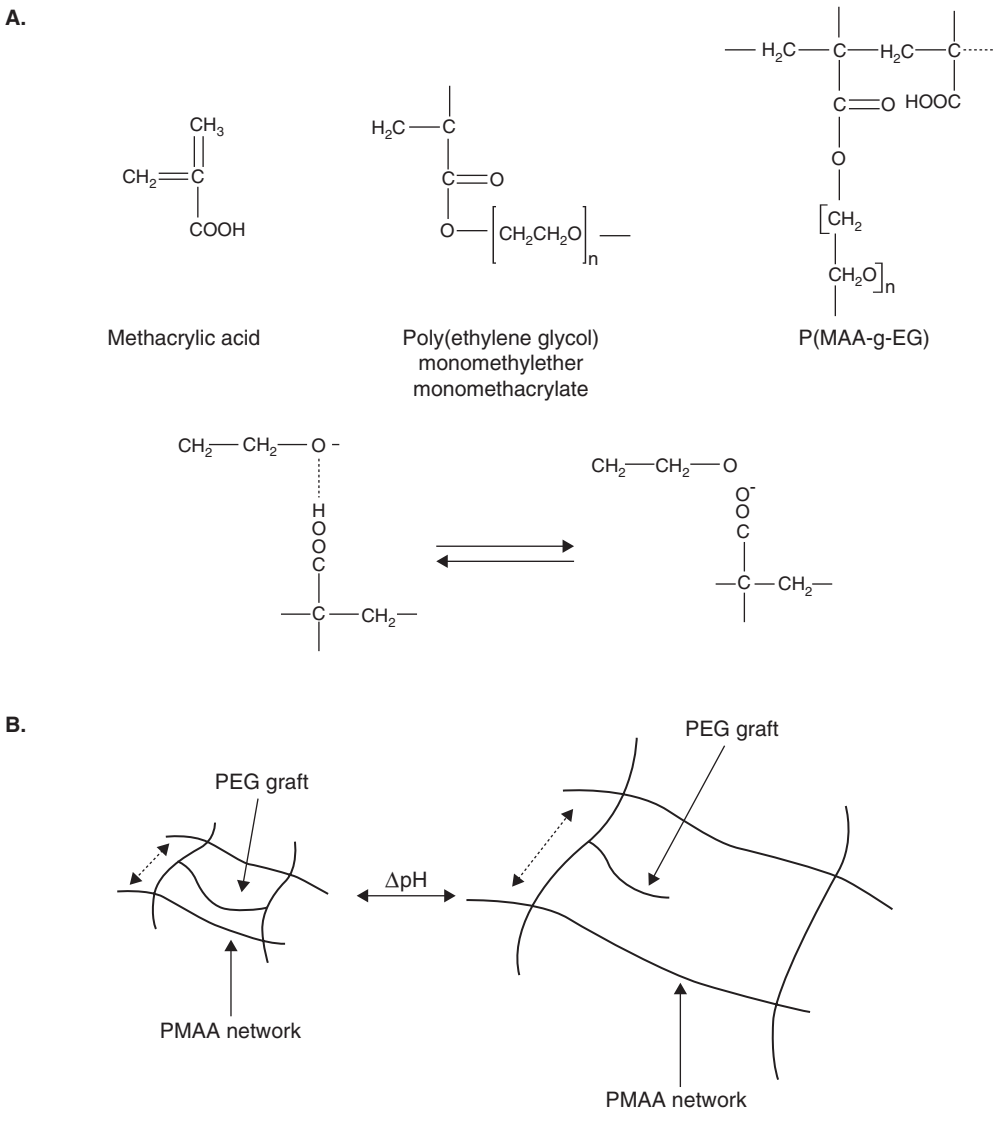

Figure 3. Inter-polymer complexes (A) between protonated pendant acid groups of PMAA and ethereal groups of PEG and (B) pH-dependent network swelling.

precipitation/dispersion method. The size of particles increased dramatically with increasing pH of the medium above the pK_a of the network. For instance, the size ranged from 200 nm at pH 2 to as high as 2 mm at pH 6. Insulin was encapsulated into these copolymers at 9.33 and 9.54 mg/140 mg of solid hydrogel matrix by partitioning from the concentrated insulin solutions. Insulin was encapsulated in acidic pH and was released in neutral pH. Further, *in vivo* experiments were conducted to investigate the ability to influence serum glucose levels in rats. The serum glucose level of diabetic rats was lower than control values for animals that received insulin-loaded polymers, which lasted for 6 h. These systems caused a significant reduction of serum glucose level with respect to control animal, suggesting their efficacy in oral insulin delivery.

3.3 Methacrylic/acrylic acid and chitosan-based hydrogels

Apart from the PAA/PMAA-based graft copolymers of PEG discussed above, systems containing MAA or PAA with other biopolymers have also been explored. One biopolymer that is widely studied is chitosan (CS), which has created great research interest in drug delivery applications due to its nontoxic nature and biocompatibility [40,41]. CS helps the transport of hydrophilic drugs across the intestinal epithelium and also increases the permeability of epithelial tissues by disrupting intercellular tight junctions [42,43]. Polyelectrolyte complexes of CS with anionic polymers like sodium carboxy methylcellulose, sodium alginate, PAA/PMAA, etc., have attracted greater interest in drug delivery applications [44-47], since these devices can be

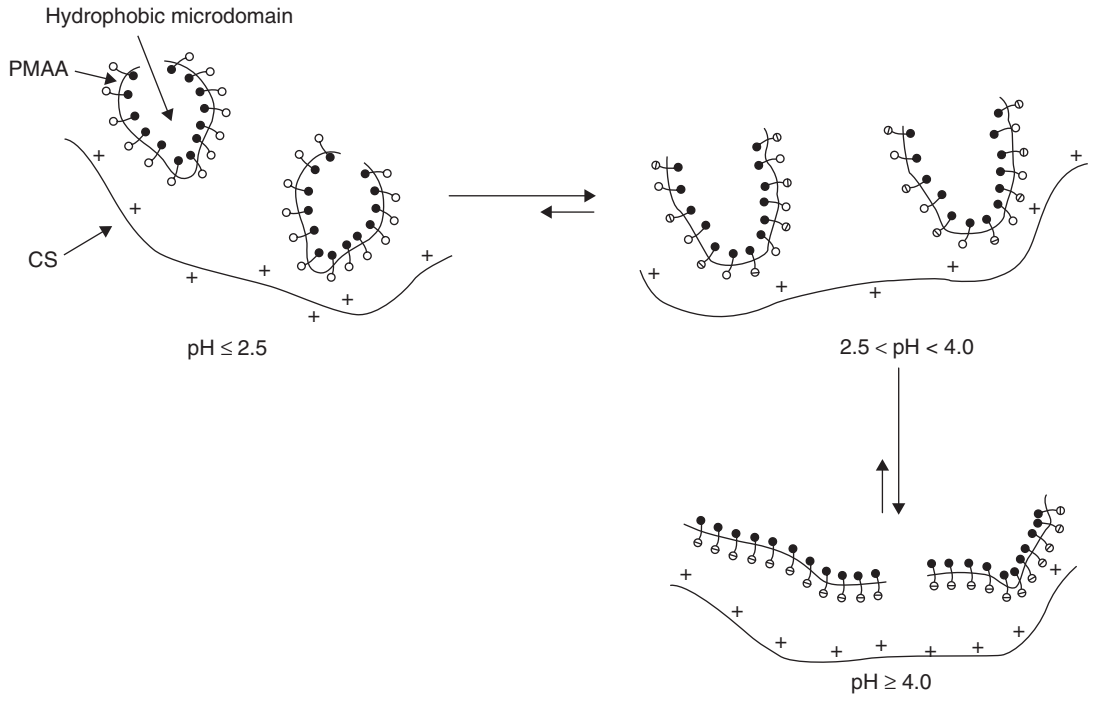

Figure 4. Conformational changes in PMAA chains due to complex formation between CS and PMAA in different pH media. Carboxylate group (⍉), carboxyl group (○) and methyl group (•).

prepared under mild conditions without surfactants, organic solvents or steric stabilizers. MAA can be polymerized in the presence of CS and PEG and the composition of component polymers can be optimized for better encapsulation/release properties. PEG helps to stabilize insulin and also induces the formation of inter-polymer complexes with better insulin retention capability. Insulin-loaded systems are subjected to *in vitro* release at pH 1.2 and 7.4 after loading it by the diffusion filling method. Their trypsin enzyme inhibition capability was studied using casein substrate with carbopol as the standard reference polymer [48]. The pH-sensitive PMMA-CS-PEG systems obtained by free radical polymerization of MAA in the presence of CS and PEG using a water soluble initiator, without organic solvents, surfactants or steric stabilizers, exhibited good encapsulation efficiency and pH-responsive release profiles. The trypsin inhibitory effect of these systems was also studied using casein substrate, wherein the systems exhibited a lesser inhibitory effect than the reference carbopol polymer, suggesting their potential for oral delivery of insulin.

Hydrogels of PMMA-CS [49] were prepared by free radical polymerization using a water soluble initiator and evaluated for oral insulin delivery after loading it by the diffusion filling method. It was observed that insulin-loaded microparticles displayed pH-dependent release profiles at alkaline/acidic pH. Microparticles exhibited sustained release of insulin for 3 – 4 h at neutral pH, and the enzyme-linked immunosorbent assay (ELISA) proved that encapsulated protein maintained 100% biological activity at neutral pH. Preliminary studies suggested that these microparticles could serve as good candidates for the oral insulin delivery. The polymerization techniques used in this study are similar to those used in preparing PAA/PMAA hydrogels, which included suspension, inverse emulsion, reverse emulsion and dispersion/precipitation polymerization processes [50,51] using surfactants or steric stabilizers for stabilizing the resultant particles. Ionic interaction between PMMA and CS has lead to the formation of polyelectrolyte complexes. This method is well suited for the preparation of polyion systems (complex coacervation) that can be used in oral insulin delivery.

Further, the mechanism of formation of inter-polymer complexes between PMAA and CS was investigated by UV and fluorescence measurements and is displayed in **Figure 4**. At higher pH, the majority of -COOH groups of PMAA are dissociated into -COO- and PMAA chains and these exist in an expanded conformation due to stronger Coulombic repulsion between carboxylate groups than the hydrophobic interaction between methyl groups. At lower pH, the reduced Coulombic repulsion leads to the formation of hydrophobic microdomain among the hydrophobic methyl groups. Hydrophilic -COOH and -COO- groups on the PMAA chain are exposed outside the hydrophobic micro-domain such that the PMAA chain would exist stably as a special conformation of a micellar-like structure. The $-NH_2$ groups of the CS chain is protonated fully at pH ≤ 6.2. The CS chain exists stably in an expanded conformation due to

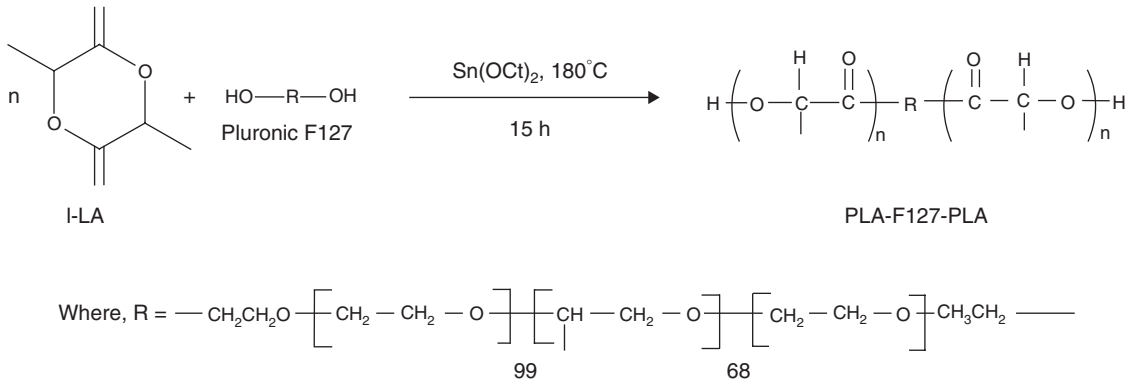

Figure 5. Synthetic scheme for the formation of PLA-F127-PLA block copolymers.

electrostatic repulsion among the amino ion groups (-NH_3^+). PMAA existing as a non-charged structure is unfavorable for the formation of ionic bonds between PMAA and CS. At higher pH, the dissociation energy of PMAA decreases when it is mixed with poly-cation, leading to an increase of apparent dissociation constant for PMAA. This means that the -COOH group of PMAA in the mixed system dissociates more than in pure PMAA solution, a fact that is favorable to the formation of ionic bonds. However, increasing the pH of the mixed solution increases the charge density along the PMAA chain, which would expand the polymer chain due to the ionic repulsion.

3.4 Biodegradable polymeric devices for oral insulin delivery

Hydrogels are water soluble, but some of the most widely used polymers, such as polylactic acid (PLA), polyglycolic acid (PGA) and poly(lactide-*co*-glycolide) (PLGA) are not water soluble. This will pose problems for the encapsulation of insulin by the conventional methods of double emulsion (w/o/w) and solvent evaporation. Also, when the particle size of the formulation is reduced to the submicron level, the insulin loading capacity of the carriers is reduced markedly due to the rapid migration and hence, the loss of insulin into the external aqueous medium. Many efforts have been made in this direction. Cui *et al.* [52] developed NPs loaded with insulin–phospholipid complexes for the oral delivery of insulin using a novel reverse micelle–solvent evaporation, wherein soyabean phosphatidylcholine (SPC) was used to improve the liposolubility of insulin along with PLGA to control its release. The effects of key parameters like polymer/SPC weight ratio, organic phase and polymer type on the properties of NPs of 200 nm size have been studied. *In vitro* drug release was characterized by an initial burst effect and subsequent delayed release in both pH 1.2 and pH 6.8 dissolution media. The pharmaceutical effects of PLGA 50/50 (mol. wt. ≈ 9500) NPs were evaluated to confirm their suitability. Intragastric administration of 20 IU/kg NPs reduced the fasting plasma glucose levels to 57% within the first 8 h of administration, which continued up to 12 h with 7.7% of oral bioavailability compared to s.c. injection.

Block copolymers of commercial pluronic® F127 (PEO-PPO-PEO) and biodegradable PLA were synthesized [53], exhibited good biocompatibility and were evaluated as carriers for oral insulin delivery. Pluronic block copolymers are one of the very few synthetic polymers approved by the US Food and Drugs Authority for use as food additives and pharmaceutical ingredients. These copolymers exhibited a strong affinity toward the small intestine due to PEO blocks and high permeation characteristics to the cell membrane, due to their amphiphilic properties [54,55]. The morphology of PLA-F127-PLA block copolymers in aqueous solutions resembles those of vesicular NPs and these are the potential candidates for oral insulin delivery. The *in vitro* release of insulin loaded in PLA-F127-29 vesicles was studied to monitor hypoglycemic effects after oral administration to the diabetic mice. The reaction scheme of their preparation is shown in **Figure 5**.

The release kinetics of insulin from polymeric micelles was affected by particle size and morphology, block composition, molecular weight, degradation rate, etc [37]. The hydrophobic PLA blocks in PLA-F127-29 are biodegradable and their hydrolytic degradation has been reported [53]. Compared with the fast release of insulin-loaded PLA-F127-29 vesicles, degradation of PLA blocks did not significantly affect the release of insulin due to a relatively slow degradation rate. Therefore, the release of insulin from PLA-F127-29 vesicles was mainly dominated by a diffusion-controlled mechanism. The unusual biphasic release of insulin from PLA-F127-29 vesicles was related to the distribution of insulin within PLA-F127-29 vesicles. Apart from insulin loaded inside the hydrophilic core of PLA-F127-29 vesicles, insulin was also present on the surface of PLA-F-127-29 vesicles. These findings demonstrated that PLA-F127-29 vesicles are the most promising polymeric devices for oral insulin delivery, since they are able to maintain a prolonged hypoglycemic effect after oral

administration. Compared to liposomes and coated liposomes, these polymeric vesicles are advantageous due to their small particle size; their bilayer thickness can be varied by the choice of amphiphile molecular weight as well as the chemistry.

Bovine insulin (5%)-loaded PLA and PLGA MPs prepared [56] by water-in-oil-water multiple emulsion solvent evaporation technique showed encapsulation efficiencies up to 75 and 80%, respectively, in PBS (pH 7.4) at 37°C. MPs are spherical with relatively porous surfaces and sizes ranging from 40 – 53 µm. Insulin release followed an initial burst, due to insulin being located on the surface of particles. The total ion chromatogram (TIC) of insulin samples extracted after 18 days of erosion revealed that deamidation was the major mechanism of instability, but no acylation products were found. Control experiments performed in concentrated lactic acid solutions confirmed the minimal reactivity of insulin.

3.5 Miscellaneous types of polymeric devices

Insulin-loaded calcium pectinate NPs were prepared [57] by ionotropic gelation with calcium ions and the effect of pectin molecular weight and pH on the release rates was studied. Commercially available LM101 and LM104 pectins, having degrees of esterification of 36% and 28%, respectively, were depolymerized by mechanical milling to give molecular weights in the range of 89 – 5.6 kDa. Increase of pH from 2 to 3 enhanced the encapsulation efficiency threefold, that is from 33 to 93% at an insulin loading concentration of 80 U/ml. This increase was correlated with the charge density on pectin as a function of pH. Subsequent release of insulin from the NPs was dependent on the extent of dilution of NPs and pH of the dissolution medium.

NPs prepared from the blend of a biodegradable, poly(ε-caprolactone) and a polycationic non-biodegradable acrylic polymer (Eudragit® RS) were used as carriers for the oral administration of insulin [58]. Encapsulation efficiency was around 96%. The therapeutic efficiency of oral insulin NPs (25, 50 and 100 IU/kg) in diabetic rats and the intestinal uptake of FITC-labelled insulin were studied. These devices increased the serum insulin levels and improved the glycemic response to oral glucose levels for a prolonged time. These NPs preserved the biological activity of insulin.

Martins *et al.* [59] prepared the MPs from alginate, chitosan and dextran sulfate by ionotropic gelation. Parameters like particle size, swelling, encapsulation efficiency, loading capacity and release profiles were compared in simulated gastric and intestinal fluids. Insulin was well protected and there was improvement in its release from the MPs after reinforcing the alginate matrix with chitosan and/or dextran sulfate. Dextran sulfate did not release insulin at pH 1.2, but released partially at pH 6.8. This was explained by the interaction between negatively charged groups of dextran sulfate and insulin.

Insulin-loaded alginate–dextran NPs, in the size range of 267 – 276 nm, with insulin encapsulation efficiency of 83%, were prepared by nano-emulsion dispersion method followed by triggered *in situ* gelation [60]. Insulin encapsulation efficiency and *in vitro* release data were obtained by Bradford protein assay. Bioactivity was determined *in vitro* using the newly developed Western blot immunoassay and *in vivo* using Wistar diabetic rats. Alginate–dextran particles suppressed the insulin release in acidic media, but promoted sustained release in neutral pH. The encapsulated insulin was bioactive, as demonstrated by both *in vivo* and *in vitro* bioassays.

Recent efforts [61-65] to utilize natural uptake processes of the intestine, such as vitamin B12 (VB12) transport system, for the oral delivery of insulin via chemical coupling of pharmaceuticals, has led to the development of novel carrier systems. The oral delivery of insulin in animal models using VB12 coated dextran NPs demonstrated the effectiveness of VB12 prepared at different levels of cross-linking and exhibited efficient insulin carrier properties. These NP conjugates showed higher size, high insulin encapsulation and faster release rates, with low levels of cross-linking. The VB12 NP conjugates with 150 – 300 nm size showed 70 – 75% blood glucose reductions that prolonged up to 54 h with a biphasic behavior in STZ diabetic rats.

Cyclodextrin (CD) complexation represents a unique and effective strategy to improve the protein therapy by stabilizing them against aggregation, thermal denaturation and degradation. β-Cyclodextrin (β-CD) forms non-covalent inclusion complex with a wide variety of drugs/proteins that often alter physicochemical and biological properties of the guest molecules [66]. Hydrophilic β-CD inhibits the adsorption of insulin to hydrophobic surfaces and prevents the self-aggregation of insulin at neutral pH [67,68]. The complexation would help to enhance the absorption of insulin across biological barriers. However, clinical exploitation of cyclodextrin-based systems is restricted due to safety concerns and hence not many studies investigated these systems.

In all the above-mentioned studies, different types of systems require correlations between pharmacokinetic (PK) and pharmacodynamic (PD) parameters for insulin therapy, since very little information is available in the literature on this aspect. It is important to have a PK/PD model to predict the time course of plasma concentration and the intensity of the pharmacological effect based on clinical dosage. The preferred approach to determine PK and PD properties of insulin analogues is the euglycemic glucose clamp. Currently, analytical approaches are used to analyze such data. In this respect, a novel compartmental model for analysis of data from glucose clamp studies was proposed by Osterberg *et al.* [69]. The data used in this trial involved 18 of the 20 originally treated subjects and results were obtained from a crossover trial where 18 healthy subjects each received a single s.c. dose of 1.2 nmol/kg (body weight) insulin

aspart (IAsp) or 1.2 nmol/kg human insulin (HI) during a euglycemic glucose clamp after overnight fasting. Serum insulin and glucose concentrations were measured and the glucose infusion rate (GIR) was adjusted after dosing to maintain blood glucose near basal levels. Individual model parameters were estimated for IAsp, HI to find statistically significant differences between most of the HI and IAsp pharmacokinetic parameters, including the sigmoidicity of the time course of absorption (1.5 for HI vs. 2.1 for IAsp (unit less), $p = 0.0005$, Wilcoxon Signed-rank test), elimination rate constant (0.010 min^{-1} for HI vs. 0.016 min^{-1} for IAsp ($p = 0.002$)). The PD model parameters were mostly the same, except for the rate of insulin action (0.012 min^{-1} for HI vs. 0.017 min^{-1} for IAsp ($p = 0.03$)). This model may provide a framework to account for different PK properties when estimating the PD properties of insulin and insulin analogues in glucose clamp experiments.

4. Conclusions

The past decade has witnessed a tremendous growth in the development of oral insulin delivery polymeric systems. In these efforts, the role of pH-sensitive hydrogels cannot be underestimated. Several applications pertaining to drug delivery aspects are emerging. The pH-sensitive hydrogels are particularly useful not only in protecting the sensitive insulin from enzymatic degradation, but also to provide flexibility to encapsulate insulin. Previous research efforts have suggested the development of innumerable oral insulin delivery devices and design strategies, yet the challenge still remains in creatively integrating different functionalities into a single multifaceted vector. The published literature indicates that several strategies have emerged to offer new and interesting perspectives on meeting the challenges of oral insulin delivery. One approach seeks to develop an alternative insulin delivery platform through the use of modulated ordered macromolecular structures. Another approach seeks to enhance the existing vector platforms (i.e., covalent functionalization of polymeric chains) by adopting combinatorial strategies and high throughput technology as a means to systematically evaluate the validity of the approach.

In the near future, oral insulin delivery research will witness a rapid development of more sophisticated bioanalytical techniques to quantify *in vitro* and *in vivo* aspects. Methods that can more accurately characterize the physicochemical characteristics of the multifaceted vectors, evaluate specific delivery enhancing effects of various physicochemical vector features and quantify the behavior of inter-polymer complexes within the cellular environment will offer researchers invaluable tools to probe the mechanistic aspects of insulin delivery. Another important factor involves resolving the biological landscape through which insulin delivery systems must travel. Several research groups have recently identified the involvement of previously unsuspected intracellular entities and pathways in oral insulin delivery, underscoring the complexity of the cellular environment, which still remains illusive. Resolving the biological landscape and its implications in oral insulin delivery is critical in improving the vector. Greater understanding of the mechanism of insulin release through polymeric devices, coupled with strategically engineering the polymers, is a future challenge of this technology. As such, it is critical that research efforts continue in this area so that we may be able to develop more efficient oral insulin delivery strategies that will overcome the hurdles posed by s.c. administration.

5. Expert opinion

For the past 75 years subcutaneous injections have been the only route of delivery of insulin therapy to diabetic patients. During this time, numerous attempts have been made to explore alternative routes for systemic insulin administration. However, thus far, no feasible alternative for non-invasive insulin delivery has been developed. The dream of an insulin tablet has also not become a reality yet, the main problem being digestion and a lack of a specific peptide carrier system in the gut. Oral delivery of insulin as a non-invasive therapy for diabetes mellitus is still a major challenge to drug delivery technologists, since insulin is degraded in the presence of enzymes in the acidic environment of the stomach and also its absorption through the gastrointestinal mucosa is questionable.

Alternative approaches to prepare the coated nano- and micro-particles that can protect the sensitive insulin in the stomach and efficiently transport across the intestinal membranes into the blood supply remain a difficult problem to solve. One alternative non-injectable route of insulin administration that is being researched is oral insulin administration delivered by a spray device into the oropharyngeal cavity. This has the potential to more closely resemble the meal-stimulated physiologic insulin profile and, therefore, to become an attractive, easy-to-use route for covering prandial insulin requirements. Efforts to design suitable oral delivery systems for insulin come from such diverse research disciplines as biopolymers, nanotechnology and hydrogels, and are mainly driven by the potential to overcome the psychological barrier against the use of insulin in patients with type 2 diabetes, due to the fear of s.c. injections. A greater advantage of the oral route of administering insulin is that it mimics more closely the endogenous secretion of insulin. This mode of distribution is highly desirable, since the liver plays an important role in maintaining glucose homeostasis in the bloodstream, taking up and storing the energy in carbohydrates in the form of glycogen.

For many years researchers have attempted to find a solution to the problem of oral insulin delivery and each of these strategies have merits in the development of a successful and a suitable carrier device using the combination of protease inhibitors, enhancers or a combination thereof acting together to circumvent the barriers of insulin

absorption. Certainly the most promising strategy to achieve oral insulin delivery is the use of hydrogel-based system. Even though the competitive development in the area of oral insulin delivery does not show action profiles that are different than those obtained with injections of short-acting insulin analogues, the pharmacokinetic and pharmaco-dynamic properties would offer several advantages for type 2 patients.

The short interval between administration and the observation of maximum serum insulin levels and the rapid onset of action has a beneficial effect, especially in patients with type 2 diabetes. The shorter duration of action as compared to insulin injections mimics the physiological insulin requirements to cover glucose absorption. However, the long-term safety and tolerability of such a new formulation cause some concerns in adopting this technology routinely. More research, specifically with combination approaches that exhibit greater intestinal uptake, is needed to overcome the absorption and enzymatic barrier problems to have a safe and effective means for administering insulin orally.

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