

Current Status of the Oral Delivery of Insulin

Vikas Agarwal and Mansoor A. Khan*



The authors summarize the current status of the development strategies of insulin drug delivery systems in the pharmaceutical industry, various challenges associated with the oral delivery of insulin, and how these challenges may be overcome by the use of enzyme inhibitors, permeation enhancers, and polymeric systems. The authors also discuss a novel dosage form that incorporates the dual controlled release of insulin and inhibitor and a dosage form that consists of immobilized inhibitor.

Vikas Agarwal, PhD, is an associate scientist at CIMA Labs (Brooklyn Park, MN). Mansoor A. Khan, RPh, PhD, is a professor of pharmaceuticals and the director of the graduate program at Texas Tech University HSC School of Pharmacy, 1300 Coulter, Amarillo, TX 79106, tel. 806.356.4000 ext. 285, fax 806.256.4034, khan@ama.ttuhs.edu.

*To whom all correspondence should be addressed.

In recent years, a spurt of research and development has been conducted involving a wide range of protein therapeutics for various conditions. Most of the proteins have been successfully applied in therapy, mainly as parenterals. An oral dosage form is the preferred form of delivery because of ease of administration, patient compliance, and cost. Major hurdles must be overcome before the oral delivery of a protein becomes a reality. Among the proteins, oral delivery of insulin has received the widest attention, yet no currently available oral insulin preparation exists. This article focuses on the oral delivery challenge of insulin as well as the advancements in its oral dosage form development, particularly in the past 25 years.

Insulin was isolated from bovine pancreas in 1922 by Banting and Best, who received the 1923 Nobel Prize with McLeod. Oral administration of insulin was found to be ineffective for the treatment of insulin-dependent diabetes mellitus. Since then the subcutaneous route has been the mainstay of insulin delivery until today.

In early years, insulin was isolated mostly from the pancreases of bovine cadavers. This method of isolation led to several immunological reactions as a result of impurities present in the isolated protein. The synthesis of insulin by recombinant DNA technology represented an important scientific milestone and made large quantities of protein available at an affordable price — a factor that led to insulin becoming one of the most popular proteins to be studied for oral delivery. Consequently, research data in several aspects of delivery of insulin are available.

The progress in the oral delivery of insulin could not have been possible without the help of research in areas such as analytical chemistry, immunoassays, biochemistry pharmaceutical sciences, novel methods of screening absorption in vitro by using isolated segments of animals and mammalian cells, and availability of functional polymers.

Clinical significance of the oral delivery of insulin

Physiological insulin that is secreted by the pancreas enters portal circulation and inhibits hepatic glucose production. It undergoes metabolism in the liver to a significant extent (~50%). The ratio of plasma insulin in portal circulation versus that in peripheral circulation is two. The physiological hypoglycemic effect of insulin is a result of the absence of hepatic glucose production that is enhanced by the increase in glucose use caused by lower insulin levels in peripheral circulation.

Table I: Apparent permeability coefficient of insulin across various segments of the GIT.

Experimental Model	Species	Concentration in Donor Side (μM)	$P_{\text{app}} \times 10^7$ (cm/s)	Technique	Reference
Duodenum	Rat	83.3	0.78 ± 0.54	Everted gut sac	(9)
		500	4.85 ± 0.99	Ussing technique	(59)
Jejunum	Rat	83.3	4.97 ± 1.51	Everted gut sac	(9)
		500	12.27 ± 1.73	Ussing technique	(59)
		100	0.922 ± 0.168	Ussing technique	(45)
Ileum	Rat	83.3	6.82 ± 1.87	Everted gut sac	(9)
		500	10.50 ± 2.06	Ussing technique	(59)
Colon	Rat	500	4.05 ± 1.09	Ussing technique	(59)

When insulin is injected subcutaneously, the plasma insulin concentration in portal circulation and in peripheral circulation is almost equal. The hypoglycemic effect of insulin is a result of its action on peripheral tissues. Oral delivery of insulin can mimic the physiological fate of insulin and may provide better glucose homeostasis. This also will lessen incidences of peripheral hyperinsulinaemia, which is linked to neuropathy, retinopathy, and so forth.

Challenges associated with the oral delivery of insulin

The various challenges associated with the oral delivery of proteins usually are evaluated by determining the fate of the protein in the gastrointestinal tract (GIT). The main challenges reported are enzymatic degradation and a lack of sufficient insulin permeability through the GIT. The enzymatic barrier and epithelial barrier for proteins have been reviewed in detail elsewhere (1,2). The following sections focus on the details of these barriers with respect to insulin.

Enzymatic degradation of insulin. Upon ingestion, insulin is subjected to acid-catalyzed degradation in the stomach, luminal degradation in the intestine, and intracellular degradation. The pancreatic enzymes that degrade insulin are trypsin and α -chymotrypsin (3,4). The rate of degradation was found to be ~ 10 times higher in the presence of α -chymotrypsin when compared with that in the presence of trypsin (5). The cytosolic enzyme that degrades insulin is insulin-degrading enzyme (IDE) (6). Insulin is not subject to enzymatic degradation by brush-border enzymes. The rate of degradation of insulin also depends on its associated state in solution. Insulin is a monomer at low concentration ($<0.1 \mu\text{M}$) and dimerizes in a pH range of 4–8 at higher concentrations. At concentrations greater than 2 mM, the hexamer is formed at neutral pH (7). The associated state affects the rate of degradation of insulin. In the presence of bile salts, the rate of degradation may increase close to six times (8). This correlated with complete dissociation of insulin into a monomeric form that was verified by circular dichroism spectroscopy.

Intestinal transport of insulin. Evidence of active transport for insulin was negative (9). Morpho-cytochemical and biochemical evidence for insulin absorption was demonstrated in rat GIT (10,11). This result was achieved by direct instillation of a solution of insulin into various parts of the GIT, followed by visu-

alization with gold markers and immunoassay of the insulin in blood. No evidence exists for the transport of insulin by the paracellular route. Researchers found that insulin is adsorbed to the apical plasma membrane and is internalized by endocytosis. It then reaches the basolateral plasma membrane via the endosomal pathway of small vesicles and is secreted into the interstitial space. Whether the internalization is a result of the presence of insulin receptors on the surface of the epithelial cells is unclear. The presence of insulin receptors has been demonstrated in enterocytes on both the apical

and basolateral sides (12–14).

Permeability studies of insulin across isolated segments of the GIT have been performed with an aim to evaluate the apparent permeability coefficient of insulin. The in vitro permeability studies also serve as screening tools to test the efficacy of absorption modifiers. Insulin permeability across the GIT has been studied by using isolated segments of the various regions of the intestine. Table I provides a list of the apparent permeability coefficients of various regions of the GIT. The values given in the table show the regional differences in permeability across various regions of rat GIT. These differences have been attributed to the histological differences between the various sites. Also, a significant difference exists between the permeability coefficients within the same segments. This difference can be explained by differences in the preparation of tissues, apparatus used, concentration of insulin used in the donor compartment, and duration of the study. The permeability of insulin through caco-2 cells was reported to be $6.45 \pm 1.2 \times 10^{-7}$ cm/s (15), and a recent study estimated the apical epithelial permeability of insulin to be 0.32×10^{-7} cm/s (16).

Dosage form stability issues. The activity of proteins depends on the three-dimensional molecular structure. The dosage form development of proteins may expose the proteins to harsh conditions that may alter their structure. This will have implications in the efficacy and immunogenic response to the proteins.

During dosage form development, proteins might be subjected to physical and chemical degradation. Physical degradation involves modification of the native structure of a protein to a higher-order structure, which may be a result of adsorption, aggregation, unfolding, or precipitation. Chemical degradation usually involves bond cleavage and leads to the formation of a new product. Chemical degradation is preceded by a physical process such as unfolding, which exposes the hidden residues to chemical reactions. The processes involved in chemical degradation are deamidation, oxidation, disulfide exchange, and hydrolysis.

The stability of insulin preparations has been documented in detail (17), and research data on the solid-state stability of proteins in dosage forms have been reviewed recently (18). Proteins must be characterized for change in conformation, size, shape, surface properties, and bioactivity upon formulation processing. Changes in conformation, size, and shape can be

Table II: Bioavailability of insulin in animal models from polymer systems containing absorption modifiers (AMs).

Polymer Systems with AMs	Species	Dose of Insulin (IU/kg)	Dose of AM (mg/kg)	Bioavailability Based on Hypoglycemic Effect	Reference
Insulin in microcapsule containing five methoxy salicylic acid that is coated with 10% azopolymer	Pancrea-tectomized dogs	11–66	0.54–0.32	<1%	(60)
Polyacrylic polymer (Eudragit L100) microspheres of insulin further coated with polyacrylic polymer. Administered with aprotinin and Bowman Birk inhibitor.	Normal and hyperglycemic rats	20	0.35	1–7.1% in normal rats and 0.8–4.6% in diabetic rats relative to i.v. efficacy	(61)
Insulin contained in hard gelatin capsule containing sodium salicylate coated with 10% solution of Eudragit L100 and Eudragit S100	Hyperglycemic rats	13.2–15.3	57.2–66.7	13.26–14.15% relative to i.p. injection	(62)
Polyvinyl alcohol gel spheres containing aprotinin and bacitracin	Hyperglycemic rats	287–344.4	13.33–16	2%	(63)
Pellets of insulin coated with enteric, time-release polymers. Administered with aprotinin and sodium deoxycholate.	Normal rats	60		0.017–0.062	(64)
Hard gelatin capsules containing insulin and sodium cholate coated with a 10% solution of Eudragit S100	Hyperglycemic rabbits	10	5, 12.5, and 25	15.6, 25.83, and 27.89 relative to i.p. injection	(65)

observed by the use of spectrophotometric techniques, X-ray diffraction, differential scanning calorimetry, light scattering, electrophoresis, ultracentrifugation, and gel filtration. Changes in surface properties can be detected with the use of electrophoretic and chromatographic techniques, and changes in the bioactivity of proteins can be observed by bioavailability studies. Selection of a particular technique is based on the sensitivity of the technique, the system under study, and the availability of equipment. The interference by formulation excipients also may be a factor when selecting the characterization technique. Theory about selected techniques used for the characterization of proteins has been reviewed (19,20). Some examples of characterization of insulin are discussed in the following paragraphs.

Size-exclusion chromatography with reversed phase–high performance liquid chromatography (HPLC) was used to determine the formation of covalent insulin dimers with trace amounts of high molecular-weight transformation products after microencapsulating insulin in a mixture of poly(DL-lactide-co-glycolide) and poly(L-lactide) (21). Differential scanning calorimetry was used to differentiate denaturation endotherms of amorphous and crystalline insulin (22). X-ray diffractograms of insulin have been obtained with mixtures of lactose and mannitol to evaluate the effect of spray-drying on the crystalline changes of insulin (23).

Strategies for improved oral delivery of insulin

Successful oral delivery of insulin involves overcoming the barriers of enzymatic degradation, achieving epithelial permeability, and taking steps to conserve bioactivity during formulation processing. The use of enzyme inhibitors, permeation enhancers, and polymer systems has been attempted to overcome these barriers. A synergistic approach usually works best.

Enzyme inhibitors. Researchers have evaluated the use of protease inhibitors with an aim to slow the rate of degradation of insulin. They hypothesized that the slow rate of degradation will increase the amount of insulin available for absorption. As discussed previously, enzymatic degradation of insulin is mediated by serine proteases trypsin, α -chymotrypsin, and thiol metalloproteinase IDE. Consequently, stability of insulin has been evaluated in the presence of excipients that inhibit these enzymes. Representative inhibitors of trypsin and α -chymotrypsin include pancreatic inhibitor (24), soybean trypsin inhibitor (24), FK-448 (25), camostat mesylate (26), and aprotinin (27). Inhibitors of insulin-degrading enzyme include 1,10 phenanthroline (28), p-chloromercuribenzoate (28), and bacitracin (29). Enzyme inhibitors have been associated with systemic intoxication if they are absorbed (30,31). If they are not absorbable, then the digestion of nutritive proteins may be disturbed (32,33).

Permeation enhancers. Permeation enhancers improve the absorption of proteins by increasing their paracellular and trans-

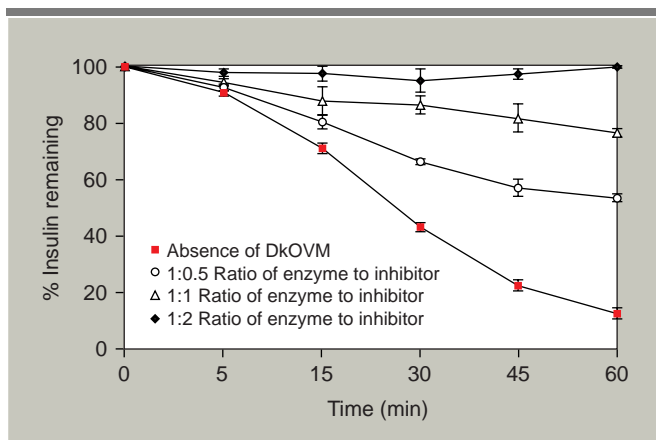


Figure 1: Chymotrypsin-mediated degradation of insulin versus time in the absence of DkOVM and in the presence of DkOVM at various enzyme-to-inhibitor ratios. Values represent the average of at least three independent experiments \pm SEM. (Reproduced with permission from *Pharmacy and Pharmacological Communications*. Copyright 2000.)

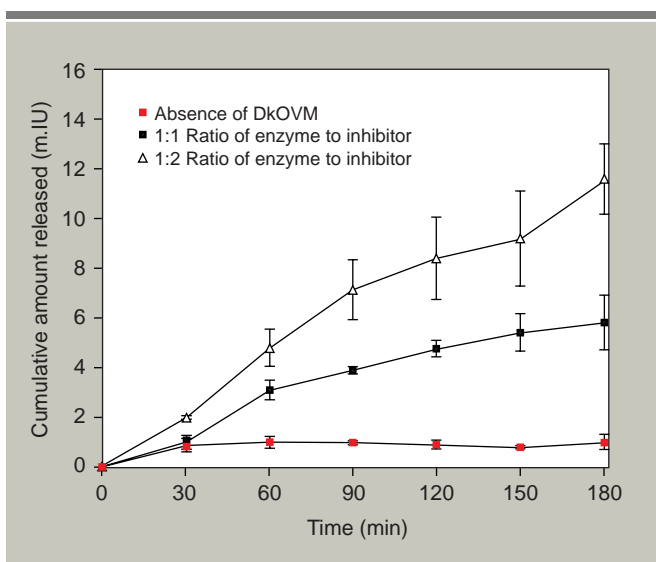


Figure 2: Cumulative amount of insulin permeated (m IU) versus time in the presence of α -chymotrypsin in the absence and presence of DkOVM at 1:1 and 1:2 ratios of enzyme to inhibitor. (Reproduced with permission from the *Journal of Pharmacy and Pharmacology*. Copyright 2001.)

cellular transports. An increase in paracellular transport is mediated by modulating tight junctions of the cells, and an increase in transcellular transport is associated with an increase in the fluidity of the cell membrane. Permeation enhancers that fall into the former category include calcium chelators, and those that fall into the latter category include surfactants. Calcium chelators act by inducing calcium depletion, thereby creating global changes in the cells, including disruption of actin filaments, disruption of adherent junctions, and diminished cell adhesion (34). Surfactants act by causing exfoliation of the intestinal epithelium, thus compromising its barrier functions (35). This raises questions about the toxicity and long-term clinical

use of permeation enhancers. Most literature studies on the use of these permeation enhancers have demonstrated that their enhancement is dose and time dependent. Examples of permeation enhancers used include sodium laurate and cetyl alcohol (36), sodium cholate (37), ethylenediaminetetraacetic acid (EDTA) (28), and zonula occludens toxin (ZOT) (38).

Use of polymer systems. The use of polymer systems both alone and concurrent with absorption modifiers such as enzyme inhibitors and permeation enhancers has been evaluated. In the former system, the drug is released after uptake of the polymer system intact from the GIT. In the latter system, the drug is released in the lumen before being absorbed.

Polymeric dosage form with absorption modifiers. Researchers have attempted to use polymers in various controlled-release applications. Depending on how the modifier is added, the reported preparations can be grouped into the following:

- Insulin has been incorporated in dosage form with an absorption modifier. The dosage form is then coated with an enteric polymer to release the contents depending on pH or enzymatic conditions of the GIT.
- Insulin has been microencapsulated with polymers and then administered with absorption modifiers.

These dosage forms have dual protective properties. The polymer protects the drug from immediate exposure to the enzymes, and the absorption modifier may increase the enzymatic stability or enhance the permeability. Table II shows some representative combinations of polymer systems with absorption modifiers. A common feature among all the systems is enteric time release of insulin. There is no uniformity in the dose of insulin studied or the absorption modifier. Dose-response studies of insulin and absorption modifiers have not been investigated in detail. The following sections discuss dual controlled-release delivery of insulin and enzyme inhibitor and immobilization of enzyme inhibitor with insulin onto a polymer matrix.

Dual controlled-release dosage form of insulin and inhibitor. This ongoing study has been conducted in our laboratory with promising results. The concept of controlled release of an inhibitor with protein was proposed to extend the stability of the protein during the absorption process in the GIT. When the inhibitor is released in an immediate fashion, it may be washed away from the site where the protein is being released. However, if the inhibitor is released together with the protein in a controlled fashion, the enzymatic stability of the protein may be enhanced. The model inhibitors used were duck ovomucoid (DkOVM) and chicken ovomucoid (CkOVM). Ovomucoids are enzyme inhibitors isolated from egg white of avian species. Extensive reviews of their source, method of isolation, and mechanism of inhibitory action have been documented (24,39–41). Their inhibitory activity is species dependent. Apart from inhibitory action, ovomucoids can interact with lectins because of the presence of carbohydrate moeity. Ovomucoids immobilized on polymers have been used for enantioselective separation on HPLC columns (42) and in the preparation of gels (43). The development of the dosage form with dual controlled-release characteristics was preceded by enzymatic stability, permeability, and dissolution stability experiments.

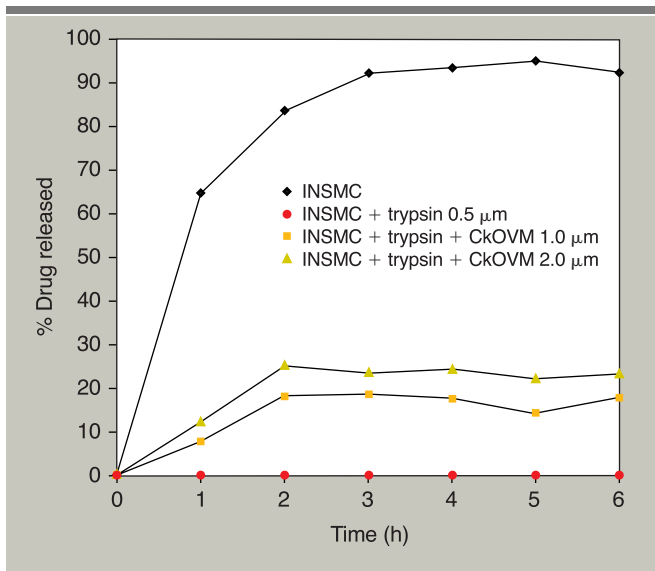


Figure 3: Dissolution stability of insulin microcapsules (INSMC) in the presence of trypsin and CkOVM at 1:2 and 1:4 ratios of enzyme to inhibitor.

Two representative inhibitors, CkOVM and DkOVM, were used to evaluate their protection against trypsin- and α -chymotrypsin-mediated degradation of insulin (44). A representative figure for α -chymotrypsin-mediated degradation of insulin in the presence of DkOVM is shown in Figure 1. The study showed that

- DkOVM offered 100% protection against α -chymotrypsin- and trypsin-mediated degradation of insulin for 1 h at an enzyme inhibitor ratio of 1:2.
- CkOVM offered protection against trypsin-mediated degradation only at a 1:1 ratio of enzyme to inhibitor.
- A concentration range of inhibitors also was established in these studies.

Transport studies with rat jejunal segments mounted in a side-by-side diffusion chamber revealed that

- Insulin permeability is decreased in the presence of CkOVM and DkOVM.
- CkOVM and DkOVM increased the permeability of a hydrophilic and lipophilic marker.
- Insulin flux was enhanced in the presence of α -chymotrypsin and DkOVM (see Figure 2) (45).

A formulation containing insulin microcapsules and CkOVM and DkOVM has been evaluated for dissolution stability in the presence of trypsin and α -chymotrypsin (46). Figure 3 demonstrates the improvement in dissolution stability of insulin in the presence of trypsin and CkOVM. Results showed that at a 1:4 ratio of enzyme to inhibitor, the availability of insulin at the end of 6 h improved from negligible to 24.70%. On the other hand, DkOVM improved the availability of insulin in the dissolution medium in the presence of α -chymotrypsin at a 1:4 ratio of enzyme to inhibitor. Insulin availability at the end of 6 h improved from negligible to 42.3% at this ratio. An optimized dosage form then was developed using response surface methodology. The dosage form consisted of a tablet matrix containing insulin microcapsules and DkOVM. Figure 4 shows the 6-h release profile of insulin and DkOVM from the dosage form. Initial bioavailabil-

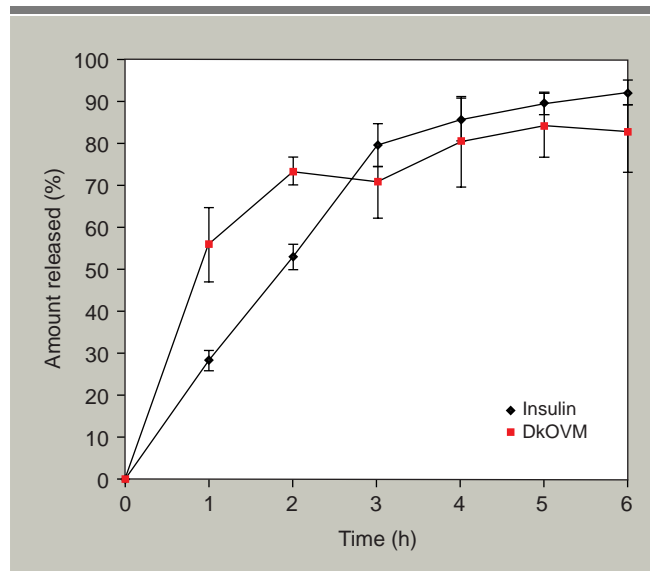


Figure 4: Dissolution profile of insulin and DkOVM from the optimized dosage form.

ity data in normal rabbits indicate that the inhibitor is effective in reducing the blood glucose levels during a period of 6 h.

Immobilization of inhibitors. Polymer inhibitor conjugates, carboxymethylcellulose–Bowman Birk inhibitor (CMC–BBI) and carboxymethylcellulose–elastin (CMC–Ela), have been shown to offer in vitro protection against trypsin, chymotrypsin, and elastase (47). These conjugates when combined with a polycarboxyl–cysteine (PCP–Cys) conjugate demonstrated a 20–40% reduction in basal glucose levels for more than 80 h (48). However, in these studies, questions remain regarding the amount of insulin absorbed and the factors affecting the long-term decrease in glucose level.

Use of polymer systems alone. Both nondegradable and biodegradable polymers by themselves have been used to prepare nanospheres that were intended for intact uptake by the GIT. This approach is supported by literature that states microspheres in the nanosize range are absorbed intact by the intestinal epithelium and travel to sites such as the liver, the spleen, and other tissues (49,50). If the nanospheres accumulate in the liver, they could act as minor depots of insulin. The extended release of insulin could decrease the elevated hepatic glucose production in diabetic patients.

A nondegradable system consisting of microspheres of isobutyl 2-cyanoacrylate loaded with insulin at 25 IU/kg when administered to streptozotocin-induced diabetic rats produced a 50% reduction in the fasted serum glucose level compared with a 60% reduction in serum glucose when the same dose was administered by subcutaneous route (51). Results showed that the same microspheres were not effective when administered in fed rats (52).

The use of biodegradable microspheres is proposed to circumvent the possible accumulation of nondegradable microspheres in the tissues that may lead to harmful effects. A biodegradable system consisting of a mixture of poly (fumaric anhydride) and poly (lactide-co-glycolide) was used to prepare nanospheres

(<1 μm) of insulin. Results showed that, upon administration to rats, the nanospheres crossed the intestinal tract intact and appeared in the liver. When these nanospheres were given to fasted rats concurrently with a glucose load, a decrease in plasma concentrations was not observed for as long as 5.5 h (53).

Commercial interest in oral delivery of insulin

Drug delivery systems for proteins, including insulin among others, have been attempted by several companies. Although a commercial product is not yet available on the market, the current state of research spans from proof-of-concept studies to late-stage clinical trials.

The Orasome technology (Endorex Corp., Chicago, IL) involves encapsulation of the proteins in liposomes. These liposomes are rendered resistant to harsh conditions of the GIT such as exposure to acidic pH, bile salts, and detergents by polymerization (54,55). Drugs are absorbed by the uptake of liposomes intact and are released in the tissues of the body.

Researchers at Emisphere Technology (New York, NY) are working with Non-Acylated α -amino acids as carriers for oral delivery of macromolecules (56,57). They claim that upon oral administration of the carrier with the protein, the carrier forms a noncovalent association with the conformation of the protein that has a higher transport rate when compared with the physiological conformation. This complex dissociates after crossing the cell membrane.

The M2 system (Nobex Corp., Research Triangle Park, NC) is based on the attachment of low molecular weight polymers to specific sites in the protein. These polymer conjugates have been reported to improve stability and absorption when compared with the performance of native protein (58).

Conclusion

The development of drug delivery systems for proteins continues to be pursued actively in academic and industry circles. The success of commercial technologies and the emergence of new ones will be demonstrated only with time. An oral delivery system of insulin will have tremendous benefits in terms of a decreased number of injections for diabetic patients and a reduced incidence of side effects.

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