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pH Responsive Polymeric Nanoparticles for Oral Insulin Delivery

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Abstract **-** Gelatin-eudragit L100 nanoparticles of wet size range 170-563nm were prepared by two step dissolvation method and the effect of different concentrations of eudragit L100 and emulsifying agent - sodium lauryl sulphate (SLS) - on the particle size were studied. Synthesized nanoparticles were characterized by attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR) and the mean size distribution. Insulin loading was done at a pH 7.4 and the in vitro insulin release studies of nanoparticles were carried out by simulating gastrointestinal tract condition which showed the minimal insulin release at pH 2.5 (20% in 90min) while appreciable release (40% in first 30min) at pH of 7.4. This pH responsive release pattern of the synthesized nanoparticles confers on the insulin protection from proteolytic degradation in acidic environment of stomach and upper intestinal part while enhancing bioavailability in the later part of intestine.

Key words: *pH responsive; nanoparticles; oral insulin delivery.*

I. INTRODUCTION

Insulin is the most effective and widely used drug in the treatment of advance-stage diabetes. Administering drug orally is by far the most widely used route of administration, although it is generally not feasible for peptide and protein drugs. The main reasons for the low oral bioavailability of insulin, a peptide drug, are inactivation in acidic pH of upper GI tract, presystamic enzymatic degradation and its poor penetration through the intestine membrane [2–3]. Insulin administration through the oral route passes through the hepatic pass and produces the similar effect as pancreas-secreted insulin [4]. Various strategies have been developed to try and achieve an oral insulin delivery which includes co-administration with absorption enhancers, enzyme inhibitors, polymeric carriers, and lipid based carriers as liposomes [5-8] After the use of macromolecules and microparticles, the researchers are now focusing on polymeric nanoparticles as drug delivery materials, owing to the larger surface:volume ratio that follows higher drug loading and release [9]. Besides the synthetic and degradable polymers the natural polymers, such as chitosan, dextran, hyaluronic acid and gelatin, are being extensively explored considering their nontoxicity and biodegradability along with their natural origin [10-13]. Gelatin forms a versatile class of naturally derived biopolymers and is indispensable in modern pharmaceuticals, the well established application being pharmaceutical dosage forms [14]. Additionally, the abundant functional groups in gelatin offer the advantage of incorporating more functionalities and introducing modifications via chemical derivatization [13]. Poly(ethylene glycol)-modified gelatin, thiolated derivatives of gelatin, chitosan conjugated gelatin and poly(DL-lactide)-grafted gelatin have already been reported as gelatin based derivatives for potential pharmaceutical applications [15-18]. Effective insulin delivery via oral route requires a polymer that would enable the release of the drug only at a pH above 7, as prevailing in the ileum and large intestine because insulin is better absorbed in this part compared to jejunum [7]. Eudragit L100 is one such anionic polymer that shows pH-dependent solubility and is therefore specifically soluble in the region of the digestive tract where juices are neutral to weakly alkaline [19].

In the present investigation gelatin-eudragit L100 nanoparticles were synthesized and evaluated in terms of insulin encapsulation efficiency and insulin release profile under physiologically relevant pH condition.

II. MATERIALS AND METHODS

Gelatin type A, from porcine skin (175 Bloom), glutaraldehyde 8% aqueous were obtained from Sigma Chemical Co (St Louis, MO, USA). Acetone, ethyl alcohol and acetonitrile were purchased from Rankem (Delhi, India). All chemicals were of analytical grade and used as received. Humuinsulin-R (r-DNA origin) of 100I.U/ml concentration purchased from Eli Lilly and Company (USA) was used as received. All the solutions were prepared in Milli Q water.

Synthesis of gelatin and gelatin-eudragit L100 nanoparticles

Gelatin and gelatin-eudragit L100 nanoparticles were prepared by a two step desolvation method, as described previously, with certain modifications [20]. In brief, 1.25g of gelatin was dissolved in 25mL water under gentle stirring. Acetone (25mL) was then added in the solution to precipitate the high molecular weight gelatin. Supernatant containing low molecular weight gelatin was discarded, while, the precipitated gelatin fraction was obtained as sediment. The sediment thus obtained was again dissolved in 25mL water by continuous stirring at an elevated temperature (37°C) and the pH was adjusted to 7. Different concentrations of eudragit L100 and sodium lauryl sulphate - surfactant - were added to the solution. The gelatin-eudragit L100 nanoparticles were formed in-situ during the second desolvation step where 50mL acetone was added drop wise under continuous stirring (500rpm). At the end of the desolvation process, glutaraldehyde solution (250µL) was added as the crosslinking agent and stirring was continued for 12h. Finally, the nanoparticles obtained were centrifuged (16000g for 20min), washed, redispersed in a solution of acetone:water (30:70) and stored at 4-8°C for future use.

III. CHARACTERIZATION

Fourier transform infrared spectroscopy

ATR–FTIR spectra (attenuated total reflectance–Fourier transform infrared spectroscopy) of vacuum-dried samples of gelatin and gelatin-eudragit L100 nanoparticles were recorded on a Perkin-Elmer spectrum one spectrometer.

Dynamic light scattering

The particle size (mean diameter) of nanoparticles was determined by particle size analyzer (Malvern zetasizer nano, Malvern Instruments, U.K). Light scattering measurement was performed for 200s per sample after suspending the particles in buffer solution for 2h at 37°C and the obtained data was analysed.

Insulin loading

Insulin loading of nanoparticles was performed by using an insulin solution of 100I.U/mL maintained at pH 7.4 by adding 1N NaOH solution. 0.02mg of Tween 80 was also added to ensure that the insulin did not adsorb onto the glass surface. One gram of nanoparticles of different size were dispered separately in 6mL of insulin solution at 37°C for 6h at pH 7.4 to allow maximum loading. The reverse phase high performance liquid chromatography (RP-HPLC) was used to determine the amount of insulin released from the samples at pH 7.4 for calculating insulin loading efficiency. To determine the insulin concentration, Kromasil C18 column was employed and the wavelength of instrument detector was set at 214nm [21]. The mobile phase was a mixture of acetonitrile and sodium sulphate buffer of pH 2.3 in the ratio 24:76 with a flow rate of 1.0mL/min. Insulin loading efficiency was calculated from the following equations:

Loading efficiency (%) $= M_{bound}/W_{theoretical} \times 100$ **(1)**
where, M_{bound} ***is the amount of insulin (mg) released from the particles at pH 7.4 in 24h and*** $W_{theoretical}$ ***is the theoretical amount of insulin originally added in loading mixture.***

In vitro insulin release studies

For in vitro insulin release studies, 1g each of insulin loaded gelatin and gelatin-eudragit L100 nanoparticles of different size were suspended separately in 10mL citrate-phosphate buffer solutions of pH 2.5 at 37°C for 90min while stirring the mixture 100rpm. At every 30min time interval, insulin loaded nanoparticles were centrifuge and re-suspended in 10mL of fresh buffer. After 90min, insulin release studies of the same particles was continued with fresh phosphate buffer of pH 7.4 and samples were collected again at 30min time interval in the same manner till 6h. The pH was changed from 2.5 to 7.4 during release experiments to simulate the GI tract conditions. The supernatant of each centrifuged sample was analyzed by RP-HPLC and the amount of insulin released was calculated by means of a standard calibration curve.

IV. RESULTS AND DISCUSSION

Characterization

Fourier transform infrared spectroscopy

The ATR-FTIR spectra of pure gelatin, eudragit L100 and gelatin-eudragit L100 nanosparticles are presented in Fig. 1. FTIR spectrum of gelatin exhibits characteristic absorption peak at 1540cm^{-1} for the gelatin backbone [22]. Other characteristic absorption bands of gelatin appear at 1650cm^{-1} and around 3000cm^{-1} because of the -C=O stretching and -NH stretching vibrations

respectively (Fig. 1d). The spectra of eudragit L100 (Fig. 1e) showed the characterstic -C=O absorption at 1721cm^{-1} whereas CH_X vibration were noted at 1385 and 1485cm^{-1}, and ester vibration could be discerned at 1160 and 1260cm^{-1}[23]. The spectra of crosslinked gelatin-eudragit L100 (Fig. 1a-c) nanoparticles showed a broad band from 3300 to 3600cm^{-1} of the amide bond thereby confirming the reaction of eudragit L100 and gelatin. Another peak observed at 1450cm^{-1} was assigned to the aldimine group formed after the reaction.

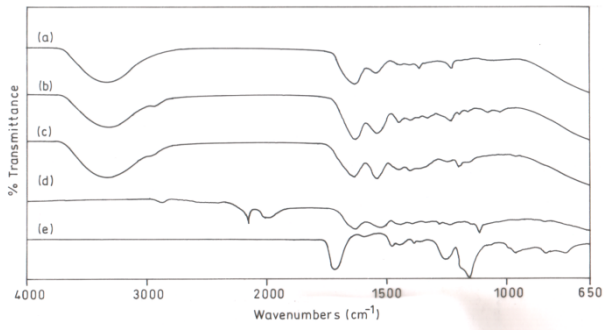

Figure 1. FTIR spectra: (a-c) gelatin-eudragit L100 nanoparticles with particle size 170nm, 218nm, 313nm, (b) gelatin and (c) eudragit L100

Dynamic light scattering

To understand the effect of eudragit L100 on particle size, varied concentration of eudragit L100 were used at a fixed gelatin concentration in the reaction mixture. It was observed through differential light scattering studies that the pure gelatin particles had the size of 234nm which increased on addition of eudragit L100 in the feeding reaction mixture (Table I). The particle size of 549nm was obtained by using eudragit L100 concentration of 0.1%. DLS studies were also performed to understand the effect of varied surfactant (sodium lauryl sulphate) concentration on the size of the particles synthesized at a constant concentrations of eudragit L100 (0.1%) and gelatin. The size of the synthesized gelatin-eudragit L100 particle decreased with an increase in the surfactant concentration from 0.5 to 2% in the reaction mixture and the results are given in Table I.

Table I Properties of gelatin-eudragit L100 nanoparticles prepared with varied constituent concentrations

Gelatin (g)	**Eudragit L100 (g)**	**Surfactant (%)**	**Particle Size(nm)**	**Loading Efficiency (%)**
1	-	-	234	54
1	0.1	-	549	-
1	0.1	0.5	313	63
1	0.1	1.0	218	72
1	0.1	2.0	170	86

Insulin loading of nanoparticles

For drug loading onto different carriers generally either of the two methods is adopted: the drug is directly added in the feed mixture during the preparation of nanoparticles or the nanoparticles are allowed to swell in the solution of drug until the equilibrium is reached. Because of the associated drawbacks with the first method, like that of the loss of chemical/biological activity of the drug, due to the direct addition of drug to the reaction mixture of carriers, the later method was adopted for insulin loading of nanoparticles in our studies. Loading efficiency of various particles was measured and is presented in Table I. The loading efficiency of pure gelatin nanoparticles was 54%. The loading efficiency of gelatin-eudragit L100 nanoparticles showed an increased trend with decrease in particle size and nanoparticles of 313, 218,170nm depicted the loading efficiency of 63, 72 and 86% respectively. The poor loading efficiency of gelatin nanoparticles compared to that of gelatin-eudragit L100 nanoparticles is because of the low swelling ratio of pure gelatin nnoparticles, allowing less penetration of insulin molecules from the solution into the particle matrix. The increase in loading efficiency in case of gelatin-eudragit L100 nanoparticles with a decrease in particle size is mainly due to varied swelling behavior of different sized nanoparticles. Smaller is the particle size higher is its surface to volume ratio and therefore higher will be the swelling and hence the loading efficiency.

In vitro *insulin release*

Insulin loaded gelatin and gelatin-eudragit L100 nanoparticles may be visualized as a three dimensional network structure with insulin molecule occupying the space available between the network strands. When these nanoparticles are allowed to swell in a release medium, the solvent (buffer) molecules diffuse into the nanoparticles matrix forming water filled permeation channels between the strands and relaxation of the polymeric chains takes place. As a result, the insulin molecules dissolve into water and diffuse out to the external receptor medium through water channels. For in vitro release studies from different sized insulin loaded gelatin and gelatin eudragit L100 nanoparticles two pH values were selected as 2.5 and 7.4, which are identical to the acidic and alkaline environment of stomach and large intestine, respectively. When insulin is administered orally, it first goes to stomach where pH is acidic and then passes to the intestine region of alkaline pH value. Thus, in order to simulate the GI tract conditions, initially, all the insulin loaded particles were

dispersed in citrate-phosphate buffer solutions of pH 2.5 for 90min followed by transfer of particles into basic pH 7.4 for further release studies. Cumulative insulin release at 37ºC from insulin loaded gelatin-eudragit L100 nanoparticles of different size as a function of pH and duration of exposure is shown in Fig. 2 and 3.

In the initial 90min, only 20% of insulin release was observed in two hours at pH 2.5 (Fig. 2) while 40% of insulin was released into the medium at pH 7.4 in first 30min (Fig. 3). Pure gelatin nanoparticles followed the same pattern of insulin release but the amount of insulin release was lower compared to any of the eudragit L100 particles studied which is because of the low swelling ratio and low insulin loading in pure gelatin nanoparticles. The highest amount of insulin release was recorded with the smallest sized gelatin-eudragit L100 nanoparticles of 170nm because of the high surface to volume ratio of these particles that allowed appreciable swelling which in turn facilitated drug loading and consequently release from these particles.

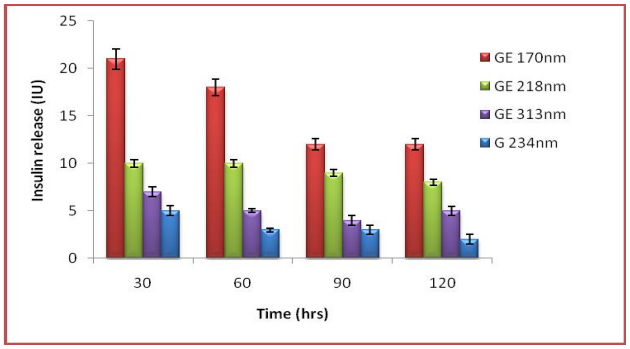

Figure 2. *In-vitro* release of insulin from gelatin-eudragit L100 nanoparticles with particle size 170nm, 218nm, 313nm and gelatin (n=3) at pH 2.5

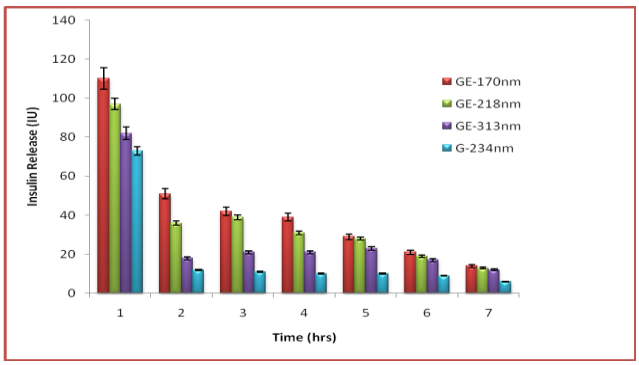

Figure 3. *In-vitro* release of insulin from gelatin-eudragit L100 nanoparticles with particle size 170nm, 218nm, 313nm and gelatin (n=3) at pH 7.4

CONCLUSION

Gelatin-eudragit L100 nanoparticles were prepared by the two step dissolvation method. Increase in eudragit L100 concentration against the constant concentration of the gelatin showed an increase in particle size. Particle size was found to be inversely proportional to the surfactant concentration. IR spectra of the prepared nanoparticles confirmed the formation of nanoparticles of gelatin and eudragit L100. Increase in insulin loading in the prepared gelatin-eudragit L100 nanoparticles was observed with decrease in the nanoparticle size. Nanoparticles showed the minimum insulin release in acidic medium while significant and sustained release was observed in basic medium as required in gastrointestinal tract. *In vitro* insulin release profile of the nanoparticles makes them a potential candidate for oral insulin delivery.

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