Eudragit S100 Entrapped Insulin Microspheres for Oral Delivery

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ABSTRACT

The purpose of this research was to investigate whether Eudragit S100 microspheres have the potential to serve as an oral carrier for peptide drugs like insulin. Microspheres were prepared using water-in oil-in water emulsion solvent evaporation technique with polysorbate 20 as a dispersing agent in the internal aqueous phase and polyvinyl alcohol (PVA)/polyvinyl pyrrolidone as a stabilizer in the external aqueous phase. The use of smaller internal aqueous-phase volume (50 μL) and external aqueous-phase volume (25 mL) containing PVA in the manufacturing process resulted in maximum encapsulation efficiency (81.8% ± 0.9%). PVA-stabilized microspheres having maximum drug encapsulation released 2.5% insulin at pH 1.0 in 2 hours. In phosphate buffer (pH 7.4), microspheres showed an initial burst release of 22% in 1 hour with an additional 28% release in the next 5 hours. The smaller the volumes of internal and external aqueous phase, the lower the initial burst release. The release of drug from microspheres followed Higuchi kinetics. Scanning electron microscopy of PVA-stabilized microspheres demonstrated spherical particles with smooth surface, and laser diffractometry revealed a mean particle size of 32.51 ± 20 μm. Oral administration of PVA stabilized microspheres in normal albino rabbits (equivalent to 6.6 IU insulin/kg of animal weight) demonstrated a 24% reduction in blood glucose level, with maximum plasma glucose reduction of 76 ± 3.0% in 2 hours and effect continuing up to 6 hours. The area under the percentage glucose reduction-time curve was 93.75%. Thus, our results indicate that Eudragit S100 microspheres on oral administration can protect insulin from proteolytic degradation in the gastrointestinal tract and produce hypoglycemic effect.

KEYWORDS: insulin, oral, Eudragit S100, microspheres, hypoglycaemic

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INTRODUCTION

Peptides exhibit the widest structural and functional variation and are integral to the regulation and maintenance of all biological processes. The increased biochemical and structural complexity of proteins compared with conventional drug-based pharmaceuticals makes formulation design for delivery of therapeutic proteins a very challenging and difficult task. The key to the success of proteins as pharmaceuticals is to have in place an efficient drug delivery system that allows the protein drugs to gain access to their target sites at the right time and for the proper duration. Four factors that must be considered to fulfill this goal are route of administration, pattern of drug release, method of delivery, and fabrication of formulation.[1]

The delivery of insulin by nonparenteral routes has gained significant attention over last 2 decades. The alternate routes explored are ocular,[2,3] nasal,[4] buccal,[5,6] rectal,[7] pulmonary,[8,9] and oral.[10,11] Among all of the alternative routes of administration of insulin, the oral route offers the maximum advantage in terms of patient compliance. However, there are several limitations to the oral route. These include low oral bioavailability because of degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, poor permeability across intestinal epithelium because of its high molecular weight, and lack of lipophilicity.

Researchers have attempted to deliver insulin orally using poly(alkyl cyanoacrylate)[12] and poly(lactide-coglycolide)[11] nanospheres, poly(vinyl alcohol)-gel spheres with protease inhibitor,[13] bioadhesives, like hydroxypropyl cellulose, with permeation enhancers, like salicylate,[14] permeation enhancers, like bile salt-fatty acid-mixed micelles,[15] hydroxypropyl methylcellulose phthalate enteric microspheres with sodium *N*-(8-[2 hydroxy benzoyl] amino) caprylate (SNAC),[16] and Eudragit S100-coated insulin hard-gelatin capsules with sodium salicylate as a permeation enhancer.[10] Poly(alkyl cyanoacrylate) nanospheres without the assistance of surfactants (like poloxamer 188 and deoxycholic acid) or surfactants and miglyol 812 cannot protect insulin against in vivo proteolytic degradation,[12] polylactide-coglycolide being a nonenteric polymer would have pH-independent release, and the released insulin would be degraded by proteolytic enzymes.[11] Poly(vinyl alcohol)-gel microspheres also suffer from a

similar drawback and, thus, need the protection of a protease inhibitor.[13] Hydroxypropyl methylcellulose phthalate dissolves at a pH between 5 and 5.5; thus, it would release insulin in the small intestine itself, where it will be degraded by trypsin and chymotrypsin. In fact, insulin-loaded hydroxypropyl methylcellulose phthalate microspheres made by double-emulsion solvent evaporation, given orally with SNAC (a permeation enhancer), have been reported to be weakly hypoglycemic in normal rats compared with an oral insulin solution and SNAC.[16] Insulin is better absorbed from the ileum and large intestine compared with jejunum.[13] Thus, a polymer that would release the drug at pH > 7 appears to be suitable for oral insulin delivery. Eudragit S100 is such a polymer. It is an anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester and has a pH-dependent solubility. It is slowly soluble in the region of the digestive tract where juices are neutral to weakly alkaline. When used to entrap insulin in microspheres, it is expected to protect insulin from degradation by gastric juice and allow it to be released in the region of the gastrointestinal tract of pH > 7, that is, the large intestine or colon where proteolytic enzymes are low in concentration. It is widely known that permeation enhancers affect not only the cell membrane but also the intercellular route with resultant increased permeability. One of the most commonly used permeation enhancers, salicylate, besides damaging the cell membrane also acts on the protein components of the plasma membrane, red blood cell membrane, and small intestine brush border membranes[17,18] and decreases the levels of nonprotein thiols,[19] which are believed to play a major role in maintaining cell integrity. Moreover, salicylate is a potent inhibitor of prostaglandin (PGE_2 and PGI_2), which has marked cytoprotective activity; therefore, it tends to irritate the mucosa causing epigastric stress, ultimately leading to ulceration.[20] Hence, considering the ill effects caused by these agents on long-term use, it appears reasonable to formulate insulin microspheres without any permeation enhancer. Thus, the purpose of this research was to investigate whether Eudragit S100 microspheres have the potential to serve as an oral carrier for peptide drugs, like insulin. Attempts were made to prepare insulin-entrapped Eudragit S100 microspheres and to study drug loading and in vitro release profile. Efforts were also made to evaluate the oral hypoglycemic activity of microspheres having maximum insulin encapsulation.

MATERIALS AND METHODS

Methacrylic acid copolymer (Eudragit S100) was supplied as a gift by Röhm Pharma (Weiterstadt, Germany). Porcine insulin injection, conforming to Indian Pharmacopoeia, (Abbott India Ltd, Mumbai, India) was subjected to purification and concentration as mentioned subsequently. Polysorbate 20, polyvinyl alcohol ([PVA] cold, M_w 30 to 70,000), polyvinyl pyrrolidone ([PVP] grade K 29-32), potassium dihydrogen phosphate, and sodium hydroxide were obtained from Central Drug House (Mumbai, India). Ethanol, dichloromethane, isopropyl alcohol, and hydrochloric acid (HCl) were obtained from Merck (Darmstadt, Germany). Deionized water was used for all of the experiments.

Purification and Concentration of Insulin

Porcine insulin injection conforming to Indian Pharmacopoeia (40 IU/mL) was used as starting material. A concentrated solution of insulin was obtained by using a Centricon pressure filtration unit (Amicon, Beverly, MA). It contained a filter capable of removing particles having a molecular weight $\leq$3,500 daltons. Additives in the commercial product, such as m-cresol, glycerin, and hydrochloric acid, were removed. All of the excess water was removed to yield a concentrated insulin solution. The aqueous insulin solution was centrifuged in SORVALL Evolution RC centrifuge, in a Centricon unit at 3,000 rpm until the volume of insulin solution reduced considerably (from 40 to 1 to 2 mL). The temperature was maintained at 4°C throughout centrifugation. The concentrated insulin solution was stored in an Eppendorf in a refrigerator between 2 and 8°C for future use. The protein content of the concentrated insulin solution was estimated using Micro BCA Protein Assay Reagent kit (Pierce, Rockford, IL).

Preparation of Microspheres

Insulin-loaded microspheres were prepared by double-emulsion solvent evaporation technique. Rosa et al[21] encapsulated insulin in poly(lactide-coglycolide) (PLGA) microspheres by double-emulsion solvent evaporation. The authors reported that polysorbate 20 at 3% w/v concentration was most effective in giving regular-shaped particles with good insulin loading and improved insulin stability in the microspheres. Hence, polysorbate 20 was used in our study. In a typical experiment, an insulin aqueous solution of 32-mg/mL (ie, 800 IU/mL) concentration was taken in a 5-mL beaker. The dispersing agent polysorbate 20, was added to this "internal aqueous phase" ([IAP] W_1) at a concentration of 3% v/v and mixed well. IAP (W_1; 1.0, 0.5, 0.2, 0.1, or 0.05 mL) was emulsified with 5 mL of organic phase for 1 minute using an ultrasonic disruptor (30-W output power, 40% duty cycle; Branson Sonifier 450, Danbury, CT). The temperature was maintained at 4°C using an ice bath. The organic phase (O) consisted of 300 mg of Eudragit S100 polymer in 5 mL of a "mixed solvent system" of dichloromethane-to-ethanol-to-isopropyl alcohol in a ratio of 5:6:4.[22] The resulting "primary emulsion" (W_1/O) was added drop by drop to "external aqueous

Table 1. Different Batches of Eudragit S100 Microspheres*

S No.	Formulation Code	Vol of IAP (W_1) (mL)	W_1:O	Vol of EAP (W_2) (mL)	O:W_2
1	S-α 1	1.0	1:5	100	1:20
2	S-α 2	0.5	1:10	100	1:20
3	S-α 3	0.2	1:25	100	1:20
4	S-α 4	0.1	1:50	100	1:20
5	S-α 5	0.05	1:100	100	1:20
6	S-β 1	0.05	1:100	50	1:10
7	S-β 2	0.05	1:100	25	1:5
8	S-γ 1	0.05	1:100	25	1:5

*Volume of organic phase (O) = 5 ml; Stabilizer S-α 1 to S-β 2 = PVA; S-γ 1 PVP. Vol indicates volume.

phase" ([EAP] W_2; 100, 50, or 25 mL) of 2% w/v PVA solution. The aqueous PVA solution acts as an emulsion stabilizer. Emulsification was continued using a homogenizer (750 W; Virtis, SENTRY Microprocessor) at 10,000 rpm for 4 minutes to form "multiple emulsion" ($W_1/O/W_2$). The resulting $W_1/O/W_2$ emulsion was stirred at room temperature for 16 to 18 hours with a magnetic stirrer to allow the solvent to evaporate. The microspheres were collected and washed 3 times with distilled water by centrifugation at 10,000*g* for 10 minutes. The microspheres were resuspended in distilled water and lyophilized for 24 hours. The final product was stored in a desiccator at 2 to 8°C. Microspheres made using 1.0, 0.5, 0.2, 0.1, or 0.05 mL of IAP, 5 mL of O, and 100 mL of EAP were designated as *S*-α1, *S*-α2, *S*-α3, *S*-α4, and *S*-α5, respectively. Similarly microspheres made with 0.05 mL of IAP, 5 mL of O, and 50 or 25 mL of EAP were designated as *S*-β1 and *S*-β2. One batch was made with 0.05 mL of IAP, 5 mL of O, and 25 mL of EAP containing 2% PVP, and the same was designated as *S*-γ1 (Table 1).

Particle Size Measurements

Particle size was measured for a selected batch of microspheres. Freeze-dried microspheres were dispersed in 2-methyl propane-1-ol (Iso-butyl-alcohol) after treatment in an ultrasonic disperser (Seishin) for 5 minutes to bring about disaggregation of the microspheres. The microspheres were sized by "laser diffractometry" using laser particle size analyzer, GALAI, CIS-1. Particle size was expressed as volume mean diameter in microns (±SD) of values collected from 2 different batches.

Microsphere Morphology and Surface Characteristics

Microsphere shape and morphology were analyzed by scanning electron microscopy (Leo, VP-435, Cambridge, United Kingdom) for selected batches. Samples mounted on aluminum stubs were sputter-coated with carbon under reduced pressures and 30-nm to 40-nm thick carbon coat was applied using Agar sputter carbon coater B-7367. The sample assembly was placed in the microscope, and a vacuum was created. The microspheres were observed under the scanning electron microscope at an accelerating voltage of 15 KV.

Encapsulation Efficiency

Twenty milligrams of microspheres were accurately weighed. They were added to 5 mL of ethanol. After the microspheres dissolved completely, 5 mL of phosphate buffer (pH 7.4) was added to this solution and mixed thoroughly. The resulting solution was analyzed for insulin content by measuring absorbance in a UV-spectrophotometer[23] (1601, Shimadzu, Kyoto, Japan) at 276 nm using phosphate buffer (pH 7.4) and ethanol mixture (1:1) as blank. Results were expressed as mean (±SD) of 3 experiments. Encapsulation efficiency (%) was calculated using the following formula:[21]

$$\text{Actual loading } (\%) = \text{mg of encapsulated insulin/100 mg microspheres}$$

$$\text{Encapsulation efficiency } (\%) = (\text{actual insulin loading/theoretical insulin loading}) \times 100$$

Enteric Nature of Microspheres

This test was performed to determine whether the drug would be released in the acidic environment of the stomach (ie, pH between 1 and 3). Twenty milligrams of microspheres were soaked in 10 mL of 0.1 N HCl that was equilibrated at 37°C ± 0.5°C in a water bath. After the immersion of the microspheres for 2 hours, the sample was centrifuged (3,000 rpm, 15 minutes, at room temperature), and the insulin content of the supernatant was analyzed by measuring absorbance in UV spectrophotometer[23] at 276 nm against 0.1 N HCl blank.

Table 2. Effect of Formulation Variables on Yield and Encapsulation Efficiency of Eudragit S100 Microspheres Prepared by Double-Emulsion Solvent Evaporation Technique*

S No.	Formulation Code	Yield % (Mean ± SD)	Theoretical Loading (%)	Actual Loading (%)	Encapsulation Efficiency % (Mean ± SD)
1	S-α1	82.5 ± 2.2	9.63	0.79	8.22 ± 1.5
2	S-α2	64.0 ± 1.4	5.06	0.88	17.6 ± 0.9
3	S-α3	46.5 ± 4.9	2.0	0.60	29.5 ± 2.8
4	S-α4	36.5 ± 2.1	1.0	0.44	44.3 ± 0.4
5	S-α5	42.5 ± 3.5	0.53	0.38	71.7 ± 1.8
6	S-β1	55.5 ± 0.7	0.53	0.40	76.7 ± 1.0
7	S-β2	58.3 ± 2.3	0.53	0.43	81.8 ± 0.9
8	S-γ1	57.4 ± 3.5	0.53	0.42	79.8 ± 1.6

In Vitro Drug Release

In vitro release of insulin from microspheres was evaluated in phosphate buffer (pH 7.4). The buffer was prepared by mixing 5.0 mL of 0.2 M KH_2PO_4 and 3.9 mL of 0.2 M NaOH and volume made up to 100 mL with water. The pH was adjusted to 7.4 with 2 M NaOH or 2 M HCl. Microspheres equivalent to 10 IU of insulin were transferred to the prewarmed dissolution media (20 mL) and maintained at 37°C ± 0.5°C under stirring at 50 rpm. Samples were withdrawn every hour up to 6 hours, and the volume was replaced immediately by fresh phosphate buffer. The sample withdrawn was centrifuged (3,000 rpm, 15 minutes, at room temperature). Insulin content of the supernatant was estimated by measuring absorbance in a UV-spectrophotometer[23] at 276 nm against a phosphate buffer (pH 7.4) blank. Results were expressed as mean (±SD) of 3 experiments.

In Vivo Studies

Healthy albino rabbits of either sex, weighing 2.5 ± 0.2 kg, were housed in the "animal house" of the institute. All of the animals used in this study were caged according to the principles established for care and use of laboratory animals. Animals were fasted over 18 hours before starting the test, but water was provided ad libitum. The animals were divided into 3 groups of 3 animals each. The treated group was administered oral insulin-loaded microspheres (equivalent to 6.6 I.U. insulin per kg of animal weight) by accurately weighing them into dry syringes followed by flushing with 10 mL of water through a stomach tube. The second group was administered oral insulin solution (equivalent to 6.6 I.U. insulin per kg of animal weight) by flushing with 10 mL of water through a stomach tube. The control group was given 10 mL of distilled water using a stomach tube. Insulin absorption was monitored by its effect on the blood glucose level. Blood samples were obtained from the external marginal ear vein at 0, 30, 60, 90, 180, 240, and 360 minutes after microsphere or insulin solution or distilled water administration. Blood glucose level was determined immediately after sample withdrawal, using Accutrend Glucometer (Roche Diagnostics, Mannheim, Germany) and was expressed as a percentage of the initial level.[14]

RESULTS AND DISCUSSION

The effect of formulation variables on yield and encapsulation efficiency of insulin-loaded Eudragit S100 microspheres is shown in Table 2. It was observed that as the volume of IAP was decreased from 1.0 to 0.05 mL (or 50 μL), the encapsulation efficiency increased from 8.22% to 71.7%. The results suggest that for higher encapsulation of insulin, a smaller volume of IAP is desirable. Uchida et al[24] have reported a similar finding while encapsulating insulin in polylactic acid microspheres by double-emulsion solvent evaporation method. The batch with formulation code *S*-α5 showed maximum encapsulation efficiency. The optimized ratio of IAP to O was 1:100, that is, 50 μL of IAP and 5 mL of O. The yield of microspheres obtained from batches *S*-α1 to *S*-α5 varied from 82.5% to 42.5%. The low yield in some cases could be attributed to the losses occurring during various steps of processing, such as sticking of the polymeric solution to the glass container, loss of microspheres during the washing step, and so forth. Loss because of sticking could be minimized by using an apparatus made of plastic or polyethylene.

Keeping the ratio of IAP to O constant (ie, 1:100), the effect of changing the volume of EAP on encapsulation efficiency of microspheres was studied next. It was observed that as the volume of EAP was decreased from 100 to 25 mL, the encapsulation efficiency increased from 71.7% to 81.8%. Use of PVP in the EAP (instead of PVA) also provided high-encapsulation efficiency of 79.8%. The yields of these batches of microspheres (*S*-β1, *S*-β2, and *S*-γ1) were between 55% and 58% (Tables 1 and 2).

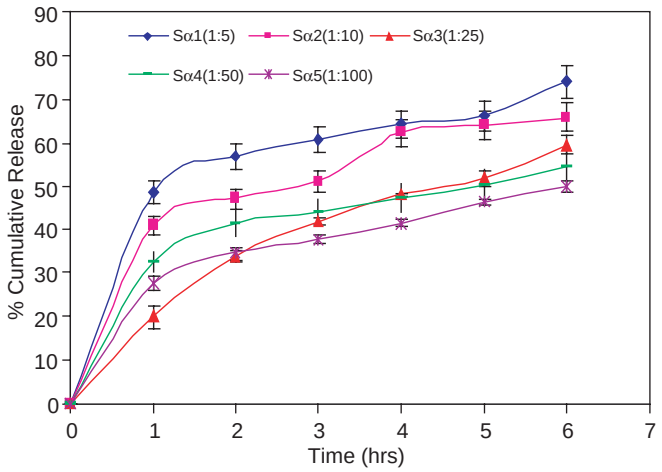

Figure 1. Effect of ratio of volume of IAP to O (organic phase) on in vitro release profile of Eudragit S100 microspheres.

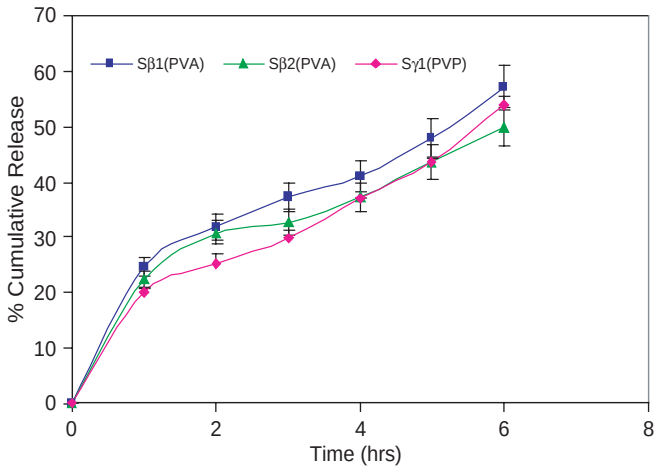

Figure 2. Release profiles of *S*-β1, *S*-β2, and *S*-γ1 batches of microspheres.

Microsphere formulation *S*-β2 showed maximum encapsulation efficiency (81.8%) where the ratio of O: EAP was 1:5. Thus, for maximum encapsulation of insulin in microspheres, the optimized ratio of IAP (W_1) to O to EAP (W_2) was 1:100:500 (ie, 50 μL IAP to 5 mL O to 25 mL of EAP). Rosa et al[21] encapsulated insulin in PLGA microspheres by multiple-emulsion solvent evaporation using a IAP (W_1) to O to EAP (W_2) ratio of 1:10:200 and 2% PVA in EAP and 3% polysorbate 20 as the surfactant for primary emulsion. The authors reported an encapsulation efficiency of 33%.

The transit time of a drug through the absorptive area of the gastrointestinal (GI) tract is between 9 and 12 hours,[25] whereas γ scintigraphy studies confirm a short GI transit time from mouth to cecum of 4 to 6 hours.[26] Thus, assuming a maximum GI tract transit time of 12 hours, a formulation in the cecum is expected to release its drug load within 6 hours. Considering the same, in vitro drug release from all the batches of microspheres was studied for a duration of 6 hours. An initial burst release was observed in the release profile. Smaller IAP was advantageous for controlling the initial burst release. It was noticed that as the ratio of IAP to O was changed from 1:5 to 1:100, the burst release decreased from 48.5% to 27.7% (Figure 1). The reason could be that during formation of primary emulsion, sonication of a lesser volume leads to smaller drug droplet formation with less surface protein; therefore, relatively little quantity of drug will be released during the first hour. For microspheres made with larger IAP (1000 or 500 μL), large droplets are formed during sonication, which could account for high burst release because of surface-associated protein. It would be appropriate to mention here that Rosa et al,[21] while studying insulin release from PLGA microspheres, observed that microsphere formulations having a high quantity of insulin located on or near the surface of microspheres showed higher burst release.

The cumulative percentage release of insulin after 6 hours was found to be higher for batches made with a larger IAP (*S*-α1 = 74%) in comparison to batches made with smaller IAP (*S*-α5 = 49.8%). These results are consistent with those obtained by Uchida et al,[24] who used polylactic acid to encapsulate insulin. Keeping IAP-to-O ratio as 1:100, when EAP was reduced to 50 or 25 mL (*S*-β1 and *S*-β2), burst release was reduced from 27.7% to 24.6% and 22.3%, respectively, and cumulative release after 6 hours was 57% and 50%, respectively (Figure 2). The addition of PVP in the EAP (*S*-γ1) showed 20% release in 1 hour and 54% in 6 hours. The effect of stabilizers (PVA and PVP) added to EAP was studied, keeping other parameters constant. The stabilizer molecules present in the EAP associate with the surface of the protein-containing droplets produced in the primary emulsion and, because of resultant steric and charge effects, prevent their coalescence. Polymer precipitation and gradual diffusion of solvent from the polymer solution droplets into the aqueous continuous phase (W_2) subsequently lead to microparticle hardening. The PVA-stabilized and PVP-stabilized microspheres

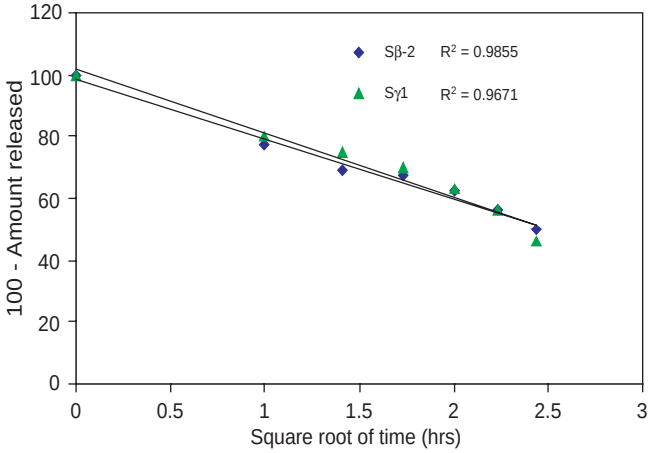

Figure 3. Release of drug from Eudragit S100 microspheres.

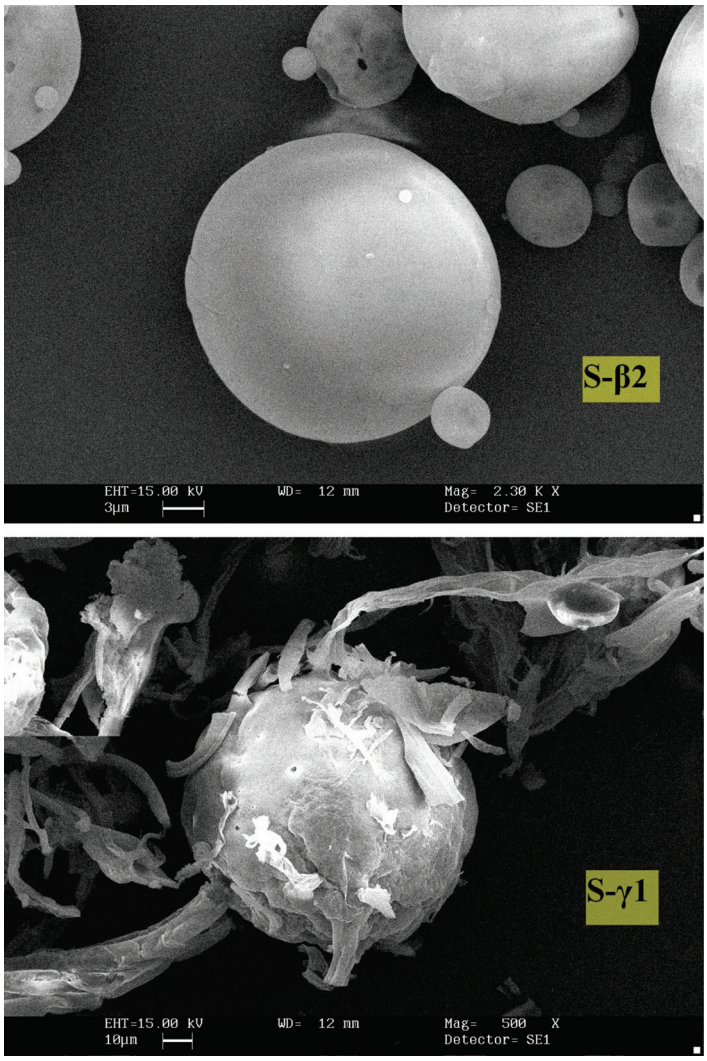

Figure 4. Scanning electron micrographs of insulin-loaded Eudragit S100 microspheres.

(*S*-β2 and *S*-γ1) did not show any significant difference in encapsulation efficiency. The in vitro drug release profiles of the batches revealed a burst effect of 22.3% for *S*-β2 and 20% for *S*-γ1. These results are contrary to those obtained by Coombes et al,[27] who reported that PVP-stabilized PLGA microparticles exhibited higher encapsulation efficiency of ovalbumin (70% for PVP-stabilized and 30% for PVA-stabilized system) and a reduced burst release (20% for PVP-stabilized and 60% for PVA-stabilized system). The release mechanism of drug from microspheres (*S*-β2 and *S*-γ1) was evaluated. A plot of 100-amount released vs $\sqrt{t}$ for the formulations was linear, which indicates a diffusion controlled release, following Higuchi kinetics (Figure 3).

The PVA-stabilized and PVP-stabilized microspheres were subjected to scanning electron microscopy. The shapes of microspheres were mostly spherical, as visible from the photographs. Figure 4 reveals good spherical forms for PVA-stabilized (*S*-β2) microspheres with smooth surface and fiber/thread-like structures in the PVP-stabilized batch (*S*-γ1), indicating PVP as an inferior stabilizer for Eudragit microspheres. Furthermore, PVA-stabilized microspheres (*S*-β2) were tested for enteric nature of the coating. Absolute enteric coating could not be achieved; 2.5% of the drug was released in 0.1 N HCl medium (pH 1) in 2 hours. This could be attributable to adsorption of insulin chains on the surface of microspheres. Another reason could be leaching of drug from the microspheres, where inefficient coating could have occurred. Recently Sajeesh and Sharma[28] have reported that insulin-loaded polymethacrylic acid and polymethacrylic acid-alginate microparticles released around 30% of loaded insulin within 2 hours at pH 1.2. Polymethacrylic acid alginate microparticles showed burst release of 90% loaded insulin in 1 hour at pH 7.4, whereas polymethacrylic acid microparticles exhibited sustained release of insulin for >5 hours, at the same pH. Similarly, insulin-loaded hydroxypropyl methylcellulose phthalate enteric microspheres made by double emulsion solvent evaporation released 20% insulin in 2 hours at pH 1.2 and released the entire drug in 10 minutes in phosphate buffer (pH 6.8), whereas enteric microspheres made by O_1/O_2 emulsion solvent evaporation did not release insulin at pH 1.2 for 2 hours, but released the entire drug at pH 6.8 in 75 minutes.[16] Thus, based on the available literature, we can say that the performance of PVA-stabilized microspheres (*S*-β2) was satisfactory with respect to drug release. The formulation would protect insulin from gastric degradation and would release its drug load slowly at pH 7.4 in the colon. The mean particle size of microspheres (*S*-β2) was 32.51 ± 20 µm. The frequency distribution curve showed Gaussian distribution profile with 98% of microspheres in the size range of 4 to 70 µm.

Mesiha and Sidhom[14] evaluated the hypoglycaemic effect of orally given insulin in rabbits using 2 different absorp-

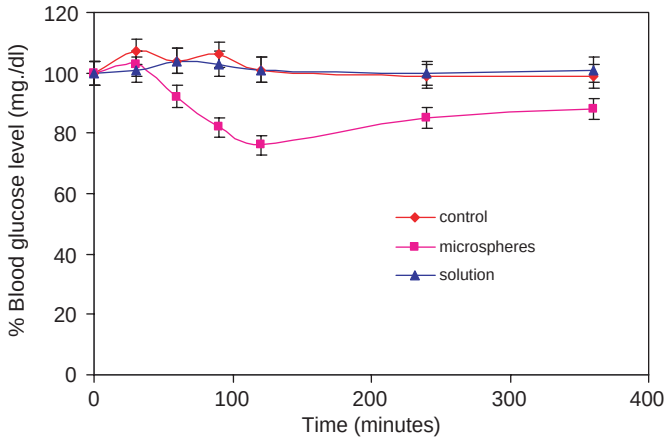

Figure 5. Blood glucose level (% of initial) of albino rabbits after oral administration of insulin-loaded Eudragit S100 microspheres (6.6 IU/kg), insulin solution (equivalent to 6.6 IU/kg), and water to control group. Results are shown as mean ± SD of 3 observations.

tion promoters and 2 different carriers, wherein insulin solution with no additive was given orally as control. Damge et al,[12] while evaluating the biological effect of insulin-loaded poly(alkylcyanoacrylate) oral nanospheres in diabetic rats, administered the control, that is, insulin in miglyol 812 containing 1% poloxamer 188 and 0.01 M deoxycholic acid, by oral route. Recently, Qi and Ping,[16] while evaluating the GI absorption enhancement of insulin from hydroxypropyl methylcellulose phthalate enteric microspheres by SNAC in rats, also used an oral solution of insulin containing SNAC as control. In the same line, in vivo studies were performed in albino rabbits after oral administration of insulin-loaded Eudragit S100 microspheres or aqueous solution of insulin at a dose of 6.6 IU/kg. Formulation *S*-β2 was chosen for in vivo study based on encapsulation efficiency, enteric nature, and in vitro drug release. The hypoglycaemic effect was taken as a monitor for insulin absorption in its physiologically active form. The results are shown in Figure 5. A prominent hypoglycaemic effect was noticed with *S*-β2 formulation from 30 to 120 minutes, and the effect was visible up to 360 minutes. The maximum plasma glucose reduction (% of initial) was found to be 76% ± 3% and the time to reach the maximum plasma glucose reduction was 2 hours. The area under the percentage glucose reduction-time curve, as determined by trapezoidal rule, was 93.75%. Orally administered insulin solution at the same dose showed no reduction in blood glucose level similar to control experiments, which appear to be because of degradation of insulin by proteolytic enzymes in the GI tract or presystemic metabolism. It would be worthwhile to mention here that insulin-loaded poly(alkyl cyanoacrylate) oral nanospheres dispersed in miglyol 812 containing surfactants (1% poloxamer and 0.01 M deoxycholic acid) on oral administration to diabetic rats decreased glycemia by 50% to 60%, but insulin-loaded nanospheres dispersed in water alone did not modify significantly fasted glycemia over the whole experiment similar to control animals, which indicates that poly(alkylcyanoacrylate) nanospheres, as such, cannot protect the insulin against in vivo proteolytic degradation.[12] Similarly, insulin-loaded hydroxypropyl methylcellulose phthalate enteric microspheres (made by double-emulsion solvent evaporation) and SNAC on oral administration to normal rats showed a very weak hypoglycaemic effect compared with that observed with an oral insulin solution containing SNAC, whereas enteric microspheres made by O_1/O_2 emulsion solvent evaporation showed a hypoglycaemic effect only in the presence of SNAC.[16] The data suggest that hydroxypropyl methyl cellulose phthalate enteric microspheres also could not protect insulin against proteolytic degradation in the GI tract and needs the assistance of SNAC for insulin absorption. Insulin-loaded Eudragit S100 microspheres, on the other hand, when administered by oral route along with water produced a hypoglycaemic effect visible from 30 to 360 minutes, indicating that the microspheres protect insulin against proteolytic degradation in the GI tract.

CONCLUSION

Insulin-loaded Eudragit S100 microspheres retard the release of insulin at low pH and release insulin slowly at pH 7.4 in the colon. In vivo study with microspheres demonstrated a prominent hypoglycaemic effect, suggesting that the polymer could protect insulin against proteolytic degradation in the GI tract. Eudragit S100 microspheres, thus, have the potential to serve as an oral carrier for peptide drugs like insulin.

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