
Microspheres as Oral Delivery System for Insulin

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Microspheres of eudragit L100 and S 100 containing insulin, protease inhibitor and bile salts were prepared by solvent diffusion technique. The chemical interaction between the drug, polymers and adjuvants was evaluated by FTIR and found that there was no interaction between them. *In vitro* release studies were carried out using metabolic shaker. The microspheres showed delayed release. Based on the *in vitro* release profile, ideal batches of microspheres (prepared with Eudragit L100 alone and S100 alone as carriers and 1% aprotinin and 1% sodium glycocholate) were selected and used for *in vivo* evaluation of hypoglycemic effect. The *in vivo* hypoglycemic effect was determined in rats by measuring the blood glucose level using a glucometer. In the *in vivo* evaluation, insulin microspheres prepared with Eudragit L-100, 1% aprotinin and 1% sodium glycocholate showed prolonged hypoglycemic effect for 3 h when compared with intravenous injection of bovine insulin.

The treatment of diabetic patients with insulin requires parenteral injection. Since injections are poorly accepted by most patients, other routes for non-parenteral administration are highly desirable. This has led to many investigations of alternative ways such as oral, transdermal, nasal and rectal routes for insulin delivery¹. An oral form of insulin administration is convenient and could encourage good patient-compliance. As it is absorbed through the intestinal wall, it also mimics the physiological transport of this hormone through the enterohepatic pathway².

The major problems encountered with oral dosage forms of insulin are rapid enzymatic degradation in the gastrointestinal tract and poor permeation. Recent studies have shown that some proteases amplify the biological effect of insulin injected directly into lumen of intestine³. The absorption of insulin can be improved by using absorption promoters like bile salts. These studies suggest that it is possible to enhance the absorption and reduce the degradation of insulin by the use of bile salts and protease inhibitors. Oral uptake of colloidal particles suggests the possibility of using

microspheres as oral delivery system for insulin. Hence, in the present study, an attempt was made to develop an oral dosage form for insulin, which is designed to deliver insulin into intestine in the presence of protease inhibitor and bile salts.

MATERIALS AND METHODS

Soluble bovine insulin was procured from Knoll Pharmaceuticals, Mumbai. Aprotinin was obtained as a gift sample from Alkem Labs, Mumbai. Eudragit L100 and S100 were purchased from Rohm Pharma, Germany. Sodium glycocholate was purchased from Loba, Mumbai, and gelatin was purchased from Sigma, Mumbai. All other reagents used were of AR and HPLC grade. Waters-HPLC Systems (Micron Bondapak C 18 column), FTIR (Perkin-Elmer, Japan), were the equipments used.

Preparation of microspheres:

Insulin microspheres were prepared by modification of the method described by Morishita *et al.* (1991)⁴. Insulin (40 U) and aprotinin (1 mg) were dissolved in 300 ml of 0.1 N hydrochloric acid. Sodium glycocholate (1mg) was added and mixed. To this solution, 4 ml of ethanol (90% v/v) and polymer(s) were added with stirring at 1200 rpm for 10 min.

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The resultant solution was added into 30 ml of light liquid paraffin. The microspheres were formed by the addition of gelatin solution (0.5% w/v). The microspheres were separated from solution by decantation and rinsed twice with 100 ml portions of water. The microspheres were vacuum filtered, and traces of solvent removed by placing on paper and drying at room temperature. Finally, the microspheres were disaggregated by passing through a sieve of 0.5 mm aperture. Six batches of microspheres were prepared with composition as shown in Table 1. The particle size of prepared microspheres was determined by optical microscopy.

Incorporation efficiency of microspheres:

Twenty milligrams of insulin-loaded microspheres were completely dissolved in 10 ml of phosphate buffer of pH 7.5, shaken in a metabolic shaker for 1 h at 37° and then analyzed by reverse phase HPLC.

In vitro release study⁵:

The *in vitro* release study was carried out using metabolic shaker. The medium employed was 70 ml of phosphate buffer of pH 6.5. The temperature was kept at 37° and constant shaking was done for 4 h. Before addition of microspheres, 0.001%w/v of methylcellulose was added to the buffer solution to avoid the adsorption of insulin on glass surface. Samples of 5 ml were withdrawn at 10, 20, 30, 60, 120, 180 and 240 min, filtered using a membrane filter of

pore size 0.45 μ m and analyzed by reverse phase HPLC. After each withdrawal, 5 ml of fresh buffer was added to the dissolution medium.

Chromatographic conditions⁶:

A Waters® HPLC system was used for the analysis. Micron Bondapak C₁₈ column (10 μ , 30 cm 3.9 mm id) was used. A mixture of acetonitrile (HPLC grade) and sodium dihydrogen orthophosphate buffer of pH 2.5 in the ratio 25:75 was used as mobile phase for insulin with an operating pressure of 5000 Psi. The flow rate was maintained at 1 ml/min. Detection was done at 214 nm.

In vivo hypoglycemic effect⁷:

The estimation of hypoglycemic effect of insulin-loaded microspheres was carried out using diabetic rats. Diabetes was induced in 30 rats, weighing between 180-250 g, by intraperitoneal injection of 150 mg/kg body weight of alloxan, dissolved in water for injection. The rats were divided into 10 groups of 3 animals each. All the diabetic rats were fasted for 16 h before the experiment and were allowed water *ad libitum*. The treatment given to different groups of animals is shown in Table 2. (Institutional Animal Ethic committee approval JSSCP/IAEC/Ph.Cog/03/2001-02, CPCSEA registration No. JSSCP65, 99-01/118199/CPCSEA/19-5 -99).

Insulin microspheres were administered orally by force-feeding with water (1 ml) via a rubber tube, under non-anesthesia. The dose of insulin was kept at 50 U total insulin/kg body weight of animal. Rats were restrained in a supine position during administration. A 0.2 ml aliquot of blood sample was collected from the jugular vein on the opposite side to the injection 5 min before insulin dosing and at 30 min, 1, 2, 3, 4 and 5 h interval after dosing. Blood glucose level was determined by using a glucometer. The relative hypoglycemic activity of micro spheres was compared with that of insulin intravenous injection.

RESULTS AND DISCUSSION

Six batches of insulin-loaded microspheres were prepared using Eudragit L-100 and S-100 as carriers and coating them with gelatin. The particle size of micro spheres was determined by optical microscopy and the values are shown in Table 1. Among the six batches, microspheres prepared with Eudragit L-100 as carrier showed uniformity of particle size. The microspheres prepared with Eudragit S-100 showed particle size range almost nearer to those prepared with Eudragit L-100. Both were free flowing in nature, but the microspheres prepared with combination of Eudragit L-100

TABLE 1: COMPOSITION OF INSULIN MICROSPERES AND THEIR CHARACTERIZATION.

Batch Code	Composition	Percentage of Insulin Entrapped	Average Particle Size (μ m)
I	Eudragit L-100, Insulin	42.6	39.5 \pm 0.26
II	Eudragit S-100, Insulin	35.7	24.6 \pm 0.24
II	Eudragit L-100, Insulin, Aprotinin	52.1	42.3 \pm 0.20
IV	Eudragit S-100, Insulin, Aprotinin	39.8	29.3 \pm 0.27
V	Eudragit L-100, Insulin, Aprotinin,	77.6	61.0 \pm 0.30
VI	Eudragit S-100, Insulin, Aprotinin, Sod. Glycocholate	62.7	43.1 \pm 0.27

TABLE 2: TREATMENT GIVEN TO DIFFERENT ANIMAL GROUPS IN THE *IN VIVO* HYPOGLYCEMIC EFFECT STUDIES

Group	Treatment
1	Microspheres containing Eudragit L-100 and insulin
2	Microspheres containing Eudragit S-100 and insulin
3	Microspheres containing Eudragit L-100, insulin and aprotinin
4	Microspheres containing Eudragit S-100, insulin and aprotinin
5	Microspheres containing Eudragit L-100, insulin, aprotinin and sodium glycocholate
6	Microspheres containing Eudragit S-100, insulin, aprotinin and sodium glycocholate
7	Insulin alone (intravenous injection)
8	Dummy microspheres of Eudragit L- 100
9	Dummy microspheres of Eudragit S-100
10	Solvent (water for injection)

In vivo hypoglycemic effect of prepared insulin micro spheres was carried out in alloxaninduced diabetic Wistar rats and was compared with insulin injection.

and S-100 showed agglomeration.

The entrapment efficiency of different batches of microspheres was determined and is shown in Table 1. In general, the incorporation efficiency of microspheres prepared with Eudragit L-100 was higher than those prepared with Eudragit S-100. The efficiency was found to increase when aprotinin and sodium glycocholate were added to the formulation.

In vitro release studies were carried out for a maximum period of 4 h. the results are shown in fig. 1. The insulin microspheres prepared with Eudragit L- 100 alone as carrier and 1% aprotinin and 1% sodium glycocholate showed a maximum release of 99.3% at the end of 4th h, whereas, those prepared with Eudragit S-100, 1% aprotinin and 1% sodium glycocholate showed a release which was less than those prepared with L-100. The release from other batches of microspheres was less than that from these two batches. From the cumulative percentage release Vs time plot, it is

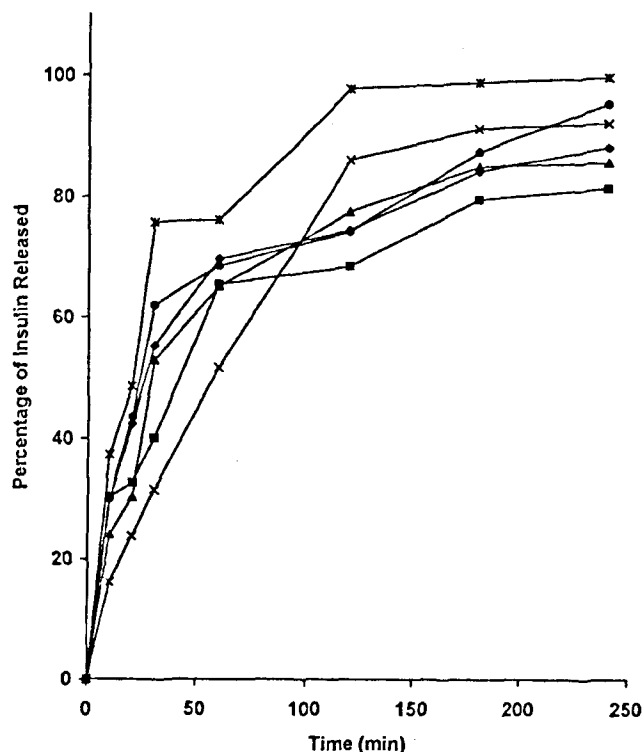


Fig. 1. *In vitro* insulin release profiles from microspheres. *In vitro* release profiles of insulin from various batches of microspheres such as batch I (-◇-), batch II (-□-), batch III (-▲-), batch IV (-X-), batch V (-*-) and batch VI (-●-) was determined using metabolic shaker method.

apparent that the release of insulin from the microspheres follows first order kinetics. Based on these results, the insulin microspheres prepared with Eudragit L-100 and S-100 alone as carriers and containing 1% each of aprotinin and sodium glycocholate were selected as ideal batches for the study of *in vivo* hypoglycemic effect.

In the *in vivo* hypoglycemic effect study, it was observed that in general, the hypoglycemic effect of insulin microspheres prepared with Eudragit L-100 as carrier was better than those prepared with Eudragit S-100 as carrier. This may be because of the fact that Eudragit L-100 is a pH sensitive polymer soluble at pH above 6 (upper intestinal region), where protease activity is reported to be less and absorption of insulin is reported to be more.

The use of aprotinin along with insulin in the microspheres improved the hypoglycemic effect. However, the effect was less when compared with bovine insulin injection. This is because of reduced enzymatic degradation of insulin, which promotes insulin absorption. The use of

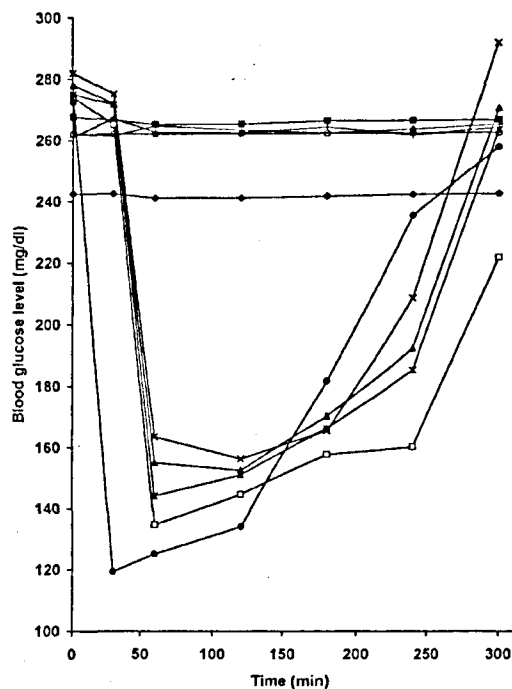


Fig. 2. *In vivo* hypoglycemic effect of insulin loaded microspheres.

In vivo hypoglycemic effect of insulin loaded microspheres on different groups of diabetic Wistar rats such as group I (-◇-), group II (-□-), group III (-▲-), group IV (-X-), group V (-□-), group VI (-*-), group VII (-●-), group VIII (- + -), group IX (- - -) and group X (-○-) was determined by measurement of blood glucose level using a glucometer.

sodium glycocholate along with aprotinin exhibited maximum hypoglycemic effect compared with other microspheres. Sodium glycocholate, being a bile salt, improves the penetration of insulin through micellar transportation. The insulin microspheres prepared with Eudragit L-100 as carrier and containing aprotinin and sodium glycocholate showed a uniform, prolonged hypoglycemic effect up to 4 h, which was not even observed with bovine insulin injection.

This study clearly showed that the microspheres prepared with Eudragit L-100 and aprotinin protect insulin degradation by pepsin, trypsin and chymotrypsin and promote absorption rate of insulin by the use of sodium glycocholate. The use of Eudragit L-100 as carrier for microspheres gives a site-specific release of insulin in the upper intestine. Hence, the development of oral insulin using Eudragit L-100 as carrier, aprotinin as protease inhibitor and sodium glycocholate as penetration enhancer is worthwhile for treatment of type-II diabetes mellitus by oral route.

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