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Drug Development and Industrial Pharmacy, 28(5), 563–570 (2002)

RESEARCH PAPER

Effect of Bioadhesive Polymers, Sodium Salicylate, Polyoxyethylene-9-lauryl Ether, and Method of Preparation on the Relative Hypoglycemia Produced by Insulin Enteric-Coated Capsules in Diabetic Beagle Dogs

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ABSTRACT

The hypoglycemic effect of oral insulin capsules coated with pH-dependent Eudragit® S100 and containing various absorption promoters was studied in hyperglycemic beagle dogs. The absorption enhancers used were bioadhesive polymers, sodium salicylate, and non-ionic surfactants. A comparative study of the bioadhesive polymers, polycarbophil (PC), hydroxypropyl methylcellulose (HPMC), and carbopol 934 in insulin-coated capsules revealed no significant difference between the insulin capsules containing these polymers, giving relative hypoglycemia (RH) values ranging from 4.3±2.3% to 6.5±5.1%. It was also found that the method of preparation of the mixture of the bioadhesive polymer with insulin either by physical mixing or freeze-drying did not affect the RH values obtained. Sodium salicylate, when used in insulin enteric-coated capsules (50 mg) mixed with insulin as a physical mixture, or prepared by wet granulation using 10% polyvinyl pyrollidone (PVP), or by freeze-drying, produced RH values ranging from 7.3±2.9% to 9.4±3.7%. When sodium salicylate (100 mg) was used with insulin in freeze-dried granules an RH value of

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10±2.6% was produced. As the dose of insulin increased from 6 to 9 U/kg, the area under curve (AUC) of the enteric-coated capsules containing 50 mg sodium salicylate increased from 73.2±27.8% to 121.4±102.7% reduction, but the RH did not change significantly. Insulin capsules containing polyoxyethylene-9-lauryl ether (POELE) used in its optimum concentration (2%), found in these experiments, produced RH of 9.5±6.8% when prepared as granules by wetting with a few drops of absolute alcohol in the presence of PC (50 mg). Insulin capsules containing lower (1%) or higher (3%) concentrations of POELE and prepared with PC, 50 mg by wet granulation produced lower RH of about 6%. The enteric-coated oral insulin capsules containing insulin (6 or 9 U/kg) and sodium salicylate (50 mg) as an absorption promoter, together with the bioadhesive polymer polycarbophil (50 mg), and prepared either by wet granulation using ethanol or by freeze-drying, are the best formulations to be used. They achieved a reduction in plasma glucose levels of about 25–30% and RH of about 10%. Also insulin (9 U/kg) capsules containing 2% POELE produced a 28% reduction in plasma glucose levels and RH of 9.6±6.8%.

Key Words: *Bioadhesive polymers; Sodium salicylate; Polyoxyethylene-9-lauryl ether; Method of preparation; Insulin capsules*

INTRODUCTION

Many attempts have been made to increase the absorption of insulin after oral administration either by avoiding its destruction (1–3) or by increasing its rate of absorption (4).

For the development of oral insulin, Loehry et al. (5) studied the permeability of small intestine to substances of high molecular weight. They found that the intestinal permeability was inversely proportional to the molecular weight of the water-soluble substances.

Soft (6) and hard (7) gelatin capsules coated with polyacrylic polymer (Eudragit®) having pH-dependent properties and containing surfactant mixture, sodium laurate:cetyl alcohol 2:8 in arachis oil, or sodium salicylate gave significant ($P < .01$) hypoglycemia when compared with controls.

The use of pH-responsive, poly(methacrylic-g-ethylene glycol) hydrogels as oral delivery vehicles for insulin was investigated (8). Insulin was loaded into polymeric microspheres and administered orally to healthy and diabetic Wister rats. Within 2 hr of administration of the insulin-containing polymers, strong dose-dependent hypoglycemic effects were observed in both healthy and diabetic rats. These effects lasted for up to 8 hr following administration.

Poly(alkyl cyanoacrylate) nanocapsules have been used successfully for oral administration of insulin in diabetic rats (9). These nanospheres protected insulin from the degradation by proteolytic enzymes in vitro, especially when they were dispersed in an oily medium (Miglyol® 812) containing surface-active agents (Poloxamer® 188 and deoxycholic acid). When dispersed in the same medium, insulin-loaded nanospheres (100 U/kg of body weight) administered perorally in streptozotocin-induced diabetic rats provoked a 50% decrease of fasted glycemia from the second hour up to 10–13 days.

Protection of insulin using water-in-oil-in-water (w/o/w) multiple emulsions was studied (10,11). Although oral w/o/w insulin emulsions are still of low efficiency, the results would indicate that diabetes can be controlled by effective oral insulin preparations.

The objective of this study is to formulate insulin capsules for oral administration so as to obtain an alternative route to parenteral insulin administration. Therefore, the influence of oral insulin capsules coated with Eudragit S100 and containing different concentrations of various additives on the reduction of plasma glucose levels is studied using overnight food-deprived hyperglycemic beagle dogs. The additives tried are some bioadhesive polymers [polycarbophil (PC), hydroxypropyl methylcellulose (HPMC), and carbopol 934], sodium salicylate, and the non-ionic surfactant (polyoxyethylene-9-lauryl ether in different concentrations). The hypoglycemia resulting from these capsule formulations was

calculated and compared to that produced after subcutaneous injection of soluble insulin.

EXPERIMENTAL

Materials

Insulin crystals HM (ge) were a generous gift from Novo Nordisk A/S (Novo Alle, 2880 Bagsvaerd, Denmark). Polycarbophil was from Lee Laboratories (Petersburg, VA). Hydroxypropyl methylcellulose was from Aldrich Chemical Co. (Milwaukee, WI). Carbopol 934 was from Winlab Ltd. (Maidenhead, Berkshire, UK). Polyoxyethylene-9-lauryl ether was from Sigma Chemical Co. (St. Louis, MO). Glucose GOD-PAP was from Randox Laboratories Ltd. (Antrim, UK).

Methods

Induction of Hyperglycemia

Male beagle dogs weighing between 9.5 and 16.5 kg were overnight food-deprived and rendered diabetic with an intravenous injection of a cocktail containing alloxan and streptozotocin (35 mg/kg each). This cocktail was injected on two occasions. On the first day, as 40 mg/kg, and 2 days later, as 30 mg/kg. The diabetic dogs were managed by daily subcutaneous (SC) injections of 2 U/kg of regular insulin and 1 U/kg of NPH insulin (Eli Lilly & Co. Indianapolis, IN). This work was approved by King Abdulaziz City for Science and Technology (KACST).

Subcutaneous Injection of Insulin

Regular human insulin injection, USP (100 U/mL) was injected as 3 U/kg body weight subcutaneously to hyperglycemic overnight food-deprived beagle dogs.

Preparation of Insulin Capsules

The oral dosage form design is based on the incorporation of insulin with one or more of the absorption promoters as a physical mixture or as freeze-dried granules into hard gelatin capsules. The capsules are coated in a coating pan rotated at 50 rpm by spraying with 10% solution of Eudragit S100 in acetone. Eudragit S100 has pH-dependent solubility properties (pH 7), so it will protect the capsules against gastric degradation and deliver insulin to the last part of the small intestine that has low protease activity.

Capsule Administration

Eudragit S100-coated insulin capsules each containing the required amount of insulin were administered to fasted hyperglycemic dogs orally by opening the mouth, pressing down the tongue, placing the capsule at the end of the mouth, and pushing it with the finger. The capsule was flushed down the dog's throat by 50 mL of water.

Blood Sampling

Blood samples (2 mL) were taken into heparinized tubes for glucose measurement in plasma before and every 1 hr after oral or SC administration for six consecutive hours by inserting a disposable intravenous (IV) cannula (20G-o.d. $1 \times 32\,mm^2$ luer lock, Casnate Co., Viadana, Italy) into the cephalic vein of each dog. The blood samples were immediately centrifuged and aliquots of plasma aspirated and stored at 4°C for subsequent glucose measurement at the end of the experiment.

Plasma Glucose Measurement

Plasma (10 μL) was added to 1 mL glucose reagent (GOD-PAP). After vortexing for 10 sec, the tubes were incubated for 25 min at room temperature. The absorbency of the standard and plasma glucose samples was measured within 60 min against reagent blank at 500 nm using a spectronic 21D spectrophotometer (Milton Roy, Rochester, NY). The plasma glucose concentration was calculated as milligrams per deciliter (mg/dL).

Calculations of the Hypoglycemic Effect

The maximum reduction in plasma glucose concentration (C_{max}) was obtained from the plasma glucose concentration–time curves (percentage of initial) of each dog, while the time to reach this reduction (t_{max}) was obtained from the mean data. The area under the percentage glucose reduction–time profile (AUC_{0-6h}) was determined using the linear trapezoidal rule. The relative hypoglycemia (RH) of insulin formulations was calculated by comparing their AUCs relative to that

after subcutaneous injections and taking dose differences into consideration. All the data are expressed as mean±SD.

Statistical Analysis

Plasma glucose levels (0–6 hr) after oral or SC administration of insulin capsules or injection, respectively, were compared in each group with the respective initial values using repeated measures analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. Differences between groups in C_{max}, AUC, and RH were carried out using Student's *t*-test to compare two values, or by one-way ANOVA followed by Tukey–Kramer multiple comparison tests in case of more than two values. These statistical calculations were performed using the Graph Pad Instat computer program (1990–1993; Graph Pad Software, V2.04, San Diego, CA).

RESULTS AND DISCUSSION

Figure 1 shows the hypoglycemic effect of insulin capsules containing 50 mg of any of the bioadhesive polymers (PC, HPMC, and carbopol 934), physically mixed with insulin (6 U/kg) using the geometric dilution method.

These capsules were enterically coated with pH-dependent polymer (Eudragit S100) and orally administered to six hyperglycemic beagle dogs. The results show that there is no significant difference ($P > .05$) among the insulin capsules containing any of the three polymers with respect to C_{max}, AUC, and RH. From the reduction in plasma glucose levels, it can be stated that these polymers are effective to some extent in protecting the insulin from degradation, and also in promoting insulin absorption. The insulin capsules containing these polymers produced a C_{max} of 82–88% at t_{max} of 3 hr, RH of 4.3±2.3% to 6.5±5.1%, and AUC of 34.1±16.9% to 51.6±40.7% reduction compared to that of SC injection of 3 U/kg regular human insulin. The insulin enteric-coated capsules containing no polymers did not produce any hypoglycemic effect.

Figure 2 shows the plasma glucose levels (percentage of initial) of six hyperglycemic beagle dogs after oral administration of enteric-coated capsules containing insulin (6 U/kg) and 50 mg of PC or HPMC prepared by freeze-drying. The resulting freeze-dried particles were sieved to produce a particle size range of 180–315 μm, filled into capsules. The results show that preparing insulin and the bioadhesive polymers by freeze-drying did not significantly ($P > .05$) improve the hypoglycemic effect of these insulin capsules when compared to that effect produced by capsules containing insulin and these polymers physically mixed. It produced a C_{max} of 78.3±8.2% and 80.3±7.7%, an AUC of

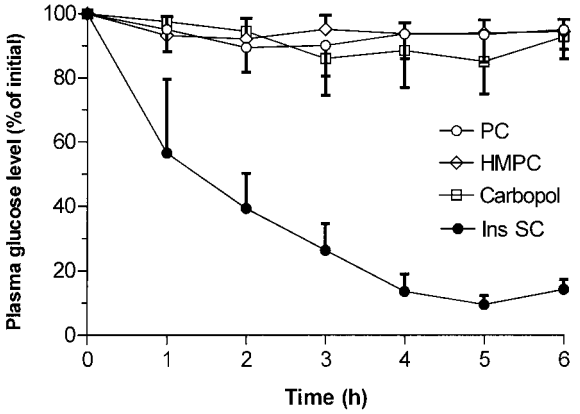

Figure 1. Plasma glucose level (% of initial) of six hyperglycemic beagle dogs after oral administration of enteric-coated capsules containing insulin (6 U/kg) physically mixed with 50 mg of either polycarbophil (PC), hydroxypropyl methylcellulose (HPMC), or carbopol 934 compared with insulin SC injection (Ins SC, 3 U/kg).

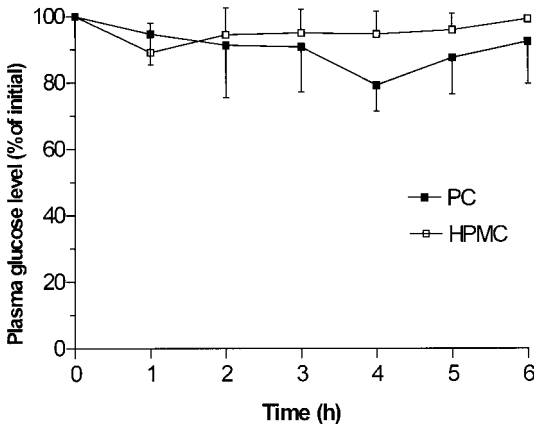

Figure 2. Plasma glucose level (% of initial) of six hyperglycemic beagle dogs after oral administration of enteric-coated insulin capsules (6 U/kg) containing 50 mg of polycarbophil (PC) or hydroxypropyl methylcellulose (HPMC) and prepared as freeze-dried granules.

59.6±60.5% and 30.7±33.8% reduction, and an RH of 7.5±7.6% and 3.9±4.3%, respectively.

Bioadhesive polymers are used primarily in oral preparations to increase the length of stay of drugs in the gastrointestinal tract (12). They could improve therapy by enhancing the drug absorption and increasing the intimacy and duration of contact of the dosage form with absorbing tissues (13). The bioadhesive polymers are able to increase the permeability of epithelial tissues and to simultaneously inhibit proteolytic enzymes (14). These polymers, because of their large molecular weight, are not absorbed and are not expected to exert any undesirable effects. The choice of these bioadhesive polymers, in the present study, was based upon numerous reports (15–19) indicating that polycarbophil and carbopol 934 have the best bioadhesive qualities, and have proven to be useful for drug delivery. They are also safe and approved by the Food and Drug Administration for use in humans, where polycarbophil is approved for use as an antidiarrheal and laxative product (20). Carbopol 934 is also used extensively within the pharmaceutical industry as a thickening agent, a base for wound dressing, an ophthalmic vehicle, and in transdermal systems (21).

Figure 3 shows the effect of enteric-coated capsules containing sodium salicylate (50 mg/capsule) added to insulin as a physical mixture or by wet granulation using 10% polyvinyl pyrollidone (PVP) as wetting agent. The results show that the enteric-coated capsules containing the physical mixture of sodium salicylate and insulin reduced plasma glucose level (18.5±8.2%) by the sixth hour, producing an AUC of 69.4±48.6% reduction and RH of 8.7±6.12% compared to that of SC injection of insulin (3 U/kg). The wet granulation of insulin and sodium salicylate using 10% PVP did not improve the hypoglycemic effect of insulin enteric-coated capsules. However, it significantly reduced the plasma glucose level from 4–6 hr post-administration.

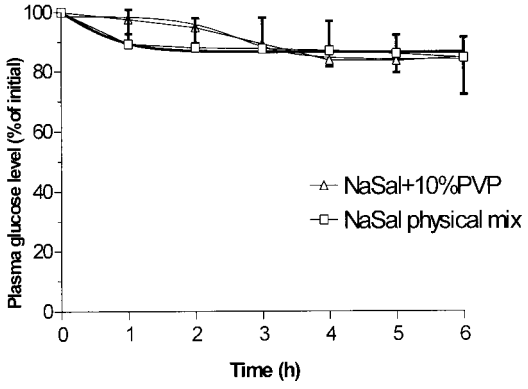

Figure 3. Plasma glucose level (% of initial) of six hyperglycemic beagle dogs after oral administration of enteric-coated insulin capsules (6 U/kg) containing 50 mg of sodium salicylate (NaSal) and prepared by physical mixing or by wet granulation using 10% polyvinyl pyrollidone (PVP).

Insulin was dissolved in sodium salicylate (50 or 100 mg) solution (2 mL) in distilled water, and polycarbophil was added and allowed to swell. The mass was freeze dried. The purpose of these preparations was to include insulin in PC to further protect it from the intestinal proteolytic enzymes. Insulin was dissolved in sodium salicylate aqueous solution as it has been shown (22) that 1.5 M sodium salicylate increased insulin solubility 7875 times. Salicylate was also found to promote absorption by acting on both the apical cell membrane and the tight junctions between cells (23–25). Salicylates may also act on protein components of plasma membranes and small intestine brush border membranes (26). Salicylates can also affect the non-protein thiols (25,27), which are believed to play an important role in maintaining cell integrity (23) and in preventing uptake of hydrophilic compounds. Nishihata et al. (25) and Suzuka et al. (27) showed that salicylates decreased the levels of non-protein thiols in intestinal tissues and isolated enterocytes. The decrease in plasma glucose levels of the diabetic dogs after oral administration of enteric-coated capsules containing the freeze-dried granules with 50 or 100 mg sodium salicylate, or 50 mg in freeze-dried form with 50 mg free sodium salicylate, is shown in Fig. 4. The capsules containing 100 mg sodium salicylate produced a significant ($P < .05$) reduction of the initial plasma glucose levels by the fifth hour, producing a C_{max} of 68.1±3.2%, an AUC of 79±21.0% reduction, and an RH of 10±2.6%. These values of AUC and RH are not significantly different from those of insulin capsules containing freeze-dried granules with 50 mg sodium salicylate, or those containing 50 mg with another 50 mg free sodium salicylate as physical mixture. These results show that sodium salicylate is an efficient absorption promoter that enhances insulin absorption after oral administration of insulin enteric-coated capsules.

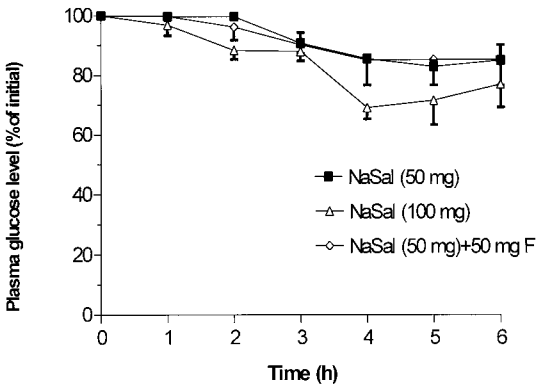

Figure 4. Plasma glucose level (% of initial) of six hyperglycemic beagle dogs after oral administration of enteric-coated insulin capsules (6 U/kg) containing 50 mg polycarbophil (PC) and 50 or 100 mg sodium salicylate (NaSal) and prepared as freeze-dried granules, or containing 50 mg NaSal as freeze-dried granules and 50 mg free NaSal (F).

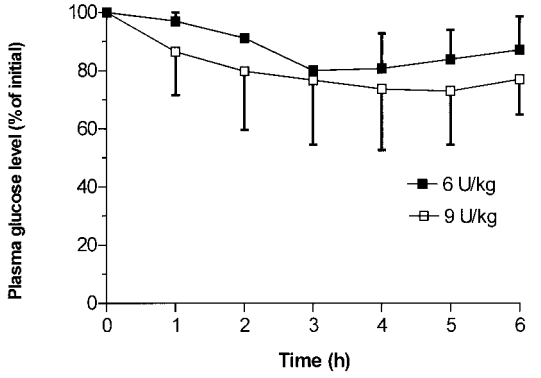

Figure 5. Plasma glucose level (% of initial) of six hyperglycemic beagle dogs after oral administration of enteric-coated insulin capsules (6 and 9 U/kg) containing 50 mg sodium salicylate (NaSal) and 50 mg polycarbophil (PC) and prepared as granules by wetting with alcohol.

Figure 5 shows the effect of enteric-coated capsules containing different doses of insulin (6 and 9 U/kg), mixed with 50 mg sodium salicylate and 50 mg PC, and granulated with a few drops of absolute alcohol. Alcohol was allowed to evaporate at room temperature. These capsules containing insulin 6 U/kg resulted in a significant ($P<.01$) lowering in plasma glucose levels, producing C_{max} of 74.9±4.7% at t_{max} of 3 hr and lasted to the end of the experiment, producing an AUC of 73.2±27.9% reduction and an RH of 9.2±3.5%. The capsules containing 9 U/kg produced a gradual significant ($P<.05$) lowering in plasma glucose levels by the second hour, which increased ($P<.01$) at the third and fourth hour and reached its C_{max} of 71.2±16.5% at the fifth hour ($P<.001$).

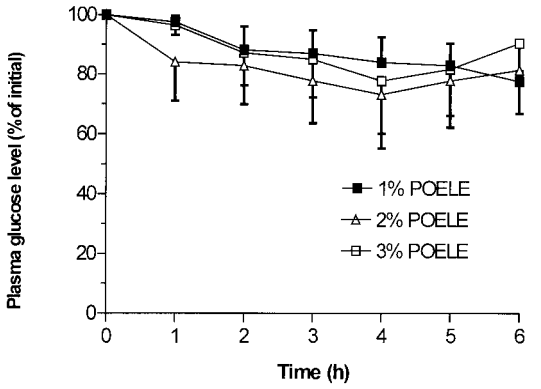

Figure 6. Plasma glucose level (% of initial) of six hyperglycemic beagle dogs after oral administration of enteric-coated insulin capsules (9 U/kg) containing 50 mg polycarbophil (PC) and different concentrations (1%, 2%, 3%) of polyoxyethylene-9-lauryl ether (POELE) and prepared as granules by wetting with alcohol.

The increase in insulin dose from 6 to 9 U/kg resulted in an increase in AUC from 73.2±27.9% to 121.4±102.66% reduction, but the RH remained almost the same, producing a value of 10.2±8.6%. The higher dose of insulin, however, showed higher variability between dogs; the AUC was 121.4±102.7% reduction.

The effect of enteric-coated insulin (9 U/kg) capsules containing different concentrations of polyoxyethylene-9-lauryl ether (POELE) (1, 2, and 3%) and 50 mg PC on the hypoglycemic response in diabetic beagle dogs is shown in Fig. 6.

The results show that the 2% concentration of POELE is the optimum one to be used. It produced a significant lowering in plasma glucose levels from the first hour, reaching a C_{max} of 72.8±15.2% by the fourth hour and remained significantly lower than the initial value (0 hr) until the end of the experiment. This resulted in an AUC of 113.5±80.8% reduction compared to 71.4±30.9% and 76.4±63.2% reduction when 1% and 3% POELE were used, respectively. It is clear from these experiments that increasing or decreasing the POELE concentration from 2% results in decreasing the AUC and RH. Non-ionic surfactants like

POELE might influence drug absorption from the gastrointestinal tract (GIT) by potentially disrupting the integrity and function of the membrane, which will lead to enhanced absorption across the GIT barrier. While inhibition of drug absorption may occur as a consequence of a drug being incorporated into surfactant micelles, surfactants can also exert an influence on drug metabolizing enzymes. It has been shown that POELE reduces the rate of insulin proteolysis in the nasal homogenates of albino rats (28).

From the previous results, it could be concluded that insulin absorption might be accomplished by oral administration of a suitably designed product containing insulin and sodium salicylate or POELE, provided that insulin was protected against degradation by a suitable coating during its passage to the absorption site. The studies presented here indicate that the problems of delivering insulin by the oral route can be minimized; although delivery by this route is not bioequivalent to the parenteral routes, the convenience to the patient will, in this case, outweigh the demand for complete bioequivalency.

ACKNOWLEDGMENTS

The investigators would like to acknowledge the financial support of the King Abdulaziz City for Science and Technology (KACST) to carry out this project through Grant No. AT/17/11.

We are also grateful to Mr. Abdullah A. Abdulrazig for his expert technical assistance throughout this work.

REFERENCES

1. Murlin, J.R.; Hawley, E.E. Absorption of Insulin from the Alimentary Tract of Depancreatized Dogs when Protected by Blood Serum. Am. J. Physiol. **1927**, *83*, 147–161.
2. Patel, H.M.; Harding, H.G.L.; Logue, F.; Kesson, C.M.; MacCuish, A.C.; McKenzie, J.F.; Ryman, B.E.; Scobie, I.N. Intra-jejunal Absorption of Liposomally Entrapped Insulin in Normal Man. Biochem. Soc. Trans. **1978**, *6*, 784–785.
3. Arrieta-Molero, J.F.; Aleck, K.M.; Sinha, M.K.; Brownscheidle, C.M.; Shaprio, L.J.; Sperling, M.A. Orally Administered Liposome-Entrapped Insulin in Diabetic Animals. Hormone. Res. **1982**, *16*, 249–256.
4. Taniguchi, K.; Muranishi, S.; Sezaki, H. Enhanced Intestinal Permeability of Macromolecules. Int. J. Pharm. **1980**, *4*, 219–223.
5. Loehry, C.A.; Axon, A.T.R.; Hilton, P.J.; Hider, R.C.; Creamer, B. Permeability of the Small Intestine to Substances of Different Molecular Weight. Gut **1970**, *11*, 466–470.
6. Touitou, E.; Rubinstein, A. Targeted Enteral Delivery of Insulin to Rats. Int. J. Pharm. **1986**, *30*, 95–99.
7. Hosny, E.A.; Khan Ghilzai, N.M.; Al-Dhawalie, A.H. Effective Intestinal Absorption of Insulin in Diabetic Rats Using Enteric-Coated Capsules Containing Sodium Salicylate. Drug Dev. Ind. Pharm. **1994**, *21*, 1583–1589.
8. Lowman, A.M.; Morishita, M.; Kajita, M.; Nagai, T.; Peppas, N.A. Oral Delivery of Insulin Using pH-Responsive Complexation Gels. J. Pharm. Sci. **1999**, *88*, 933–937.
9. Damge, C.; Vranckx, H.; Balschmidt, P.; Couvreur, P. Poly(alkyl cyanoacrylate) Nanospheres for Oral Administration of Insulin. J. Pharm. Sci. **1997**, *86*, 1403–1409.
10. Cunha, A.S.; Grossiord, J.L.; Puisieux, F.; Seiller, M. Insulin in w/o/w Multiple Emulsions: Biological Activity After Oral Administration in Normal and Diabetic Rats. J. Microencaps. **1997**, *14*, 321–333.
11. Shichiri, M.; Kawamori, R.; Goriya, Y.; Oji, N.; Shigeta, Y. A Model for Evaluation of the Peroral Insulin Therapy: Short-term Treatment of Alloxan Diabetic Rats with Oral Water-in-Oil-in-Water Insulin Emulsion. Endocrinol. Jpn. **1976**, *6*, 493–498.
12. Longer, M.A.; Ch'ng, H.S.; Robinson, J.R. Bioadhesive Polymers as Platforms for Oral Controlled Drug Delivery. III. Oral Delivery of Chlorthiazide Using a Bioadhesive Polymer. J. Pharm. Sci. **1985**, *74*, 406–411.
13. Ch'ng, H.S.; Park, H.; Kelly, P.; Robinson, J.R. Bioadhesive Polymers as Platforms for Oral Controlled Drug Delivery. II. Synthesis and Evaluation of Some Swelling, Water Insoluble Bioadhesive Polymers. J. Pharm. Sci. **1985**, *74*, 399–405.
14. Lue Ben, H.H.; Lehr, C.M.; Rentel, C.O.; Noach, A.B.; de-Boer, A.G.; Verhoef, J.C.; Junginger, H.E. Bioadhesive Polymers for Peroral Delivery of Peptide Drugs. J. Contr. Rel. **1994**, *29*, 329–338.
15. Hosny, E.; Niazy, E.; El-Dardiri, M. Effect of Polycarbophil Concentration on Diclofenac Sodium Bioavailability from Suppositories in Beagle Dogs. Int. J. Pharm. **1996**, *136*, 37–41.
16. Hosny, E.; Niazy, E.; El-Gorashi, S. Effect of Polycarbophil Concentration on In-Vitro Release and In-Vivo Availability in Beagle Dogs of Dihydroergotamine Mesylate Suppositories. Int. J. Pharm. **1995**, *117*, 147–150.

17. Park, H.; Robinson, R. Physico-chemical Properties of Water-Insoluble Polymers Important to Mucin/Epithelial Adhesion. J. Contr. Rel. **1985**, *2*, 47–57.
18. Deasy, P.; O'Neill, C. Bioadhesive Dosage Form for Peroral Administration of Timolol Base. Pharm. Acta Helv. **1989**, *64*, 231-235.
19. Hosny, E. Polycarbophil as a Controlled Release Matrix. Int. J. Pharm. **1993**, *98*, 235–238.
20. Reynolds, J. *Martindale: The Extra Pharmacopoeia XXX*; The Pharmaceutical Press: London, 1993; 870.
21. Craig, D.; Tamburic, S.; Bucklon, G.; Newton, J. An Investigation into the Structure and Properties of Carbopol 934 Gels Using Dielectric Spectroscopy and Oscillatory Rheometry. J. Contr. Rel. **1994**, *30*, 213–223.
22. Touitou, E.; Alhaique, F.; Fisher, P.; Memoli, A.; Riccieri, F.M.; Santucci, E. Prevention of Molecular Self-association by Sodium Salicylate: Effect on Insulin and 6-Carboxyfluorescein. J. Pharm. Sci. **1987**, *76*, 791–793.
23. Szabo, S.; Trier, J.S.; Frankel, P.W. Sulfhydryl Compounds May Mediate Gastric Cytoprotection. Science **1981**, *214*, 200–202.
24. Kajii, T.; Hori, T.; Hayashi, M.; Awazu, S. Effect of Salicylic Acid on the Permeability of the Plasma Membrane of the Small Intestine of the Rat: Fluorescence Spectroscopic Approval to Elucidate the Mechanism of Promoted Drug Absorption. J. Pharm. Sci. **1986**, *75*, 475–478.
25. Nishihata, T.; Nghiem, B.T.; Yoshitomi, H.; Lee, C.S.; Dillsaver, M.; Higuchi, T.; Choh, T.; Suzuka, T.; Furuay, A.; Kamada, A. Changes in Intestinal Mucosal Permeability Caused by Non-protein Thiol Loss in Rats. Pharm. Res. **1986**, *3*, 345–351.
26. Nishihata, T.; Higuchi, T. Ortho-hydroxybenzoates May Act at the Protein Fraction to Enhance Membrane Permeability. Biochim. Biophys. Acta **1984**, *775*, 269–271.
27. Suzuka, T.; Yata, N.; Sakai, K.; Nishihata, T. The Effects of Salicylate Concentration on the Uptake of Salicylate and Cefmetazole into Rat Isolated Small Intestine Epithelial Cells. J. Pharm. Pharmacol. **1988**, *40*, 469–472.
28. Hayakawa, E.; Yamamoto, A.; Shoji, Y.; Lee, V.H. Effect of Sodium Glycocholate and Polyoxyethylene-9-lauryl Ether on the Hydrolysis of Varying Concentrations of Insulin in the Nasal Homogenates of the Albino Rabbit. Life Sci. **1989**, *45*, 167–174.