EUDRAGIT COATED MICROPARTICULATE DELIVERY OF BOVINE INSULIN FOR ORAL DELIVERY

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ABSTRACT
Composite polymeric bovine insulin loaded microparticles were prepared using double emulsion solvent evaporation technique. Firstly, bovine insulin loaded microparticles using chitosan and Eudragit L 100: Eudragit S 100 (1:1) were prepared with exclusion of PLGA. Then, bovine insulin loaded microparticles using PLGA and Eudragit L 100: Eudragit S 100 (1:1) were prepared with exclusion of chitosan. The mean diameter of the microparticles prepared with exclusion of PLGA was in the range of 1-10 μm. and those prepared with exclusion of chitosan was in the range of 9-33 μm.. Bovine insulin loaded chitosan and Eudragit L 100 : Eudragit S 100 (1:1) microparticles showed more initial burst release in 1 hr. and higher % cumulative release in 12 hr. in PBS pH 6.8 than bovine insulin loaded PLGA and Eudragit L 100 : Eudragit S 100 (1:1) microparticles. IR spectra suggest the entrapment of insulin in composite polymeric microparticles. CD spectra suggest maintenance of secondary structure of insulin. SDS-PAGE confirmed that the structural integrity of the drug was maintained during encapsulation. Stability studies results showed that insulin loaded particles stored at 4^0C were more stable than particles stored at 25 ^{0}C and at -20^0C.

Keywords: Insulin,microparticle, Eudragit L 100, Eudragit S 100.

INTRODUCTION
The rapid advances in recombinant DNA technology have fuelled enormous interest in using peptides and proteins as drug candidates. The formulation of peptide and protein drugs is more complex and demanding. Unfortunately, peptide and protein development has far outpaced and advanced more rapidly than the ability to deliver these compounds systemically using convenient and effective delivery systems.[1,2] Oral route is the most convenient and popular[3,4] and it is more natural, less invasive and less expensive[37] but most peptide drugs like insulin show low oral activity. This is due to degradation by gastrointestinal tract enzymes and poor permeability across intestinal mucosa. To prevent these problems, many protease inhibitors and surfactants were used in insulin formulations. However, protease inhibitors also prevent digestion of important nutrients present in the food[4,5]. Similarly, surfactants irritate the protective mucous membrane leads to passage of unwanted toxins and pathogens. [4, 5] so, considering these all facts, protease inhibitors

and surfactants are not used in the current formulation.

Alternative routes, such as ophthalmic,[6, 7] nasal,[8]buccal,[9, 10] rectal,[11] pulmonary,[12, 13] etc. have been investigated. It has been shown that protease activities in the homogenates of the nasal, buccal, rectal and vaginal mucosa of rabbits are substantial and quite comparable to those in the intestinal mucosa.[14] In another study, degradation of leutinizing hormone releasing hormone (LHRH) was reported in nasal, buccal and liver tissues.[15] Implantable drug delivery systems of biocompatible polymers such as ethylene-vinyl acetate (EVA$_C$),[16] cellulose acetate and polymethylmethacrylate (PMMA),[17] have been studied. These implant devices release drugs with zero-order kinetics for an extended period which is desired. However, these devices must be surgically implanted and in some cases, explanted. Consequent disadvantages of using these implants include patient discomfort, possibility of infection and medical costs.[18]

Insulin is better absorbed from the ileum and large intestine as compared to the jejunum.[40] Indeed, insulin absorbed by the intestinal epithelium reaches the liver through the portal vein and can directly inhibit hepatic glucose output.[26] Thus a polymer that would release the drug in the ileum or upper intestine has the potential for oral insulin delivery. Eudragit L 100 and Eudragit S 100 are such polymers. These are anionic polymers synthesized from methacrylic acid and methyl methacrylate and have a pH-dependent solubility. Eudragit L 100 would release the drug in the region of gastro-intestine (GIT) of pH (6-6.5) i.e. ileum or upper intestine.[4, 38]Eudragit S 100 would release the drug in the region of gastro- intestine (GIT) of pH > 7 i.e. large intestine or colon.[5]

Polymer therapeutics have confirmed that they can satisfy the stringent requirements of both industrial development and regulatory authority approval process.[32] However, only a small fraction of polymeric particles is absorbed by the intestine, because the residence time of these particles is relatively low (4-6 hr.) due to the intestinal fluid flow and peristalsis action of the small intestine.[33] Insulin instability has been regarded as a major obstacle to the development of an insulin oral dosage device aimed at attaining optimal diabetic control. A promising strategy is the use of multifunctional polymers exhibiting gastrointestinal permeation enhancing and mucoadhesive properties. Interest in using natural materials as part of drug

delivery protocols has increased in the past two decades.[23] Chitosan is such a polymer.Chitosan [(1,4)-2-amino-2-deoxy-ß-D-glucan] is a polysaccharide comprised of co-polymers of glucosamine and N-acetylglucosamine.[19] It is partially or fully deacylated derivatives of chitin. The polymer is linear consisting of β (1-> 4) linked D-glucosamine residues with a variable number of randomly located N-acetyl glucosamine groups.[34] It is a non-toxic, biocompatible, safe and efficient intestinal permeation enhancer for absorption of protein drugs and mucoadhesive.[20, 21] Chitosan is capable of improving the paracellular transport of hydrophilic macromolecular drugs.[19]

PLGA is a polymer which is used for the controlled oral delivery of the drug. It is an anionic, biodegradable, biocompatible and hydrophobic polymer used in oral delivery of proteins [22, 36, 38]Many novel polymers including biodegradable polymer backbones, dendritic architectures, block copolymer micelles and polymers containing pendent cyclodextrin are used to prepare second-generation polymer therapeutics.[31] A successful PLGA nanoparticulate system should have a high drug loading capacity as it allows a small quantity of the carrier during a single administration. Eudragit is used for colon targeting.[5] This kind of microparticles not only gives protein a proper microenvironment by hydrophilic protection but also makes the initial release be retarded.[28, 29, 30] PLGA is not water soluble and encapsulation of water soluble peptides into PLGA particles requires double emulsion (w/o/w) solvent evaporation technique or a solvent diffusion/ nanoprecipitation method.[24]The preparation of particles from polymers is based on emulsion-diffusion .[35, 39]Both double and triple emulsion solvent evaporation methods are used for this purpose.

MATERIALS

PLGA (50:50) was obtained from Birmingham Polymer Inc. USA.Methacrylic acid copolymers Eudragit L 100 and Eudragit S 100 were supplied as a gift by Rohm Pharma (Weiterstadt, Germany). Hydrophilic chitosan was obtained from Sigma-aldrich,Inc., Germany. Recombinant human insulin powder was purchased from Sigma-Aldrich Co., India. Poly vinyl alcohol (PVA, cold, mol.wt. 30-70,000 Da) was obtained from Sigma chemicals Co., USA. Dichloromethane (DCM), Isopropanol (IPA), Ethanol and Disodium hydrogen phosphate were

obtained from E.Merck India Ltd. MQ was used throughout the study.

METHODS

Internal aqueous phase (IAP) (100µl.) consists of 20 mg/ml. of recombinant bovine insulin solution, 2.5 mg BSA, 10 mg sucrose (cryoprotectant) [27] and 2 mg of disodium hydrogen phosphate. Firstly, bovine insulin loaded microparticles using chitosan and Eudragit L 100: Eudragit S 100 (1:1) were prepared with exclusion of PLGA by double emulsion solvent evaporation method. Organic phase (OP) (5 ml.) consists of 3-6% w/v Eudragit solution [L100:S100 (1:1)] dissolved in ethanol and isopropanol mixture (1:4) because more ratio of ethanol taken in ethanol and isopropanol mixture (2: 1) had resulted in about 20% release of the drug in 0.1 NHCl as performed in the laboratory. External aqueous phase (EAP) (20 ml.) consists of 0.1% w/v hydrophilic chitosan with 1% w/v PVA solution (stabilizer) and 10% w/v sucrose (cryoprotectant). Primary emulsion (IAP/OP) was prepared by probe sonicator for 1 min. The secondary emulsion (IAP/OP/EAP) was prepared by homogenizer at 8,000 rpm for 3 min. Then, bovine insulin loaded microparticles using PLGA and Eudragit L 100: Eudragit S 100 (1:1) were prepared with exclusion of chitosan by triple emulsion solvent evaporation method. The composition of internal aqueous phase (IAP) (100µl.) was same as for above method. Internal organic phase (IOP) (1 ml.) consists of 25 mg of PLGA (50: 50) in 1 ml. of DCM. External aqueous phase (EAP) (3 ml.) consists of 0.1% w/v hydrophilic chitosan with 1% w/v PVA solution and 10% w/v sucrose (cryoprotectant). External organic phase (EOP) (12 ml.) consists of 3-6% w/v Eudragit solution [L100:S100 (1:1)] dissolved in ethanol and isopropanol mixture (1:4). Primary emulsion (IAP/OP) was prepared by probe sonicator for 1 min. The secondary emulsion (IAP/OP/EAP) was prepared by homogenizer at 8,000 rpm for 3 min. Then triple emulsion (IAP/IOP/EAP/EOP) was prepared on magnetic stirring by adding eudragit solution drop by drop by pipette to secondary emulsion.   Then organic solvents were removed by overnight magnetic stirring at room temperature.

Abbreviations

IAP, Internal aqueous phase; IOP, Internal organic phase; EAP, External aqueous phase; EOP, External organic phase; DCM, Dichloromethane; PVA, Polyvinyl alcohol; PLGA,

Poly (lactide- co-glycolide); IPA, Isopropanol; S.C., Subcutaneous; BSA, Bovine serum albumin.

CHARACTERIZATION
Particle size measurements

The freeze-dried microparticles (2mg) were dispersed in 1 ml.MQ and vortexed for 2 min. to bring about disaggregation of the microparticles.Size analysis of microparticles was carried out by laser light scattering using Mastersizer 2000S.

Zeta potential

Surface charge (zeta potential) was measured using Nano Z, Malvern instruments. To determine the zeta potential, suspension of the polymeric particles was diluted with water to ensure that the signal intensity is suitable for the measurement. The suspension is then placed in the electrophoretic cell for measurement.

Microsphere Morphology and Surface Characteristics

Optical Microscopy: 20µl. of a suspension of microparticles in MQ was placed on a glass slide covered with a cover slip without an air bubble. Particles were observed at 40 times magnification under inverted microscope, Nikon Eclipse Ti100 and images were recorded by software 'Nis Elements'.

Scanning Electron Microscopy

10-20 µl.of a suspension of microparticles was spread uniformly as a thin film on a round cover-slip mounted on an aluminium stub using a double-sided carbon adhesive tape and lyophilized for 1-2 hours for the complete removal of water. Silver conductive paint was applied between the cover slip and the stub. Stubs were sputter-coated with conductive Gold-Palladium. Samples were viewed under Scanning Electron Microscope (SEM).

Encapsulation Efficiency

Known quantities of microparticles (10 to 15mg) were taken in an eppendorff tube, 1ml of Isopropanol (IPA) was added, vortexed well and centrifuged (13,000rpm, 5 minutes at 4°C). The supernatant was discarded; pellet was resuspended in Isopropanol (IPA) and centrifuged again (13,000rpm, 5 minutes at 4°C). The process was repeated thrice; the pellet finally obtained was dried in the desiccator at room temperature and then resuspended in

1ml. MQ. Insulin content was estimated by micro BCA assay method.

Enteric nature of Microparticles
This test was performed to determine whether the drug would be released in the acidic environment of the stomach (i.e. the pH between 1 and 3). Twenty milligrams of microparticles were soaked in 1 ml. of 0.1N HCl in an eppendorff tube that was put on the incubator shaker at 37^0C ±0.5^0C. After 2 hr., the sample was centrifuged (13,000 rpm, 10 min., 4^0C) and the insulin content of the supernatant was measured using micro BCA protein assay method.

In-Vitro Drug Release
In-vitro release of insulin from microparticles was evaluated in 1X phosphate buffer saline (PBS, pH 6.8) for Eudragit L 100: Eudragit S 100 (1:1) particles. The particles were evaluated in PBS, pH 6.8 throughout 12 hr. The pH was adjusted with 2M NaOH or 2M HCl. Insulin microparticles were transferred to the prewarmed dissolution media (100 ml.) and maintained at 37 ± 0.5^0C under stirring at 100 rpm in incubator shaker. Samples were withdrawn at regular intervals (0, 1, 4, 6 and 12 hr.) and volume was replaced by fresh PBS. The sample withdrawn was centrifuged (5,000 rpm, 10 min., 4^0C). Insulin content of the supernatant was estimated by micro BCA protein assay method.

SDS-PAGE
Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of bovine insulin and optimized bovine insulin loaded microparticles was performed according to the method of Laemmli on a slab gel containing 20% resolving gel and 5% stacking gel. Vertical minigel apparatus (Pharmacia, GE Healthcare, Uppsala, Sweden) was used to check for protein integrity. Samples of insulin released from the microparticles in Tris-HCl buffer, insulin solution and a molecular weight reference marker (m.w. range 14.4-97 KDa) were dissolved in 1X running buffer containing SDS, Tris-HCl and glycine, loaded onto a vertical slab gel and subjected to electrophoresis at 30 mA. Protein bands were fixed and stained with Coomassie Brilliant Blue R-250 (0.1% w/v) in water: methanol: acetic acid (5:4:1).

CD Spectroscopy
Circular dichroism (CD) spectra were obtained with a Jasco J-720 spectropolarimeter (Tokyo, Japan) equipped with a temperature controller to examine the secondary structure of insulin in particles. Spectra were collected at 25^0C using a 0.1 cm cell over the wavelength range of 200–260 nm. A resolution of 0.2 nm and scanning speed 50 nm /min. with a 4 s response time were employed. Each spectrum obtained represents an average of three consecutive scans. . Samples for CD analysis were prepared by dissolution in Tris-HCl buffer. The spectra of insulin samples (insulin loaded microparticles) with concentrations about 200µg/ml. were compared with that of fresh insulin solutions with same concentration.

Fourier transform infrared spectroscopy
Fourier transform infrared spectra of insulin, polymers and optimized insulin loaded microparicles using different polymers were obtained using ATR -FTIR using a Perkin Elmer, Spectrum BX, FTIR spectrophotometer (Tokyo, Japan) as KBr pellets. Samples were placed on the probe. The scanning range was 4000-400 cm^{-1} and the resolution was 2 cm^{-1}.

X-ray diffraction
The X-ray diffraction patterns for bovine insulin, Eudragit L 100: Eudragit S 100 (1:1) and bovine insulin loaded Eudragit L 100: Eudragit S 100 (1:1) particles were recorded using a X-ray diffractometer using $CuK\alpha_1$ radiation of wavelength 1.5406 ^{0}A, by measuring the angle of diffraction over the range of 3.0^0-45.0^0 2Θ at the scanning rate of 2.0^0/ min.

Stability Testing
It is the capacity of a drug product to remain within the specifications established to ensure its identity, strength, quality and purity.
Accurately weighed parent insulin (bovine insulin) and selected batches of insulin microparticles were kept in falcon tubes in self-sealing cover at different temperatures (-20^0C, 4^0C and 25^0 C). They were checked for their drug encapsulation efficiency (% EE). Samples were withdrawn at months 0, 1.5, 3, 6 and 12 and % EE was estimated.

RESULTS AND DISCUSSION
Formulation variables
The effect of the formulation variables on the encapsulation efficiency and size of chitosan, PLGA, Eudragit L 100 and Eudragit S 100 microparticles prepared by triple emulsion solvent evaporation technique is shown in Table 1. The microparticles prepared with more

concentration of Eudragit polymer encapsulated a larger amount of insulin compared with lower concentration of Eudragit polymer. This could be due to more coating of the Eudragit polymer and hence less leakage of the drug occurs due to less number of pores left. It was observed that small volume of IAP and EAP provides the increased encapsulation efficiency and decreased particle size. The mean diameter of the microparticles prepared with exclusion of PLGA was in the range of 1-10 μm. and those prepared with exclusion of chitosan was in the range of 9-33 μm.

It has been reported that the droplet size of a primary emulsion may increase with an increase in the volume of the internal aqueous phase which, in turn may be responsible for the increase in size of particles. Accordingly, during formation of a primary emulsion, sonication of a small volume leads to smaller droplet formation, which could account for a smaller particle size. A higher rate of leaching, poor physical stability of the primary emulsion and an increase in number of pores on the surface of the microparticles may be responsible for the decrease in encapsulation efficiency associated with increase in volume of internal aqueous phase of the primary emulsion. Thus, a smaller volume of the internal aqueous phase is desirable for higher encapsulation efficiency of insulin and particles of smaller size. This increased encapsulation efficiency may be simply due to a relatively greater proportion of polymer with respect to the amount of the drug. The increase in polymer load leads to a shorter time for the composition of the polymer solution to reach the viscous (gelation) boundary, resulting in rapid formation of a film-like membrane on the periphery of the droplets. If the film-like polymeric membrane is quickly solidified, the microparticle structure is more fixed and thus solvent and nonsolvent counter diffusion is delayed. As a consequence, less water may be able to diffuse into the dispersed phase and less drug would be carried by the solvent into the aqueous phase.

Enteric nature of microparticles
Studies of the optimized formulations revealed that the release of insulin from insulin-loaded microparticles was mainly influenced by the nature of the polymer as well as pH of the surrounding media. Complete enteric coating could not be achieved because 5.45%-9.42% of drug release in 0.1NHCl (pH 1.2) in 2 hr. The release of the protein from the microparticles in acidic medium may be due to insufficient coating

or film defects created during lyophilization. Since small amounts of chitosan and PLGA were used in the formulation, the particles were only slightly swollen and remained intact in this case. As a result, insulin would remain almost inactive as well as lose its structural integrity at low pH in the stomach (pH 1.2). Hence, a significant amount of insulin wouldn't reach the target site (colon) for digestion of proteins. Thus, use of enteric polymer matrix system for oral delivery of insulin would be satisfactory (because the protein is not released from the optimized formulation in an adequate amount in the stomach) for maintaining the structural integrity of the protein during the transit through the stomach. The microparticles had a negative charge contributed by the carboxylic groups of phthalic acid (pKa about 4.47) and methacrylic acid (pKa about 4.23) residues in the enteric polymer backbone.It is known that the carboxylic groups of anionic particles become protonated if pH is below the pKa of the carboxylic acid, leading to a decrease in the surface charge of the particles. Reduction in the surface charge diminishes the electrostatic repulsion and increases aggregation. Thus, it appears quite natural that the microparticles would aggregate in 0.1NHCl which has a pH 1.2 below the pKa of .phthalic acid (pKa about 4.47) and methacrylic acid (pKa about 4.23). It has been reported that the stomach retains food particles until these are fragmented into particles smaller than 0.5 mm in diameter. Subsequent to gastric emptying, the particles would enter the duodenum where the pH is > 5.0. Accordingly, as the pH of the dispersion of the microparticles in 0.1NHCl was raised to 5.3, deaggregation of the particles was observed due to pH- induced deprotonation of the carboxylic groups and restoration of negative charge. The result suggests that the particles, on being emptied by the stomach into the duodenum, which has a pH > 5.0, would undergo deaggregation, resulting an increase in the effective surface area of the particle.

The characterization of Eudragit L 100: Eudragit S 100 (1:1) particles with exclusion of PLGA and Eudragit L 100: Eudragit S 100 (1:1) particles with exclusion of chitosan is summarized in Table 1 and Table 2 respectively.

In vitro release profile
In vitro release from all the optimized batches of bovine insulin loaded chitosan and Eudragit L 100 :Eudragit S 100 (1:1) microparticles showed 5.45%-8.12% of drug release in 0.1NHCl (pH 1.2) in 2 hr. while bovine insulin loaded PLGA

and Eudragit L 100 : Eudragit S 100 (1:1) microparticles showed 6.55%-9.42% of drug release in 0.1NHCl (pH 1.2) in 2 hr. Next, the drug release was studied in PBS pH 6.8. Bovine insulin loaded chitosan and Eudragit L 100 :Eudragit S 100 (1:1) microparticles (BLSY III) showed more initial burst release (20.963%) in 1 hr. and higher % cumulative release (83.896%) in 12 hr. than bovine insulin loaded PLGA and Eudragit L 100 : Eudragit S 100 (1:1) microparticles (BLS III) showing initial burst release (20.023%) in 1 hr. and % cumulative release (78.572%) in 12 hr. This may be due to contolled release property of PLGA. (Fig.1 and Fig. 2).

CD Spectroscopy
Preservation of the structural integrity of a protein drug after release is crucial for its biological efficacy. Thus, the secondary structure of insulin released from particles was investigated using CD. Effect of the the matrix materials and formulation procedure on the secondary structure of insulin is shown in Fig. 4 The circular dichroism spectrum of intact insulin showed ellipticity trough at 212-214 nm (Fig.3). The circular dichroism spectrum of insulin in prepared microparticles was close to that of intact pure insulin (ellipticity trough at 213-216 nm) demonstrating that the matrix materials and formulation procedure did not significantly influence insulin conformation.

Fourier transform infrared spectroscopy
TheFourier transform infrared spectra of bovine insulin, ,Eudragit L 100 : Eudragit S100 (1:1), bovine insulin loaded Eudragit L 100 : Eudragit S100 (1:1) microparticles are shown in Figures 6-8. The Eudragit L 100 and Eudragit S100 polymers contain both carboxylic acid and ester groups. The spectra of Eudragit L 100: Eudragit S100 (1:1) show the carbonyl stretching vibrations of carboxylic acid group at 1708.62 cm^{-1}. The spectra of Eudragit L 100: Eudragit S100 (1:1) also show the carboxylate anion stretching vibrations at 1382.71 cm^{-1}.

Bovine insulin shows a broad band in the region of 3320-3140 cm^{-1}, having a peak at 3268.75 cm^{-1} respectively due to the N-H stretch of a secondary amide. Bovine insulin shows a predominant band at 1680-1630 cm^{-1}, having a peak at 1635.34 cm^{-1} due to the carbonyl stretching vibrations of secondary amide. Bovine insulin shows the carbonyl stretching vibrations due to phenol at 1378.85 cm^{-1} while O-H bending vibrations of phenol at 1229.40 cm^{-1}.It

also shows C-S stretching of sulfides and disulfides at 660.50cm^{-1}.

The spectra of bovine insulin loaded Eudragit L 100 :Eudragit S100 (1:1) microparticle show peak at 3399.89 cm^{-1} due to the N-H stretch of a secondary amide. The particles show peak at 1710.55 cm^{-1} due to the carbonyl stretching of secondary amide.Particles show the carbonyl stretching vibrations due to phenol at 1366.32 cm^{-1} while O-H bending vibrations of phenol at 1247.72 cm^{-1}. Particles also show C-S stretching of sulfides and disulfides at 610.36 cm^{-1}.

Powder x-ray diffraction
Figure 9, 10 and 11 show the powder x-ray diffractionpatterns for bovine insulin, Eudragit L 100 : Eudragit S100 (1:1) and bovine insulin loaded Eudragit L 100 : Eudragit S100 (1:1) microparticles respectively. The diffractograms of bovine insulin and the polymer indicated an amorphous structure. The diffractogram of microparticles indicated crystalline structure with slightly amorphous structure. This may be due to slightly crystalline nature of insulin.

Particle morphology
Scanning electron microscopy was used to observe the morphology of the microparticles. (Fig. 12) Insulin loaded Eudragit L 100 microparticles, insulin loaded Eudragit S 100 microparticles, and insulin loaded Eudragit L 100: Eudragit S100 (1:1) microparticles were spherical and had smooth surface.

Stability study
Tables 3, 4 and 5 show the results of the stability studies of the optimized insulin loaded microparticles of chitosan and Eudragit L 100: Eudragit S100 (1:1) microparticles and free insulin formulations. Tables 6, 7 and 8 show the results of the stability studies of the optimized insulin loaded microparticles of PLGA and Eudragit L 100: Eudragit S100 (1:1) microparticles and free insulin formulations. From tables, it is clear that insulin loaded microparticles show more % EE than free insulin formulations on storage for 12 months. It is clear from tables that all formulations show highest % EE at 4 ^{0}C, lowest at 25 ^{0}C and formulations stored at -20 ^{0}C show higher % EE than formulations stored at 25 ^{0}C but lower than formulations stored at 4 ^{0}C. Thus, the stability of insulin entrapped in microparticles was significantly improved in comparison with the free insulin powder.

CONCLUSION
Insulin was successfully entrapped in enteric microparticles by double and triple emulsion solvent evaporation technique, optimizing the formulation parameters in order to attain the maximum encapsulation efficiency, a spherical shape and an optimum *in-vitro* release profile. The release profiles for formulations confirmed their gastroresistance, thus allowing pH-dependent release of insulin in the gastrointestinal tract. The results of in-*vitro* release studies showed that bovine insulin loaded chitosan and Eudragit L 100: Eudragit S100 (1:1) microparticles release more and quicker insulin than bovine insulin loaded PLGA Eudragit L 100: Eudragit S100 (1:1) microparticles. Thus, the particles formulated with exclusion of PLGA and the particles formulated with exclusion of chitosan have great potential as oral carriers for delivery of insulin to small and large intestine to facilitate reduction of blood glucose level.

ACKNOWLEDGEMENT

Table 1: Characterization of Eudragit L 100: Eudragit S 100 (1:1) particles with exclusion of PLGA

Formulation Code	Actual Loading (µg/mg)	Actual Loading (%)	%EE	Particle Size (d.nm)	Zeta Potential (mV)
BLSY I	11.496	1.149	49.43	6771	-16.1
BLSY II	10.846	1.084	59.65	9045	-34.7
BLSY III	11.369	1.136	80.29	4651	-37.2
BLSY IV	10.881	1.088	85.96	9238	-26.8

Abbreviations: B, Bovine insulin; L, Eudragit L 100; S, Eudragit S 100; LSY, Eudragit L 100 : Eudragit S 100 (1: 1); I, 3% w/v Eudragit; II , 4% w/v Eudragit; III, 5% w/v Eudragit; IV, 6% w/v Eudragit; EE, Drug entrapment efficiency

Table 2: Characterization of Eudragit L 100: Eudragit S 100 (1:1) particles with exclusion of chitosan

Formulation Code	Actual Loading (µg/mg)	Actual Loading (%)	%EE	Particle Size (µm)	Zeta Potential (mV)
BLSI	6.363	0.636	64.902	27.933	-32.4
BLSII	6.466	0.646	81.471	22.442	-35.9
BLSIII	5.955	0.595	89.325	28.141	-26.2

Abbreviations: B, Bovine insulin; L, Eudragit L 100; S, Eudragit S 100; LS, Eudragit L 100 : Eudragit S 100 (1: 1); I, 4% w/v Eudragit; II , 5% w/v Eudragit; III, 6% w/v Eudragit; EE, Drug entrapment efficiency

Table 3: % EE remaining at -20°C

Formulation code	0 M	1.5 M	3 M	6 M	12 M
BI	88.71%	77.14%	69.47%	65.24%	57.31%
BLSY III	76.17%	73.70%	70.98%	66.11%	60.19%
BLSY IV	85.96%	82.91%	78.88%	75.39%	69.15%

Table 4: % EE remaining at 4°C

Formulation code	0 M	1.5 M	3 M	6 M	12 M
BI	88.71%	79.14%	71.46%	68.37%	60.15%
BLSY III	76.17%	74.69%	72.91%	68.41%	62.41%
BLSY IV	85.96%	83.27%	80.72%	77.11%	70.19%

Table 5: % EE remaining at 25⁰ C

Formulation code	0 M	1.5 M	3 M	6 M	12 M
BI	88.71%	75.39%	67.45%	62.17%	55.51%
BLSY III	76.17%	72.95%	69.19%	64.08%	58.11%
BLSY IV	85.96%	81.92%	76.17%	73.13%	66.41%

Table 6: % EE remaining at -20⁰ C

Formulation code	0 M	1.5 M	3 M	6 M	12 M
BI	88.71%	77.14%	69.47%	65.24%	57.31%
BLS II	81.47%	77.42%	73.44%	69.37%	64.33%
BLS III	89.33%	85.26%	82.19%	79.18%	73.81%

Table 7: % EE remaining at 4⁰ C

Formulation code	0 M	1.5 M	3 M	6 M	12 M
BI	88.71%	79.14%	71.46%	68.37%	60.15%
BLS II	81.47%	79.44%	76.49%	71.93%	67.29%
BLS III	89.33%	87.51%	85.62%	80.47%	75.74%

Table 8: % EE remaining at 25⁰ C

Formulation code	0 M	1.5 M	3 M	6 M	12 M
BI	88.71%	75.39%	67.45%	62.17%	55.51%
BLSY III	81.47%	75.38%	71.95%	66.71%	60.49%
BLSY IV	89.33%	83.29%	78.44%	76.91%	69.13%

Abbreviations: BI, Bovine insulin; M, month
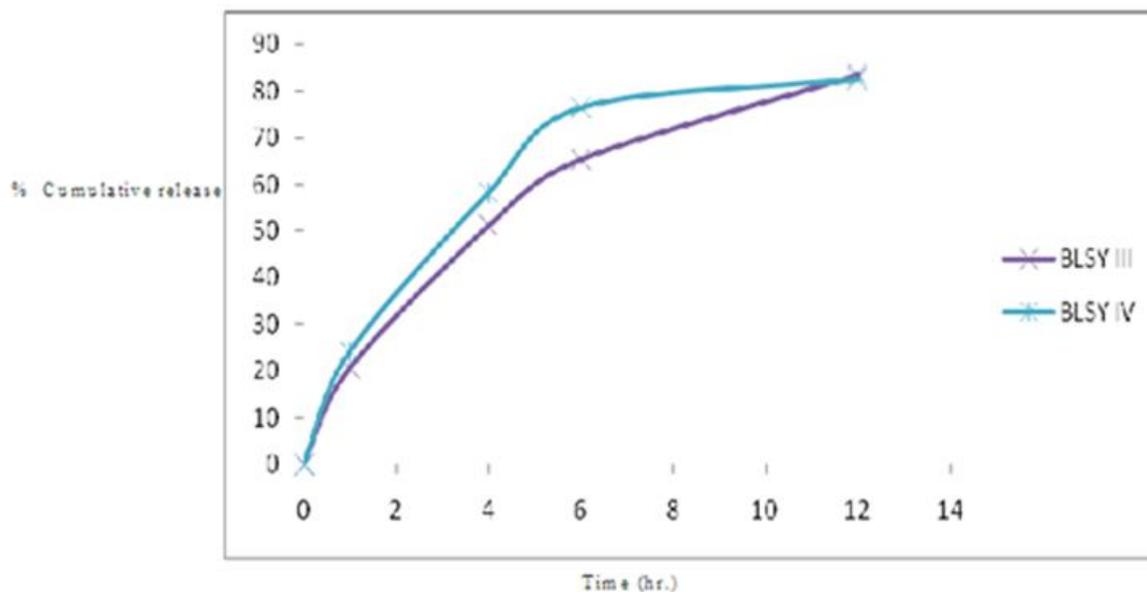
Fig. 1: Comparison of % CR of bovine insulin loaded particles using chitosan and Eudragit L 100: Eudragit S 100 (1:1)
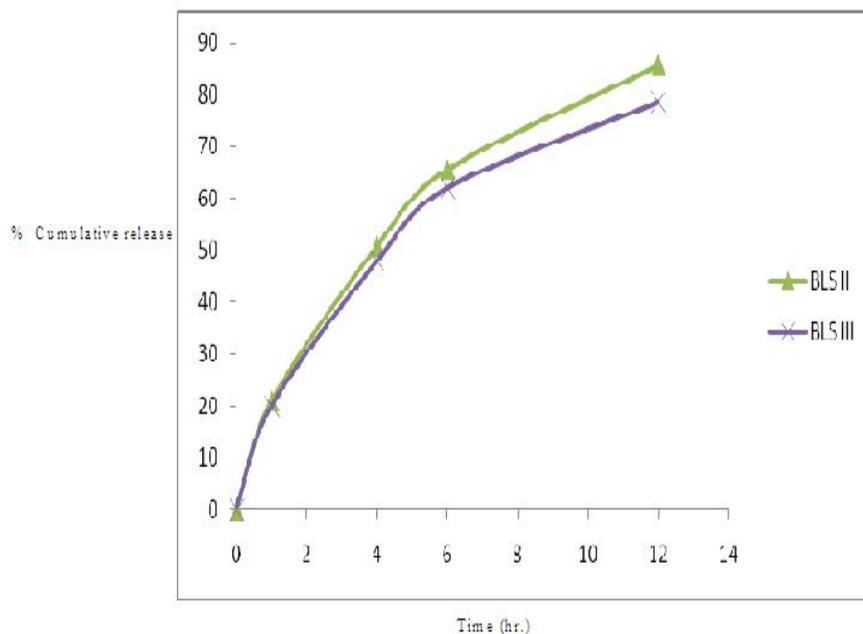
**Fig. 2: Comparison of % CR of bovine insulin loaded
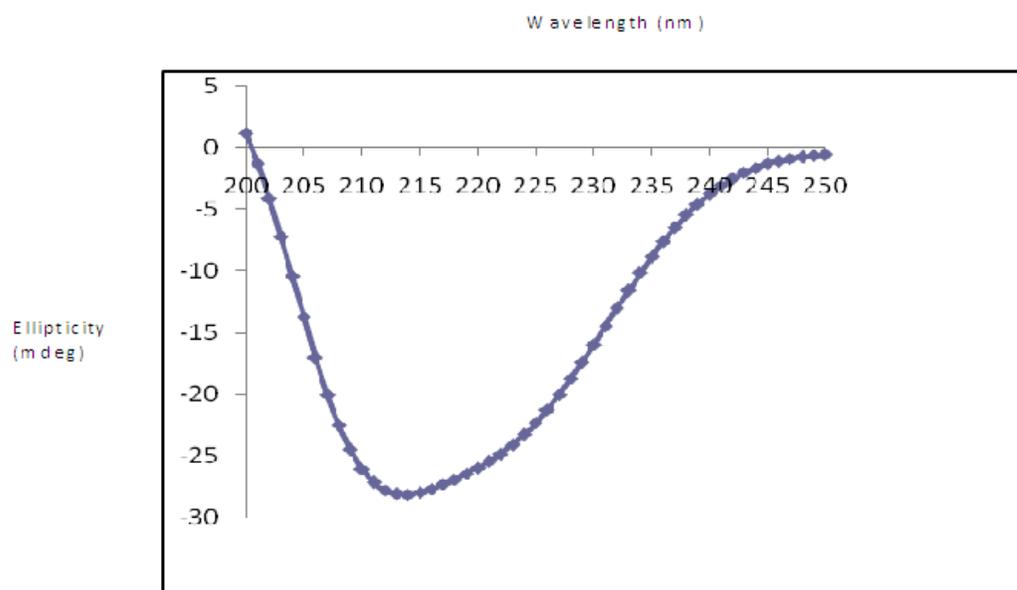particles using PLGA and Eudragit L 100: Eudragit S 100 (1:1)**
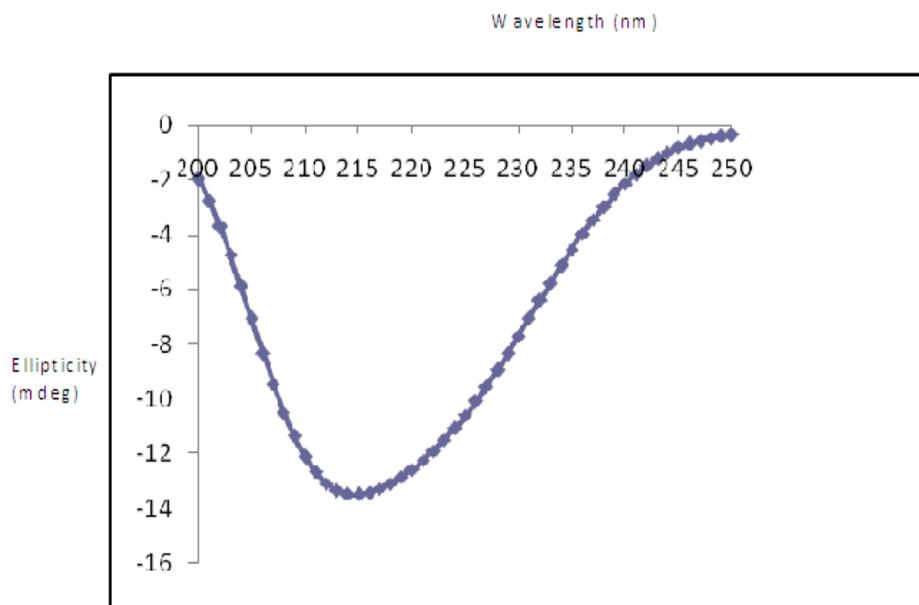

Fig. 3: CD spectra of bovine insulin

Fig. 4: CD specta of bovine insulinloaded microparticles
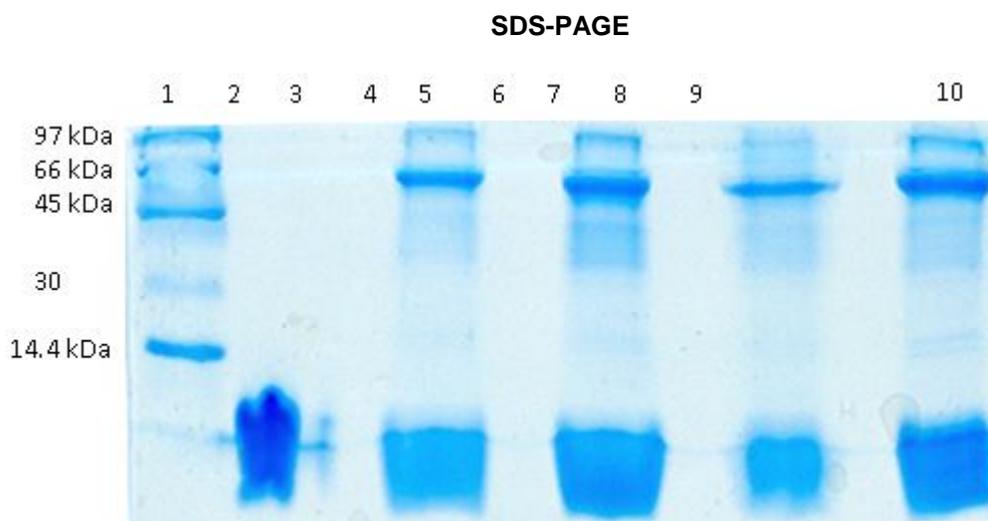
SDS-PAGE

Fig. 5: SDS-PPAGE analysis of insulin and optimized insulin loaded microparticles
Lane-1: M.W. markers; Lane-2: Bovine insulin solution; Lane-4: Bovine insulin loaded
Chitosan and 5% [Eudragit L 100 : Eudragit S100 (1:1)] microparticles (BLSY III)
Lane-6 : Bovine insulin loaded Chitosan and 6% [Eudragit L 100 : Eudragit S100 (1:1)]
microparticles (BLSY IV)
Lane-8: Bovine insulin loaded PLGA and 5% {Eudragit L 100 : Eudragit S100 (1:1)}
Microparticles(BLS II)
Lane-10: Bovine insulin loaded PLGA and 6% {Eudragit L 100 : Eudragit S100 (1:1)}
Microparticles(BLS III)
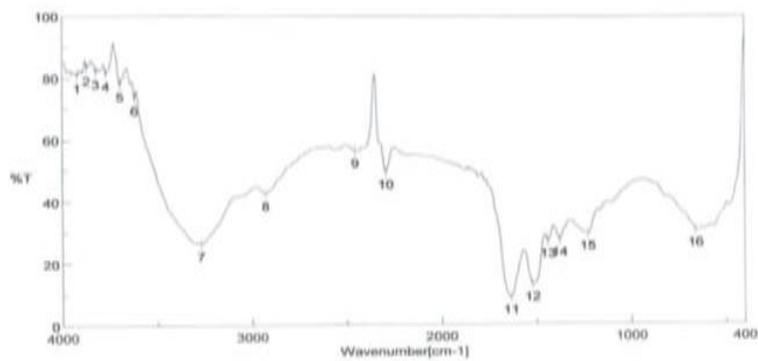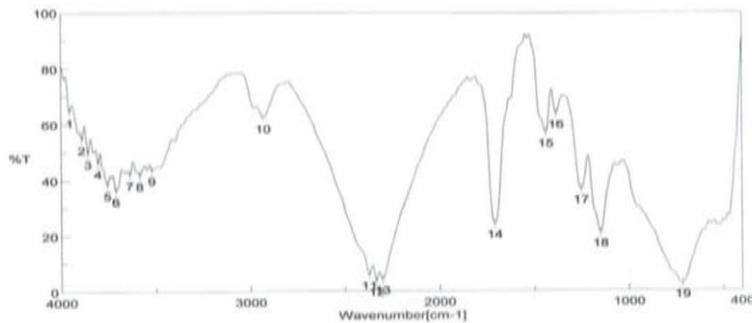
Fig. 6: FT-IR spectra of Bovine Insulin

Fig. 7: FT-IR spectra of Eudragit L 100 : Eudragit S100 (1:1)
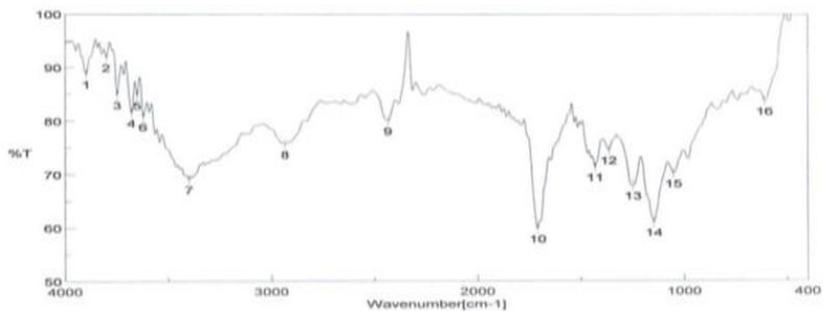

**Fig. 8: FT-IR spectra of bovine insulin loaded
Eudragit L 100 : Eudragit S100 (1:1)
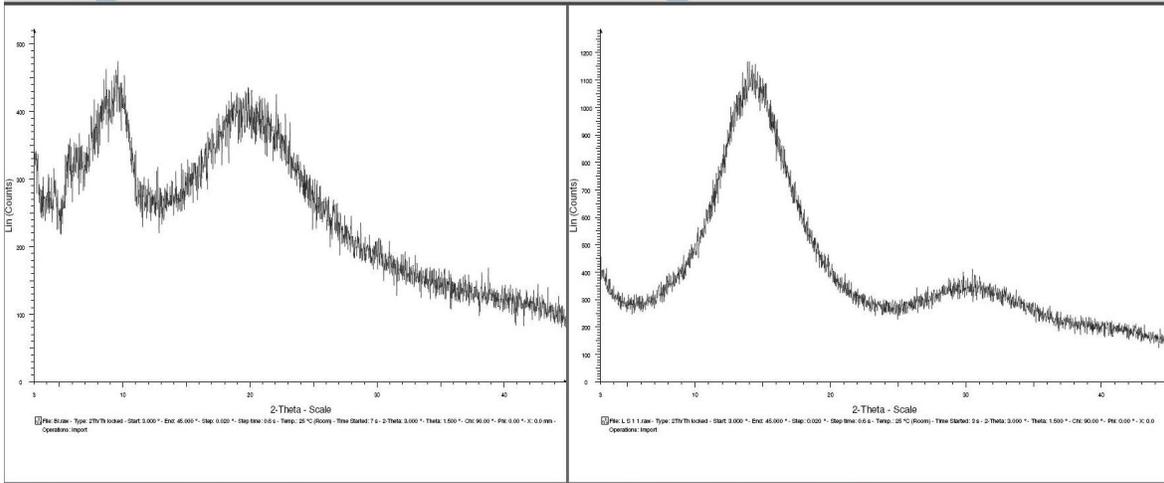Microparticles**

Powder x-ray diffraction

Fig. 9:   XRD pattern of bovine insulin powderFig. 10 XRD pattern of Eudragit S 100 : Eudragit L 100 (1: 1)
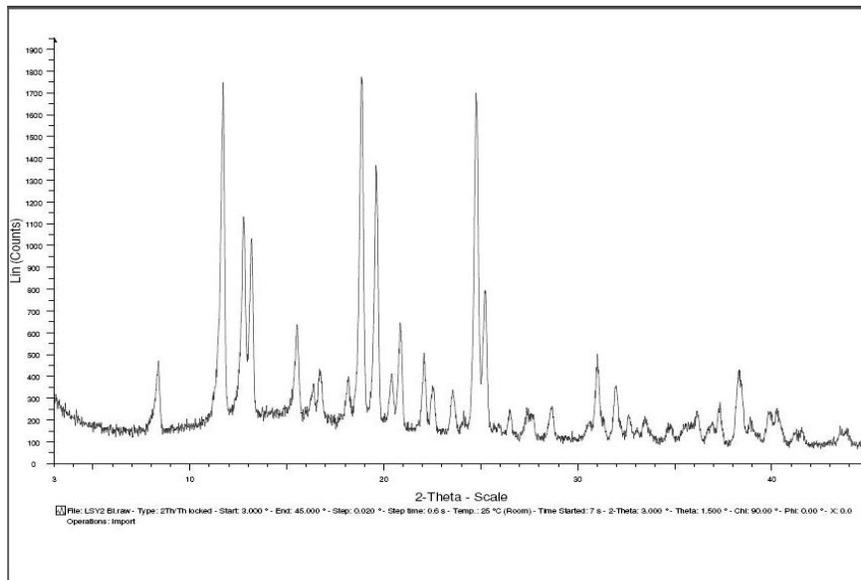

Fig. 11: XRD pattern of bovine insulin loaded Eudragit S 100 : Eudragit L 100 (1:) microparticles
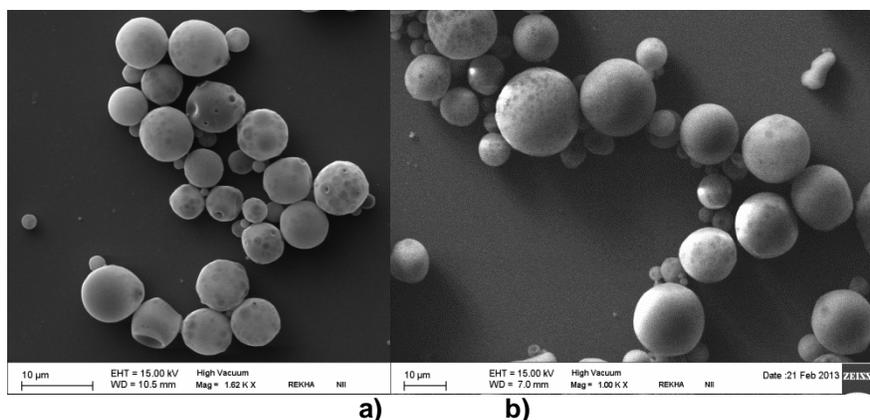
Fig. 12: SEM micrographs of optimized bovine insulin loaded a) chitosan and Eudragit L 100: Eudragit S100 (1:1) microparticles b) PLGA and Eudragit L 100: Eudragit S100 (1:1) microparticles

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