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RESEARCH ARTICLE

pH-Sensitive oral insulin delivery systems using Eudragit microspheres

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Abstract

In this paper, we present *in vitro* and *in vivo* release data on pH-sensitive microspheres of Eudragit L100, Eudragit RS100 and their blend systems prepared by double emulsion-solvent evaporation technique for oral delivery of insulin. Of the three systems developed, Eudragit L100 was chosen for preclinical studies. Insulin was encapsulated and *in vitro* experiments performed on insulin-loaded microspheres in pH 1.2 media did not release insulin during the first 2 h, but maximum insulin was released in pH 7.4 buffer media from 4 to 6 h. The microspheres were characterized by scanning electron microscopy to understand particle size, shape and surface morphology. The size of microspheres ranged between 1 and 40 µm. Circular dichroism spectra indicated the structural integrity of insulin during encapsulation as well as after its release in pH 7.4 buffer media. The *in vivo* release studies on diabetic-induced rat models exhibited maximum inhibition of up to 86%, suggesting absorption of insulin in the intestine.

Keywords: Eudragit L100, Eudragit S100, microspheres, oral delivery, insulin, pH-sensitive

Introduction

Intensive efforts over the past decade have resulted in the development of novel types of polymeric carriers for oral insulin delivery[1–5]. The main hurdles in developing oral insulin delivery devices are attributed to poor intrinsic insulin permeability across the biological membrane. Insulin has high molecular weight, and is easily degraded by proteolytic enzymes of the stomach or small intestine[6–8]. However, insulin denaturation/degradation can be overcome by designing a suitable carrier that will protect insulin from harsh environments of the stomach before it reaches gastrointestinal tract (GIT). Therefore, to protect insulin in acidic conditions, efforts have been made to develop pH-sensitive polymers or hydrogels for an effective oral formulation of insulin[9–11]. Various strategies have been adopted to achieve insulin delivery, which include co-administration with absorption enhancers[4], enzyme inhibitors[12,13], chemical modifications[14,15] or use of liposomes[2,16,17].

In the literature, poly(glycolic acid), poly(lactic acid), poly(lactic acid-*co*-glycolic acid), poly[lactic acid-*co*-poly(ethylene glycol)], dextran–PEG as well as pH-sensitive polymers like poly(acrylic acid) and poly(methacrylic acid), graft copolymers of poly(methylmethacrylate) with ethylene glycol, chitosan, cyclodextrin, etc., have been used as carriers in oral insulin delivery[6]. In addition, pH-sensitive hydrogels exhibiting reversible formation of inter-polymer complexes that are insoluble at lower gastric pH, but swell in alkaline conditions of the intestine, and dissociate the complexes to release insulin have been used. In this category of devices, one of the most widely investigated systems is that of pH-sensitive graft polymers of methacrylic acid and polyethylene glycol[7,18,19]. Other studies include the development of complexation polymers[20] and pH responsive hydrogels[21]. Acrylate and methacrylate copolymers are commercially available as Eudragit polymers in different ionic forms, since these are widely employed in the preparation of microspheres to deliver macromolecules[22–24].

In continuation of our ongoing program of research concerning the development of oral insulin devices, we

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present here a novel approach of preparing oral insulin delivery formulations based on Eudragit polymers. Microspheres were prepared by solvent evaporation technique using light liquid paraffin oil as an external phase. The developed formulations were characterized by scanning electron microscopy (SEM) to examine particle size, shape and surface morphology. *In vitro* release of insulin was performed in pH 1.2 and 7.4 buffer solutions at 37°C. *In vivo* experiments were conducted on diabetes-induced rats and by glucose tolerance tests on healthy rats. Pure insulin, encapsulated insulin and released insulin from the Eudragit matrices were evaluated by circular dichroism (CD) for assessing the structural integrity of insulin.

Materials and methods

Materials

Eudragit L100 and S100 were received as gift samples from Degussa India Ltd., Mumbai, India. Human (human recombinant expressed in yeast) and bovine insulin were procured from Sigma Aldrich, St. Louis, MO. Light liquid paraffin and Span-80 were purchased from Himedia, Mumbai, India and s.d. fine chemicals, Mumbai, India, respectively. All other chemicals used were of reagent grade. Milli Q water was used throughout this study.

Preparation of insulin-loaded Eudragit L100 microspheres by solvent evaporation method

Solvent evaporation method is one of the most popular and widely used encapsulation techniques for proteins and peptides[25]. In this work, solvent evaporation method is used to produce insulin-loaded microspheres of uniform size. Two different approaches used to prepare the insulin-loaded formulations are given below.

Method I

In this method, 500 mg of Eudragit L100 was dissolved in a mixture of 5 mL of methanol and 15 mL dichloromethane (DCM) under constant stirring in a 50 mL beaker. To this, 20 mg of human insulin dissolved in 0.5 mL of 0.1 M HCl was added and homogenized at 11,000 rpm for 2 min in an ice bath. The entire solution was transferred to a 250 mL beaker containing 100 mL of light liquid paraffin oil and 0.5% of Span-80 surfactant. The solution was stirred at 600 rpm for 3 h to evaporate the solvent. After 3 h, the microspheres were filtered and washed with 50 mL petroleum ether and 8 mL Milli Q water to remove excess paraffin oil and Span-80. The completely washed microspheres were freeze dried at–40°C for 20 h and stored at –20°C before characterization and further analysis.

Similar method as above was employed for the preparation of blend microspheres of Eudragit L100 and Eudragit S100. Here, 250 mg of Eudragit L100 was dissolved in 10 mL of methanol, whereas 250 mg of Eudragit RS100 was dissolved in 10 mL of DCM under constant stirring in a 50 mL beaker. Both the solutions were mixed with constant stirring, to which 20 mg of human insulin dissolved in 0.5 mL of 0.1 M HCl was added and homogenized at 11,000 rpm for 2 min in an ice bath. The solution was then transferred to a beaker containing 100 mL of light liquid paraffin oil and 0.5% of Span-80 surfactant. The solution was stirred at 600 rpm speed for 3 h to evaporate solvents. After 3 h, microspheres were filtered and washed with petroleum ether and Milli Q water to remove excess paraffin oil and Span-80. The washed particles were freeze dried and stored at–20°C before characterization.

Method II

In this method, 720 mg of Eudragit L100 was dissolved in 8 mL of methanol under constant stirring in 15 mL falcon tube. To this, 36 mg of bovine/human insulin dissolved in 0.5 mL of 0.1 M HCl was added and homogenized at 11,000 rpm for 70 s in an ice bath. The entire solution was transferred to 1 L plastic beaker containing 100 mL of light liquid paraffin oil and 0.5% of Span-80. The emulsion was stirred at 500 rpm for 4 h to evaporate methanol. After 4 h, the microspheres were filtered and washed with 50 mL petroleum ether (60–80°C) and 8 mL Milli Q water to remove excess paraffin oil and Span-80. The microspheres were then dispersed in 2 mL water, freeze dried at –41°C for 20 h and stored at –20°C before characterization and further analysis.

Following the same protocol as above, microspheres of Eudragit S100 were prepared by taking 720 mg of Eudragit S100 and dissolving in 8 mL of DCM at constant stirring in 15 mL falcon tube. To this, 36 mg of bovine insulin dissolved in 0.5 mL of 0.1 M HCl was added and homogenized at 11,000 rpm for 70 s. The solution was transferred to 1 L plastic beaker containing 100 mL of light liquid paraffin oil and 0.5% of Span-80. The emulsion was stirred at 500 rpm for 4 h to evaporate methanol. After 4 h, the microspheres were filtered and washed with 50 mL of petroleum ether (60–80°C) and 8 mL Milli Q water to remove excess paraffin oil and Span-80. The microspheres were then dispersed in 2 mL water, freeze dried at –41°C for 20 h and stored at –20°C before characterization and further analysis.

Characterization

Size, shape and morphology analyses

SEM was done on insulin-loaded microspheres mounted on metal stubs using double-sided adhesive tape, drying in a vacuum chamber, sputter-coating with a gold layer and viewing under SEM (JSM-840, Jeol Instruments, Tokyo, Japan) to characterize the shape and morphology as well as to confirm the particle size.

Encapsulation efficiency

Microspheres (50 mg) containing insulin were dissolved in 5 mL methanol. Insulin was extracted using 25 mL 0.1 M HCL solution in a separating funnel, shaken for few minutes and the solution was filtered using 0.22 μm filter. Insulin content was analyzed using high-performance liquid chromatography (HPLC).

HPLC method I. The amount of insulin released from the microspheres was collected by taking 100 µL samples at predetermined time intervals of 1 h up to 4–6 h and analyzed by HPLC. Insulin was separated on C18 Vydac 218MS54 column (4.6 × 250 mm) having the pore size of 300 A° and a particle size of 5 µm. The buffer solution for mobile phase was prepared by dissolving 28.4 g of anhydrous sodium sulfate in 1000 mL double distilled water and pH of the buffer was adjusted to 2.3 by adding 2.7 mL of orthophosphoric acid. The mobile phase consisted of (A) 82:18 of acetonitrile:buffer and (B) 50:50 acetonitrile:buffer solutions. HPLC experiments were performed in gradient mode (see Table 1) at the flow rate of 1 mL/min, injection volume of 100 µL and detection wavelength of 210 nm.

HPLC method II. In this method, insulin was analyzed using MN C18 column (250 × 4.6 mm). HPLC run was carried out in a gradient mode (see Table 1) at the flow rate of 1 mL/min, injection volume of 100 µL and detection wavelength of 210 nm. Mobile phase consisted of (A) water (0.05% trifluoroacetic acid, TFA v/v) and (B) acetonitrile: water (80:20) (0.05% TFA v/v).

$$\%\ Drug\ loading = \left(\frac{Weight\ of\ drug\ in\ microspheres}{Weight\ of\ microspheres} \right) \times 100 \quad (1)$$

$$\%\ Entrapment\ efficiency = \left(\frac{Drug\ loading}{Theoretical\ loading} \right) \times 100 \quad (2)$$

CD

The released insulin samples were filtered (0.22 µm; Millipore; Ireland) before subjecting to CD analysis (for understanding the insulin's integrity) to remove any particulate matter as well as possible protein aggregates. CD spectra at 25°C were obtained using a Jasco J-180 spectropolarimeter on a 1 cm path length quartz cell at protein concentration of 2 mg/mL. Analysis conditions used were: 0.5 nm bandwidth, 10-mdeg sensitivity, 0.2-nm resolution, 2s response, 10 nm/min scanning speed and 200–240 nm measuring range. Each spectrum is the average of at least three runs and the buffer baseline was subtracted from the average spectra. Final spectra are presented in mean residual ellipticity. Deconvolution of CD spectra was obtained by SELCON method[26].

Table 1. Gradient mode HPLC conditions.

Method I		Method II	
Time (min)	Conc. B (v/v)	Time (min)	Conc. B (v/v)
0	16	0.10	27
22	60	5.0	27
24	100	20	60
26	16	21	27
32	16	25	27

In vitro *release of insulin-loaded microspheres*

The *in vitro* release experiments were done on the formulated microspheres prepared by Method I by taking 100 mg of insulin-loaded Eudragit L100 and Eudragit L100/RS100 blend particles in a flask containing 25 mL of buffer solution. Dissolution was carried out in an incubator maintained at 37°C under constant stirring at 100 rpm. At regular intervals of time, aliquots (2 mL each time) were withdrawn and analyzed for insulin by HPLC at the λ_{max} value of 210 nm employing the gradient method as mentioned in HPLC method I. In order to simulate the stomach and intestinal environments, all the *in vitro* release experiments were performed in solutions of pH of 1.2 and 7.4 buffer, respectively. The formulations were kept in 1.2 pH media for the first 2 h and later, in pH of 7.4 media to follow the intestinal environment.

In vitro release experiments were done on the formulated microspheres prepared by Method II. In this case, 50 mg of insulin-loaded microspheres were separately suspended in 25 mL of pH 1.2 and pH 7.4 release media. *In vitro* release was performed in an incubator at 37°C under constant stirring condition at 100 rpm. Sample (1 mL aliquot) was withdrawn and replenished with fresh release media at different time intervals. The release samples were filtered and analyzed using HPLC as mentioned in HPLC method II.

Methods for testing in vivo efficacy of insulin-loaded microspheres on diabetic-induced rats

Male Wistar rats (250 g) were housed in a 12–12 h light–dark cycle under the constant temperature environment of 22°C and relative humidity of 55%; these were allowed free access to water and food during acclimatization. To minimize diurnal variance of blood glucose, all the experiments were performed in the morning. Diabetes was induced with intravenous injection of 150 mg/kg alloxan in saline (0.9% NaCl). Ten days after the treatment, rats with frequent urination, loss of weight and blood glucose levels (BGLs) higher than 300 mg/dL were included in the experiments and 5% dextrose solution was given in the feeding bottle for 1 day to overcome the early hypoglycemic phase. After 72 h, the blood glucose was measured by a glucometer. Then, the diabetic rats (glucose level >300 mg/dL) were separated.

In order to investigate the effects of oral insulin-loaded microspheres, 12 h fasted diabetic rats were divided into three groups, each group containing six rats were fed with insulin-loaded microspheres (20 IU) and placebo microspheres as the control. Group 1 rats received the placebo microspheres and Group 2 rats received 20 IU of insulin-loaded Eudragit L100 microspheres, whereas Group 3 rats received 20 IU of insulin-loaded Eudragit L100/RS100 blend microspheres, both dispersed in a mixture of 9 mL of 5% carboxy methyl cellulose and 1 mL of 0.1 M HCl solution through oral route using the oral feeding needle. The glucometer was calibrated as per specifications mentioned in the strips and precision of the glucometer was ±5%. Glucose was

measured on a drop of blood collected from the tail vein spread over on the marked end of the strip before and at different intervals up to 200 min after the oral administration. Results were expressed as means ± standard deviation.

Statistical analyses were done using the SPSS statistical package. Analysis of variance followed by the least significant difference procedure was used for comparison of BGL and % inhibition from control, Eudragit L100 and Eudragit L100/RS 100 blend groups; the parameter, $p < 0.05$ was considered significant.

Results and discussion

In applications involving oral delivery of insulin, pH-sensitive polymers are used as carriers to protect the insulin in acidic conditions and to release it in alkaline medium. Therefore, for developing oral insulin formulation, it is necessary to have a particulate device that will change its behavior based on the pH of the medium. In this regard, we have developed a pH-sensitive carrier system using Eudragit L100 to effectively encapsulate insulin in order to protect it from the enzymatic/proteolytic degradation and to release it by the erosion mechanism in a controlled manner, thus allowing higher concentration of insulin in the serum.

Some earlier reports are available on using Eudragit polymers[5,12,27–29] loaded with insulin for oral applications. For instance, Paul and Sharma[5] have developed insulin-loaded tricalcium phosphate microspheres coated with pH-sensitive Eudragit polymer, wherein the stability and conformational variations of insulin as well as its biological activity in diabetic rats were investigated. Agarwal et al.[27] used the co-precipitation technique to prepare insulin-loaded microspheres of Eudragit L100 and studied the effect of variables such as addition of salts in the precipitating medium and ratio of polymeric solution to volume of precipitating medium on dissolution and encapsulation efficiencies. Damgé et al.[28] developed the nanoparticles of biodegradable poly(ε-caprolactone) with non-biodegradable Eudragit® RS 100 blend using poly(vinyl alcohol) as a surfactant to achieve the encapsulation efficiency (EE) of 96%.

Suitability of Eudragit L100 microspheres prepared by double emulsion-solvent evaporation method was evaluated by Jain and Majumdar[1]. Various parameters were optimized to attain maximum EE and optimum *in vitro* release profile. The microspheres retarded the insulin release in gastric pH, but provided slow release in alkaline pH condition of the upper intestine. Gowthamarajan et al.[12] developed the microspheres of Eudragit L100 and S100 loaded with insulin, protease inhibitor and bile salts by the solvent diffusion technique and microspheres of this study have shown delayed release of insulin. The microspheres prepared from Eudragit L100 or S100 along with 1% aprotinin and 1% sodium glycocholate were used for *in vivo* evaluation of hypoglycemic effect. The *in vivo* evaluation of microspheres of Eudragit L100, 1% aprotinin and 1% sodium glycocholate showed a prolonged hypoglycemic effect for 3 h compared to intravenous injection of bovine insulin.

Surface morphology

Surface morphology and size of the microspheres were examined by SEM. Images of human insulin-loaded microspheres prepared by method I at 5000× and method II at 4000× and 2000× magnifications are depicted in Figure 1A and 1B, respectively. Microspheres prepared by Method I (2.5% polymer concentration) are spherical in shape with agglomerations having the slight rough surfaces. The size of microspheres prepared by Method I is around 1–5 µm. Microspheres prepared by Method II (9% polymer concentration) are also spherical without agglomerations and their sizes vary from 10 to 40 µm; the microspheres have a smooth surface. Process parameters like polymer concentration, insulin loading and solvent play significant roles in preparing the microspheres[29].

EE

Encapsulation of insulin using Eudragit was achieved by double emulsion-solvent evaporation technique[25], wherein insulin was first dissolved in 0.1 M HCl and emulsified in Eudragit solution to form the primary emulsion, which was then emulsified in light paraffin oil. After the solvent evaporation, microglobules of primary emulsion were precipitated and microspheres were solidified. Microspheres produced by Method I show the rough surfaces with EE of 52% and 26%, respectively for Eudragit L100 and Eudragit L100/RS100 (50:50) blend. When insulin in 0.1 M HCl was homogenized with a mixture of methanol and DCM, a low EE of 30% was observed. The solubility of insulin in the external phase was assessed by dissolving 36 mg of insulin in 0.5 mL HCl (0.1 M) and by adding the insulin solution under stirring to the beaker containing 100 mL paraffin oil with 0.5% Span-80. A white turbid solution was observed, which could be attributed to insulin precipitation in the external oil phase, revealing the loss of insulin due to surface bonding, thus resulting in loss in the washing step. Further, changing the solvent from methanol-DCM mixture to methanol and increasing the polymer concentration from 2.5 to 9 wt. % in the primary emulsion gave 95% yield with a EE of 63% in case of bovine insulin, whereas for human insulin, the yield and EE values were 92 and 64%, respectively. With bovine insulin-loaded Eudragit RS100 microspheres, the yield was 94%, whereas the EE was 33% (see results in Table 2).

CD

CD provides qualitative as well as quantitative information about the conformation of proteins[30]. In this work, CD is used to probe the unfolding and folding of protein

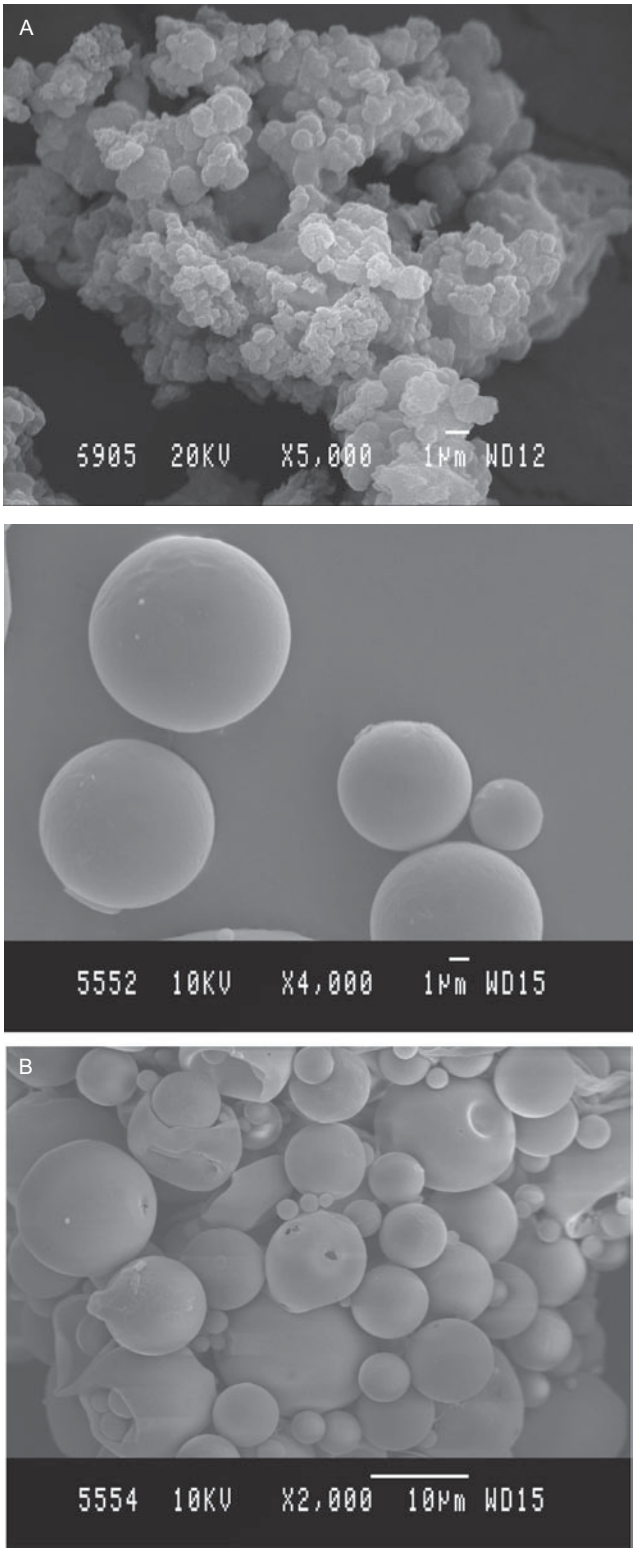

Figure 1. (A) SEM image of group of microspheres of Eudragit L100 prepared by method I. (B) SEM image of group of microspheres of Eudragit L100 prepared by method II.

secondary structure either kinetically or at equilibrium. Figure 2 displays different CD spectra of (A) pure bovine insulin; (B) released bovine insulin from the developed formulation at pH 7.4. Figure 3 shows the CD spectra of (A) pure human insulin, while (B) shows that of the released human insulin from Eudragit L100 at pH 7.4. The CD spectra in basic pH revealed no significant difference in the secondary structure of released insulin compared to native insulin.

Table 2. % Yield and encapsulation efficiency of the microspheres for different formulations.

Insulin type	Polymer type	% Yield	% EE[a]
Method I			
Human	Eudragit L100	86	52
Human	Eudragit L100 + Eudragit RS100 blend	80	26
Method II			
Bovine	Eudragit L100	95	63
Human	Eudragit L100	92	64
Bovine	Eudragit RS100	94	33

[a]EE, encapsulation efficiency.

In vitro release study

In vitro release profiles of insulin-loaded Eudragit L100 microspheres at pH 1.2 and 7.4 shown in Figure 4 indicate that at pH 1.2, the polymer shrinks, whereas at pH 7.4, it swells to release insulin. At pH 1.2, Eudragit L100 has released nearly 9% insulin, but in pH 7.4, almost 100% release of insulin occurred in about 5 h. To minimize the amount of insulin release in pH 1.2, the insulin-loaded microspheres were formulated in the form of tablet using 25 mg of microspheres and 12 mg of poly(vinyl pyrrolidone), 4 mg of magnesium stearate, and 184 mg of microcrystalline cellulose as excipients. Further, the formulated tablet was coated with 5% Eudragit L100 solution in isopropanol by dip-coating method. By following this procedure, insulin release in pH 1.2 was negligible, but maximum insulin release occurred in pH 7.4 as shown in Figure 5. On the other hand, blending of Eudragit RS100 with Eudragit L100 prevented the release of insulin at pH 1.2 followed by a maximum release in pH 7.4 as shown in Figure 6.

Figure 7 shows *in vitro* release of bovine insulin-loaded Eudragit L100 microspheres prepared by Method II. In this formulation, 0.3% of insulin is released in pH 1.2, whereas in pH 7.4, 93% is released in 3 h. In case of human insulin-loaded Eudragit L100 formulation, insulin release in pH 1.2 is 0.8%, whereas in pH 7.4, it is as high as 76% in 3 h (see Figure 8). In order to reduce the initial burst release in pH 7.4 media, another formulation was prepared using Eudragit RS100. However, the *in vitro* release of bovine insulin indicated a slow release in pH 7.4, but high release of insulin was observed in pH 1.2 as shown in Figure 9.

In vivo efficacy studies

Figure 10 displays the *in vivo* efficacy results of oral insulin-loaded formulations. These results are plotted as average BGL (mg/dL) versus time for placebo microspheres and experimental formulations *viz.*, insulin-loaded Eudragit L100 microspheres and insulin-loaded Eudragit L100/RS100 blend microspheres with dose of

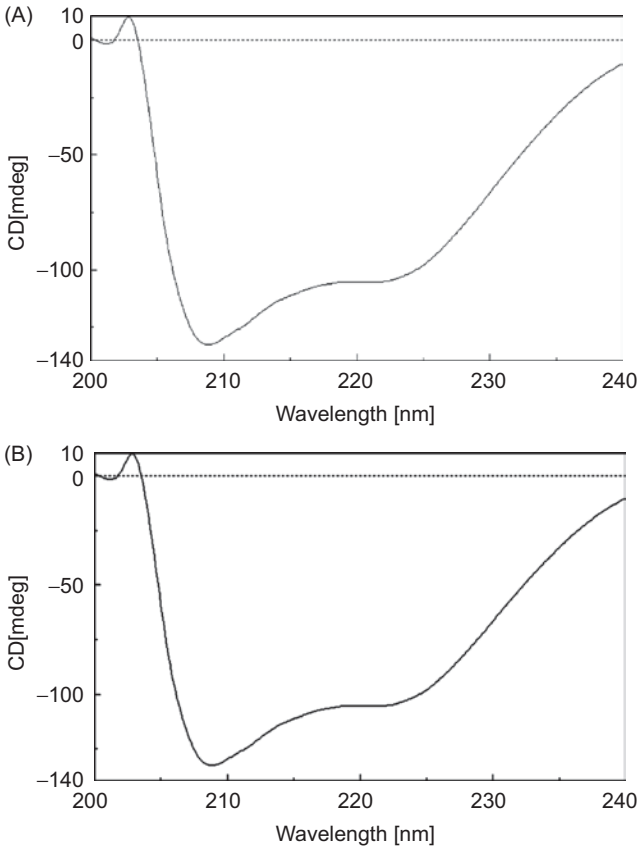

Figure 2. CD spectra of (A) pure bovine insulin and (B) released bovine insulin.

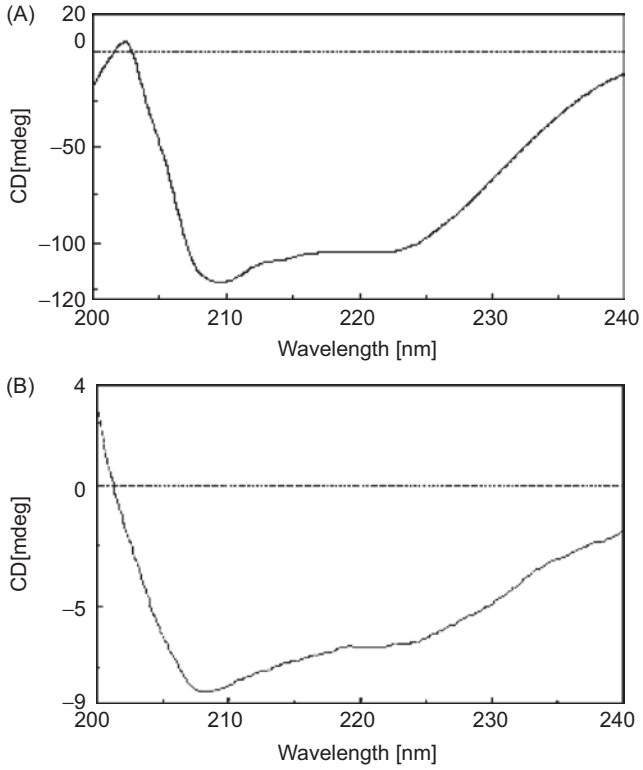

Figure 3. CD spectra of (A) pure human insulin and (B) released human insulin.

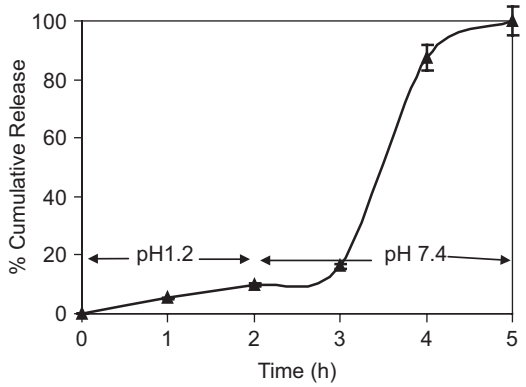

Figure 4. *In vitro* release of insulin-loaded Eudragit L100 in pH 1.2 and 7.4 ($n = 3$).

20 IU/200 g of body weight. When the placebo microspheres were administered orally, almost no change in BGL was observed until the last time point. For the insulin-loaded microspheres, BGL was low throughout the study experiments. Insulin-loaded Eudragit L100 microspheres administered by the oral route dropped BGL from 455 to 62 showing 86% inhibition, whereas insulin-loaded Eudragit L100 and RS100 blend microspheres administered by the oral route dropped the BGL from 319 to 258, giving 42% inhibition (see Figure 11). Higher reduction of BGL in diabetic rats is attributed to the continuous absorption of glucose from the GIT. One way analysis of variance results from comparison of control and Eudragit L100 groups indicates significant difference ($p = 0.00$, df = 15 and F = 22). Also control and Eudragit L100/RS 100 blend formulations indicate significant differences with p value of 0.00 (df = 15 and F = 68). The % inhibition from Eudragit L100 and Eudragit L100/RS 100 blend formulations indicates insignificant differences with a p value of 0.175 (df = 11 and F = 2).

Non-biodegradable and degradable polymers have been widely used in the oral delivery of insulin through the GIT[22–25]. The use of biodegradable microspheres is proposed to circumvent the possible accumulation of nondegradable microspheres in the tissues that may lead to harmful effects. Mainly, three possible mechanisms have been suggested for intestinal uptake of released insulin from the microspheres: (i) uptake via paracellular pathway; (ii) receptor-mediated transcytosis and transport via epithelial cells of the intestinal mucosa; (iii) lymphatic uptake via the M cells of Peyer's patches, abundant in ileum. In case of Eudragit microspheres, insulin is protected in gastric pH, but when in contact with the intestinal pH, microspheres have released the insulin and absorbed in the intestine. Additionally, absorption of insulin may be attributed to increased residence time of insulin microspheres next to the absorption surface of GIT. The present studies show that oral administration of insulin-loaded Eudragit L100 microspheres could reduce the BGL, which lasted for 300 min, confirming the sustained release of active insulin through the insulin-loaded Eudragit L100 microspheres.

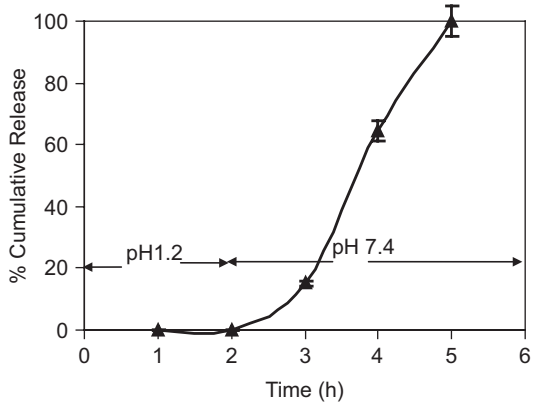

Figure 5. *In vitro* release of Eudragit L100 coated insulin-loaded Eudragit L100 tablet in pH 1.2 and 7.4 ($n=3$).

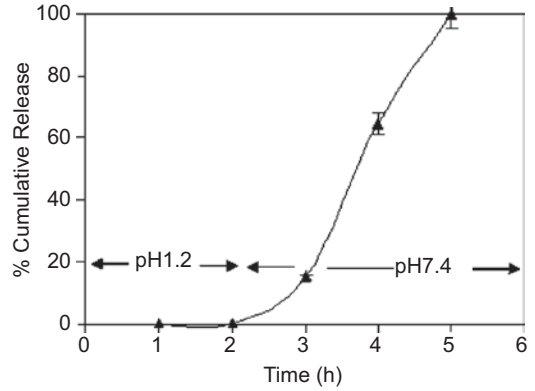

Figure 6. *In vitro* release of insulin-loaded Eudragit L100/Eudragit RS100 microspheres in pH 1.2 and 7.4 (Method I) ($n=3$).

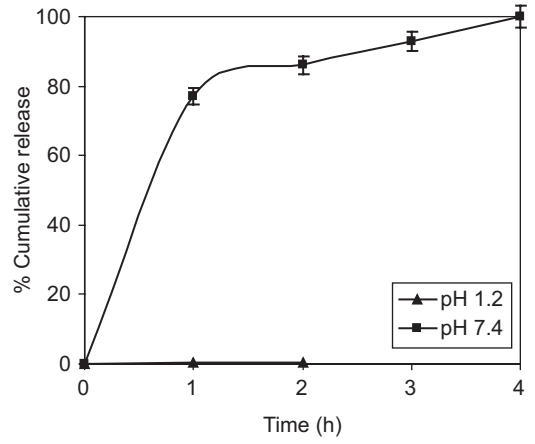

Figure7. *In vitro* release of bovine insulin-loaded Eudragit L100 microspheres (Method II) ($n=3$).

Conclusions

The present research describes development of pH-sensitive Eudragit microspheres for oral delivery of insulin. The devices were developed from Eudragit L100, Eudragit S100 and their (50:50) blend all prepared by solvent evaporation method that offered the EE ranging from 33 to 64% with the yield of 95%. Of the three devices developed, insulin-loaded formulations

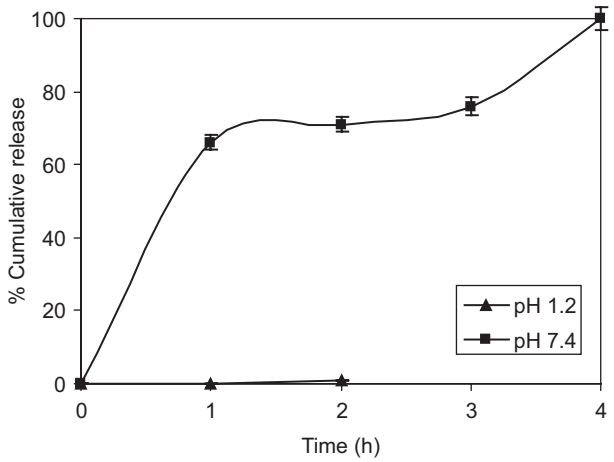

Figure 8. *In vitro* release of human insulin-loaded Eudragit L100 microspheres (Method II) ($n=3$).

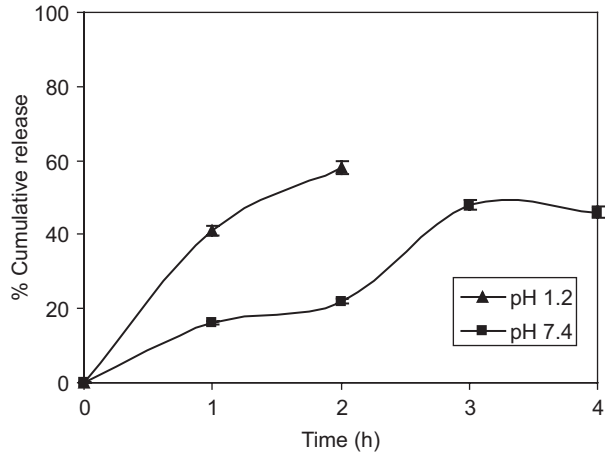

Figure 9. *In vitro* release of bovine insulin-loaded Eudragit RS100 microspheres (Method II) ($n=3$).

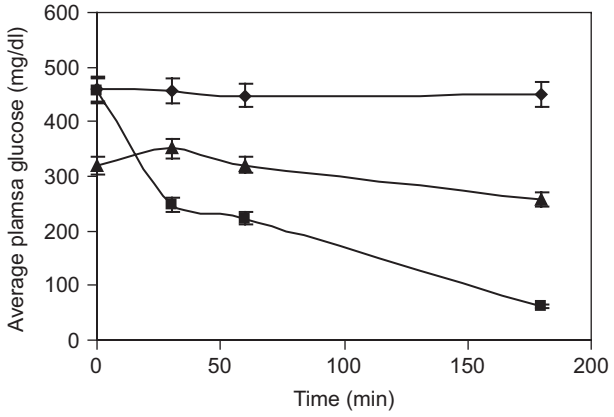

Figure 10. *In vivo* animal experiments: symbols: (◆) control, (■) insulin-loaded Eudragit L100 microspheres (20 IU/200 g) and (▲) insulin-loaded Eudragit L100/RS100 blend microspheres (20 IU/200 g).

prepared from Eudragit L100 are the most effective for oral delivery of insulin, since they could protect insulin from denaturation in acidic conditions of the stomach and release insulin in the intestinal conditions i.e.,

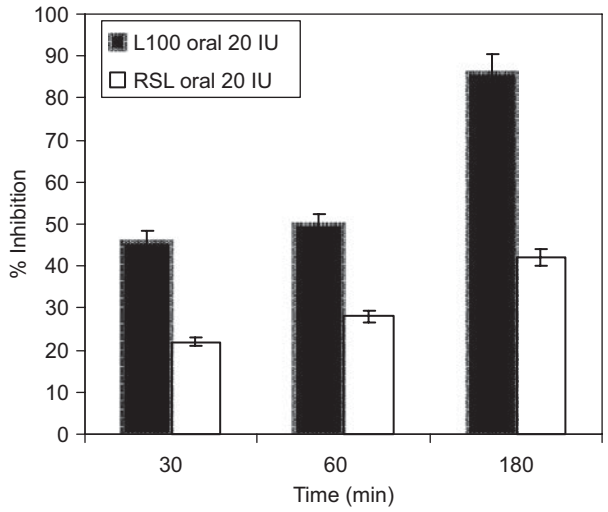

Figure 11. % Inhibition of insulin-loaded Eudragit L100 and Eudragit L100/RS100 blend microspheres.

insulin was released only in pH 7.4, but almost no insulin was released in pH 1.2. Microspheres are in the size range of 1–50 μm and the integrity of insulin after the release was assessed by CD. *In vivo* experiments performed on diabetic-induced rats indicated the inhibition up to 86%. The formulations of this study provide a novel approach to utilize pH-sensitive Eudragit polymer *viz.*, L100 for controlled release of insulin through the oral route.

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Declaration of interest

The authors declared no conflict of interest.

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