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Preparation and In Vitro/In Vivo Evaluation of Gliclazide Loaded Eudragit Nanoparticles as a Sustained Release Carriers

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ABSTRACT The aim of this study was to formulate and optimize gliclazide-loaded Eudragit nanoparticles (Eudragit L100 and Eudragit RS) as a sustained release carrier with enhanced efficacy. Eudragit L 100 nanoparticles (ELNP) were prepared by controlled precipitation method whereas Eudragit RSPO nanoparticles (ERSNP) were prepared by solvent evaporation method. The influence of various formulation factors (stirring speed, drug:polymer ratio, homogenization, and addition of surfactants) on particle size, drug loading, and encapsulation efficiency were investigated. The developed Eudragit nanoparticles (L100 and RS) showed high drug loading and encapsulation efficiencies with nanosize. Mean particle size altered by changing the drug:polymer ratio and stirring speed. Addition of surfactants showed a promise to increase drug loading, encapsulation efficiency, and decreased particle size of ELNP as well as ERSNP. Dissolution study revealed sustained release of gliclazide from Eudragit L100 as well as Eudragit RSPO NP. SEM study revealed spherical morphology of the developed Eudragit (L100 and RS) NP. FT-IR and DSC studies showed no interaction of gliclazide with polymers. Stability studies revealed that the gliclazide-loaded nanoparticles were stable at the end of 6 months. Developed Eudragit NPs revealed a decreased t_{min} (ELNP), and enhanced bioavailability and sustained activity (ELNP and ERSNP) and hence superior activity as compared to plain gliclazide in streptozotocin induced diabetic rat model and glucose-loaded diabetic rat model. The developed Eudragit (L100 and RSPO) NP could reduce dose frequency, decrease side effects, and improve patient compliance.

KEYWORDS Eudragit L100, Eudragit RSPO, Gliclazide, Nanoparticles, Sustained release

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INTRODUCTION

Gliclazide 1-(1-azabicyclo- [3,3,0]-oct-3-yl)-3-(*p*-tolylsulfonyl) urea (Fig. 1), is a potential second generation oral hypoglycemic agent widely used for the

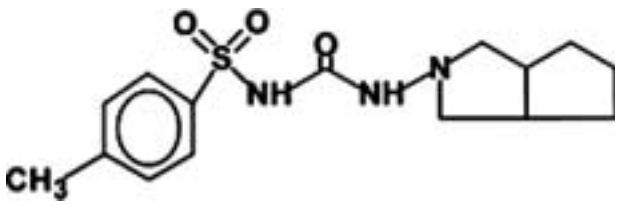

FIGURE 1 Chemical Structure of Gliclazide.

treatment of noninsulin-dependent diabetes mellitus (NIDDM) (Hong et al., 1998). Prior research work revealed that it has good general tolerability, low incidence of hypoglycemia (Palmer & Brogden, 1993; Mailhot, 1993) and low rate of secondary failure (Harrower, 1994). In addition, it has potential for slowing the progression of diabetic retinopathy (Palmer & Brogden, 1993). For these reasons, gliclazide appears to be a drug of choice in long-term sulfonylurea therapy for the control of NIDDM (Campbell et al., 1991; Harrower, 1994).

In general, rapid gastrointestinal (GI) absorption is required for oral hypoglycemic drugs, in order to prevent a sudden increase in blood glucose level after food intake in patients with diabetes mellitus. However, the GI absorption rate of gliclazide, in conventional dosage form (i.e., tablets), appears to be rather slow. Several studies using healthy volunteers or patients revealed that the time to reach peak serum gliclazide concentration ranged from 2 to 8 hr following oral administration of the gliclazide tablet (Palmer & Brogden, 1993). A slow absorption of a drug usually originates from either poor dissolution of the drug from the formulation or poor permeability of the drug across the GI membrane. The dose of gliclazide is 80 mg and could be increased to 380 mg TID, and hence there is always a need for the development of sustained release patient compliant formulation of gliclazide, as it is short acting sulfonyl urea.

Nanoparticles are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Nanoparticles can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency, and improving patient compliance (Davis & Illum, 1988; Ritschel, 1989).

Eudragit L100 and RS polymers are commonly used for enteric coating and also for preparation of controlled-release dosage forms (Bodmeier & Chen, 1989; Kawashima et al., 1993; Perumal et al., 1999; Pignatello, 2001). Pignatello et al. (2002a,b) explored Eudragit nanosuspensions for the ophthalmic controlled delivery of ibuprofen as well as for flurbiprofein. Haznedar and Dortune (2004) reported controlled release Eudragit microspheres of acetazolamide whereas Dai et al. (2004) studied Eudragit nanoparticles as a carrier for enhancing oral bioavailability of cyclosporine.

The aim of this study was to formulate and optimize Eudragit nanoparticles (Eudragit L100 and Eudragit RSPO) containing gliclazide to achieve a sustained release profile suitable for *per oral* administration with enhanced efficacy and which could overcome the drawbacks of gliclazide delivery.

We investigated the effect of formulation variables (polymer type, polymer, drug ratio, stirring speed, and homogenization) to obtain lower particle size, yield of production, increase in encapsulation efficiency, and drug loading. Further, we also investigated effect of nature of surfactants (cationic, anionic, and nonionic surfactants) on particle size, drug loading and encapsulation efficiency. In vitro drug release of gliclazide from nanoparticles with and without surfactants was also investigated. The developed ELNP and ERSNP were also evaluated for their efficacy in diabetic rats as well as in glucose-loaded model and the nanoparticulate formulations suitable to achieve our goal were determined.

MATERIALS AND METHODS

Materials

Eudragit L100 and Eudragit RSPO (Röhm Pharma) were kindly provided by Degussa (India). Gliclazide was supplied by Yasham Importers and Exporters Ltd., India. Surfactants used in the study such as Tween 80, cetrimide, and docusate sodium were purchased from Merck, India whereas Cremophor RH40 was kindly provided by BASF, India. Streptozotocin was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of reagent grade or higher.

Preparation of Nanoparticles

Eudragit L100 Nanoparticles

Eudragit L100 nanoparticles were prepared by controlled precipitation method. Briefly, Eudragit L100

and gliclazide were dissolved in 20 mL of 0.1 N NaOH and volume was made up to 100 mL with deionized water. Surfactant, if added, was dissolved in aqueous phase. Glacial acetic acid (0.1% v/v) was added slowly using syringe fitted with a 24-gauge needle to adjust pH of the system to 3.5. The formed nanoparticles were stirred for 4 hr. The formed nanosuspension was centrifuged at 20,000 rpm for 1 hr. The supernatant was separated and analyzed for free drug content. The formed ELNPs were isolated, washed three times with deionized water and freeze-dried.Placebo ELNPs were prepared following the above method without inclusion of gliclazide. All the nanoparticle formulations were prepared in triplicate.

Eudragit RSPO Nanoparticles

Eudragit RSPO nanoparticles were prepared by solvent-evaporation technique. Briefly, Eudragit RSPO was dissolved in acetone (10 mL). Gliclazide was dissolved in 10 mL of 0.1 N NaOH and volume was made up to 100 mL with ultra pure water. Surfactant if added, was dissolved in aqueous phase. The organic phase was added using syringe fitted with a 24-gauge needle to the aqueous phase slowly while stirring. Formed nanoparticles were stirred for 4 hr and kept overnight under magnetic stirring for removing residual acetone. The formed nanosuspension was centrifuged at 20,000 rpm for 1 hr. The supernatant was separated and analyzed for free drug content. The formed ERSNPs were isolated, washed three times with ultra pure water and freeze dried. Placebo ERSNPs were prepared following the above method without inclusion of gliclazide. All nanoparticle formulations were prepared in triplicate.

Physicochemical Characterization

Particle Size

The mean particle sizes of the formulations were determined by particle size analyzer (Beckman Coulter N4+ Plus, Wipro India Ltd.) equipped with software N4 Plus. Every sample was appropriately diluted with 0.22 μm filtered water and the reading was performed at a 90° angle with respect to the incident beam. The morphology of the Eudragit NP (L100 and RS) was examined by scanning electron microscopy (SEM) in a Jeol JSM-6400 scanning microscope (Philips, Japan).

Differential Scanning Calorimetry

The thermogram of gliclazide, sodium alginate, and gliclazide-loaded sodium alginate nanoparticles were obtained using differential scanning calorimetry (DSC) using a Perkin-Elmer DSC7 apparatus (Uberlingen, Germany) between 30°C and 300°C. The temperature gradient was 10°C/min.

Fourier Transform Infrared Spectroscopy

The FT-IR spectra of gliclazide, sodium alginate, and gliclazide-loaded sodium alginate nanoparticles were taken in KBr pellet using Perkin-Elmer Fourier transformed infrared (FT-IR) spectrophotometer (spectrum 2000) instrument.

Encapsulation Efficiency and Drug Loading

The amount of gliclazide entrapped within NP was determined by measuring the amount of nonentrapped drug in the supernatant recovered after centrifugation and washing of the NP by a suitable analytical method (Pignatello et al., 2001).

$$\% \text{ Encapsulation efficiency} = \frac{\text{Mass of drug added during NP preparation} - \text{Mass of free drug in supernatant}}{\text{Mass of drug added during NP preparation}} \times 100$$

For estimation of drug loading, around 10 mg of nanoparticles containing different amounts of gliclazide was dissolved in 10 mL of methanol and analyzed by HPLC method. Briefly, the HPLC system consisted of a pump (Jasco 655-A40), a UV detector (Jasco Intelligent). A reversed phase silica column (Spherisorb, Merck, India, C_{18}, 5 μm, measuring 25 × 0.4 cm) was utilized for drug separation. The mobile phase comprised a mixture of 0.04 M potassium dihydrogenphosphate (pH 4.6)–acetonitrile–isopropyl alcohol (4:5:1, v/v). The flow rate of the mobile phase was maintained at 1.0 mL/min. The eluate was monitored at 227 nm with 0.01 AUFS sensitivity. Each sample was filtered through 0.2-μ filter and, a 20-μl aliquot of the supernatant was injected onto an HPLC system. Gliclazide concentration in the sample was determined using a calibration curve. The drug loading

was determined using following formula (Govender et al., 1999):

$$\%\,\text{Drug loading} = \frac{(\text{Mass of gliclazide in NP})}{\text{Mass of NP recovered}} \times (100)$$

Optimization by Varying Processing Parameters

Drug:Polymer Ratio

Effect of various gliclazide:Eudragit (L100 and RSPO) ratio from 1:4 to 2:1 were assessed on drug encapsulation efficiency, loading, particle size, and polydispersity index.

Effect of Stirring Speed

Effect of varying stirring speed from 500–4000 rpm was observed on drug encapsulation efficiency, loading, particle size, and polydispersity index.

Effect of Surfactants

Surfactants such as cationic (cetrimide), anionic (docusate Na), nonionic (Tween 80, and Cremophor RH40) were selected for the study. Effect of surfactants on particle size, encapsulation efficiency and drug loading of nanoparticles were assessed.

In Vitro Drug Release Studies

The in vitro drug release studies were performed with some modification as described earlier (Leroux et al., 1996; Liu et al., 2003). Briefly, gliclazide-loaded Eudragit (L100 and RSPO) NPs (equivalent to 10 mg of gliclazide) were suspended in 50 mL of pH 1.2, 4.5, and 7.4 phosphate buffer (USP-XIV) in a 100 mL glass bottle. The glass bottles were placed in a mechanical shaking bath (100 cycles/min), with temperature adjusted to 37°C. At selected time intervals, 2.0 mL of the sample was removed and replaced with fresh buffer. The sample was then filtered through 0.2-μ membrane and analyzed by UV-spectrophotometry at λ_{max} of 227 nm.

Stability Study

The selected formulation (Eudragit NPs) was subjected to accelerated stability studies as per ICH guidelines to evaluate effect of stress conditions. Eudragit NPs were packed in 0.044 mm dilaminated aluminium foil and subjected to elevated temperature and humidity conditions of 40° ± 2°C/75 ± 5% RH, 30° ± 2°C/65 ± 5% RH and also 25° ± 2°C/60 ± 5% RH. Samples were withdrawn at the end of 1, 3, and 6 months and evaluated for physical properties of the NP by visual inspection, drug content by the HPLC method, and in vitro release of drug determined by the developed stability indicating HPLC method.

In Vivo Study

Animals

Experiments were performed in adult male Wistar rats weighing from 200 to 250 g. Rats were procured from Haffkin Institute, India and were housed in stainless steel cages in groups of four and housed under standard environmental conditions (23 ± 1°C, 55 ± 5% humidity and a 12 hr/12 hr light/dark cycle) and maintained with free access to water and a standard laboratory diet (carbohydrates 30%, proteins 22%, lipids 12%, vitamins 3%) *ad libitum* (Hindustan Lever, India). The protocol of the animal study was approved by Institutional Ethical Committee.

Streptozotocin (STZ)-Induced Diabetic Rat Model

Diabetes was induced by intravenous injection of streptozotocin (Sigma, St. Louis, MO) into the tail vein at a dose of 50 mg/kg body weight (Burcelin et al., 1995). STZ was extemporaneously dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. Serum glucose levels were monitored post streptozotocin injection with Glucose Estimation Kit (Autopak, Johnson and Johnson, India). No diabetic animals with glucose levels less than 350 mg/dL were used in the study. The animals were fasted overnight (12 hr) before starting the experiment. The rats were randomly assigned to five different groups ($n = 6$ in each group). The control and diabetic control group received distilled water; treated groups received plain drug, gliclazide at a dose of 1.33 mg/kg and ELNP and ERSNP (~1.33 mg/kg gliclazide) in suspension form. All experiments were realized in overnight fasted rats. The drug solutions or vehicle were administered orally by gastric intubation using a syringe.

On the day of study, Eudragit nanoparticles (L 100 and RSPO) given in the form of suspension (~1.33 mg/kg gliclazide) and gliclazide (1.33 mg/kg) *per os*. Blood was withdrawn from retro orbital plexus of rat. Blood serum was separated by centrifugation at 5000 rpm for 10 min and isolated serum was (10 µl) estimated for serum glucose level using Glucose estimation kit.

Glucose-Loaded Rat Model (Oral Glucose Tolerance Test)

The oral glucose tolerance test (Bonnet-weir, 1988) was performed in overnight fasted (12 hr) diabetic rats. The rats were randomly assigned to four different groups ($n = 6$ in each group).Treated animals received gliclazide (1.33 mg/kg), ELNP (~1.33 mg/kg gliclazide), ERSNP (~1.33 mg/kg gliclazide) with glucose (2 g/kg) *per os* in suspension form whereas control diabetic rats received only glucose solution (2 g/kg) *per os*. Blood was withdrawn from the retro orbital sinus at various time interval and serum obtained after centrifugation at 5000 rpm was estimated for fasting serum glucose levels using a glucose oxidase–peroxidase glucose estimation kit (Autopak, Johnson and Johnson, India) (Ye et al., 2002).

Statistical Analysis

The mean serum glucose levels determined in samples collected before oral administration was taken as the baseline levels. The percentage of glucose reduction at each time after dosing was calculated and plotted against time. The terms t_{min} refers to time required for maximum decrease in serum glucose levels, AOC (Area over curve) refers to total area over the percent decrease in serum glucose level whereas C_{min} refers to maximum decrease in serum glucose level after formulations administration. Data from different experimental groups were compared with the corresponding control groups by one-way ANOVA followed by Dunnett's test to determine the level of significance. Value of $p < 0.05$ was considered significant (Bolton, 1990).

RESULTS AND DISCUSSION

Effect of processing parameters on ELNP and ERSNP preparation

The importance of enhanced drug incorporation efficiency in nanoparticles has been emphasized earlier, since a high nanoparticles recovery is required for reducing manufacturing costs and its size and morphology important for quality control and biodistribution (Doughlas et al., 1987), it was necessary to study the influence of processing parameters on nanoparticles preparation. The selection of optimal formulation in our study was, therefore, based on that which provided a combination of good morphology (in terms of particle size, polydispersity index), high drug loading, and encapsulation efficiency.

Controlled precipitation method was used to prepare ELNP whereas solvent evaporation method was used to prepare ERSNP. Different gliclazide:Eudragit ratios were tried.The effect of drug:polymer ratio on encapsulation efficiency, drug loading, particle size, and polydispersity index is shown in Table 1. Various drug:polymer ratios tried were 1:4, 1:2, 1:1, and 2:1. Increase in gliclazide concentration with respect to Eudragit L100 showed significant increase in gliclazide loading ($p < 0.05$) up to 1:1 drug:polymer ratio in ELNP. Further increase in gliclazide concentration showed a marginal increase in drug loading. Increase in gliclazide concentration also revealed a marginal

TABLE 1 **Effect of Drug:Polymer Ratio on Drug Encapsulation Efficiency, Loading, Particle Size and Polydispersity Index (PI) in Gliclazide-Loaded ELNP and ERSNP at Fixed Stirring Speed (rpm = 3000)**

Ratio Drug: Polymer	Drug (mg)	Encapsulation efficiency (%)		Gliclazide loading (%)		Particle size		PI	
		ELNP	ERSNP	ELNP	ERSNP	ELNP	ERSNP	ELNP	ERSNP
1:4	50	73.12 ± 5.25	50.26 ± 4.56	10.15 ± 2.45	6.26 ± 1.47	1768 ± 50.14	505 ± 25.45	0.39 ± 0.08	0.028 ± 0.002
1:2	100	87.26 ± 6.58	64.44 ± 7.45	25.64 ± 3.47	15.98 ± 2.14	1825 ± 60.14	732 ± 32.25	0.19 ± 0.02	0.89 ± 0.01
1:1	200	88.63 ± 8.47	88.16 ± 10.25	35.31 ± 4.78	19.11 ± 1.47	1200 ± 74.15	869 ± 41.15	1.15 ± 0.09	1.25 ± 0.21
2:1	400	92.01 ± 10.45	94.07 ± 8.47	38.14 ± 5.47	34.11 ± 6.78	2000 ± 58.15	974 ± 50.14	1.51 ± 0.1	1.16 ± 0.23

increase in encapsulation efficiency. Maximum gliclazide loading and encapsulation efficiency was obtained at 2:1 ratio whereas lowest particle size was obtained at 1:1 ratio. The formulation with 1:1 gliclazide:Eudragit L100 ratio which provided 35.31% drug loading, 88.63% drug encapsulation and 1200 nm particles size was selected for further study as it showed comparatively lower particle size as compared to 2:1 drug:polymer ratio.

Initially various solvents like acetone, acetonitrile, and methanol were tried for preparation of ERSNP. However, maximum drug loading, gliclazide encapsulation efficiency with lowest particle size was obtained only when acetone was used as an organic phase. Hence, for further study, acetone was used as an organic phase. Maximum gliclazide loading and encapsulation efficiency were obtained at drug polymer ratio 2:1. As drug:polymer ratio was increased, increase in particle size was observed. As compared to Eudragit L100 NP, Eudragit RSPO NP showed lower particle size range. This could be attributed to nature of polymer and further on method of preparation (Table 1).

Previous work showed that an increase in stirring speed of the system decreased mean particle size of nanoparticles (Pongpaibul et al., 1984; Haznedar & Dortunc, 2004). Hence, we also observed effect of stirring speed on NP formation. As the stirring speed increased from 500 to 4000 rpm, increase in drug loading and encapsulation efficiency were observed for ELNP. As expected, increase in stirring speed also decreased particle size of ELNP. High gliclazide encapsulation efficiency and drug loading resulted with lowest particle size at a stirring speed of 3000 rpm (Table 2). Hence, for further study, we used stirring speed of 3000 rpm.

Similarly, tthe effect of stirring speed on formation of ERSNP was also assessed. Increase in stirring speed from 500–4000 rpm revealed an increase in gliclazide encapsulation efficiency, drug loading, and decrease in particle size (Table 2) as seen in ELNP. For further study, stirring speed of 3000 rpm was employed.

The formulation with 2:1 gliclazide:Eudragit RSPO ratio which provided 34.10% loading and 94.07% encapsulation with 974 nm particles size was selected for further study as it showed higher gliclazide encapsulation efficiency, loading, and lower particle size (Table 2).

Effect of Homogenization

Previously, Muller et al. (2001) showed the use of homogenizer for reduction of particle size of drug-loaded nanoparticles. Hence, the optimized Eudragit nanoparticles (L100 and RS) were further employed for particle size reduction using high-pressure homogenizer (APV-Gaulin lab model). Without homogenization, Eudragit L 100 NP showed particle size of 1175.90 nm and polydispersity index (PI) of 1.5. Increase in pressure up to 400 psi showed decrease in particle size and also PI in Eudragit L100 NP. Interestingly, further increase in pressure increased particle size and PI of the Eudragit L100 NP and this may be attributed to the increase in pressure and increased temperature of the nanoparticulate suspension which could aggravate the agglomeration of nanoparticles (Fig. 2). In case of Eudragit RSPO NP, initial particle size and PI obtained were 974.00 nm and 1.89, respectively. Pressure was applied from 100 psi and gradually increased up to 700 psi. Increase in pressure decreased particle size and also PI of Eudragit RSPO NP (Fig. 2).

TABLE 2 **Effect of Stirring Speed (rpm) on Drug Encapsulation Efficiency, Loading, Particle Size and Polydispersity Index (PI) in Gliclazide-Loaded ELNP (Drug Polymer Ratio 1:1) and ERSNP (Drug Polymer Ratio 2:1)**

Stirring Speed	Encapsulation efficiency (%)		Gliclazide loading (%)		Particle size		PI	
RPM	ELNP	ERSNP	ELNP	ERSNP	ELNP	ERSNP	ELNP	ERSNP
500	84.67 ± 8.11	56.06 ± 5.14	24.65 ± 3.25	14.14 ± 1.45	1996 ± 36.14	1566 ± 34.15	0.23 ± 0.09	0.56 ± 0.12
1000	85.61 ± 8.45	66.88 ± 6.15	26.89 ± 4.58	18.98 ± 2.56	1905 ± 40.18	1409 ± 36.77	1.87 ± 0.21	1.26 ± 0.34
2000	87.12 ± 9.47	82.69 ± 7.45	29.58 ± 3.48	20.89 ± 3.45	1260 ± 32.01	990 ± 32.14	1.41 ± 0.99	0.86 ± 0.12
3000	88.92 ± 10.15	94.07 ± 11.15	35.31 ± 5.47	34.11 ± 4.98	1175 ± 25.14	980 ± 30.16	1.49 ± 0.29	0.66 ± 0.12
4000	90.83 ± 11.14	93.56 ± 12.14	32.26 ± 3.14	33.68 ± 4.99	1252 ± 31.15	1000 ± 40.15	1.83 ± 0.28	0.88 ± 0.22

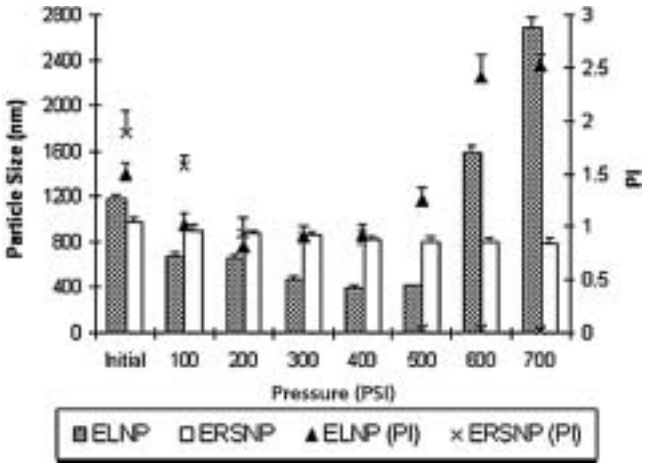

FIGURE 2 **Effect of Homogenization Pressure on Particle Size and PI of ELNP and ERSNP.**

Effect of surfactants

We investigated effect of various surfactants on particle size, gliclazide encapsulation efficiency, and loading in Eudragit (L100 and RS) NP. Lamprecht et al. (1999) used Polyvinylalcohol (PVA) as a surface-active agent for decreasing particle size and PI of PLGA nanoparticles. We selected surfactants from categories like cationic-cetrimide, anionic-docusate Na and from nonionic-Tween 80 and Cremophor RH40. All the surfactants were used in fixed concentration that is, 0.05%. Previously, Trotta et al. (2003) found that ionic surfactants decreased particle size of solid lipid nanoparticles than nonionic surfactants. Contrary to this, we observed all the surfactants under study showed decrease in particle size in case of Eudragit L100 NP, which was prepared by controlled precipitation method.

Surfactants also affected gliclazide encapsulation efficiency and loading. All the surfactants under study showed increase in gliclazide loading and marginal increase in encapsulation efficiency in Eudragit L 100 NP. Nonionics such as Tween 80 and Cremophor RH40 showed maximum decrease in particle size and PI whereas increase in drug loading and encapsulation efficiency in Eudragit L100 NP (Table 3).

In case of Eudragit RSPO NP, all the surfactants under study showed decrease in particle size, similar to that observed with Eudragit L100 NP. Cetrimide, a cationic surfactant showed a maximum decrease in particle size and increase in gliclazide loading and encapsulation efficiency. Interestingly, other surfactants that is, docusate Na, Tween 80, and Cremophor RH40 showed decrease in gliclazide loading and encapsulation efficiency (Table 3). Surfactants promisingly showed decrease in particle size in both, Eudragit L100 as well as Eudragit RSPO NP formulations. All the surfactants under study showed increase in gliclazide encapsulation efficiency and loading in Eudragit L100 NP. In case of Eudragit RSPO NP, only cationic surfactant, cetrimide, showed increase in gliclazide encapsulation efficiency and drug loading.

Dissolution study

Dissolution study of Eudragit L100 NP in pH 1.2 buffer revealed an incomplete release (~36%). Incorporation of surfactant during preparation of nanoparticles affected release of gliclazide in pH 1.2 buffer. Incorporation of Cremophor RH40 showed maximum increase in dissolution rate followed by docusate sodium and Tween 80 (Table 4). Cetrimide did not show any increase in dissolution profile (Table 4). In pH 4.5 buffer, release of gliclazide from Eudragit L100 NP was found to be less as compare to pH 1.2 (~30%) (Table 4). Maximum increase in dissolution profile was obtained by incorporation of Tween 80 in Eudragit L100 NP. Incorporation of cetrimide during

TABLE 3 **Effect of Surfactants on Gliclazide Loading, Encapsulation Efficiency and Particle Size in Gliclazide-Loaded ELNP and ERSNP**

Surfactants incorporated	Encapsulation efficiency (%)		Gliclazide loading (%)		Particle size	
	ELNP	ERSNP	ELNP	ERSNP	ELNP	ERSNP
NP Without Surfactant	88.63 ± 8.15	94.02 ± 9.46	35.31 ± 6.15	32.11 ± 3.15	1200 ± 56.48	974 ± 22.44
NP-Cet	93.86 ± 9.45	96.02 ± 8.16	36.34 ± 6.48	56.24 ± 6.78	850 ± 50.33	121 ± 10.14
NP-DSS	88.98 ± 7.45	94.25 ± 8.49	56.26 ± 7.45	25.31 ± 2.48	752 ± 40.11	193 ± 8.45
NP-Tw	90.25 ± 10.11	92.16 ± 4.78	66.14 ± 6.44	12.52 ± 3.15	522 ± 33.25	503 ± 6.45
NP-CR40	92.28 ± 9.48	90.15 ± 9.45	61.65 ± 7.15	16.38 ± 3.45	806 ± 31.15	78 ± 6.48

TABLE 4 Comparative Dissolution Profile of Eudragit L100 NP With and Without Addition of Surfactants in pH 1.2 Buffer, pH 4.5 Buffer and pH 7.4 Buffer

Time	No surfactant			NP-DSS			NP-Cet			NP-CR40			NP-TW		
(h)	pH 1.2	pH 4.5	pH 7.4	pH 1.2	pH 4.5	pH 7.4	pH 1.2	pH 4.5	pH 7.4	pH 1.2	pH 4.5	pH 7.4	pH 1.2	pH 4.5	pH 7.4
1	28.30	24.28	66.20	35.76	24.81	52.28	14.66	13.68	49.68	40.35	24.16	40.31	21.18	24.56	54.86
2	29.14	26.71	86.53	38.80	30.80	70.04	19.91	16.00	55.51	62.15	28.46	49.59	29.97	26.79	67.40
4	37.61	27.94	92.88	51.77	35.68	69.15	26.98	19.45	58.89	77.79	33.46	64.28	40.56	38.49	74.61
8	39.50	27.79	93.8	60.14	36.22	71.78	31.73	21.89	59.84	100.23	36.49	68.63	46.10	41.16	76.34
12	36.69	28.79	95.98	63.81	38.60	70.56	32.50	22.48	61.73	–	37.49	70.52	49.42	46.19	82.16
24	35.74	30.77	95.95	68.56	40.14	70.14	32.69	23.40	66.82	–	36.21	83.01	60.57	45.12	81.29

NP preparation did not revealed an increase in release of gliclazide from Eudragit L100 NP in pH 4.5 buffer. In pH 7.4 buffer, docusate sodium and Tween 80 showed maximum increase in release rate whereas Cremophor RH40 did not show any significant change in release. Release of gliclazide was decreased by incorporation of cetrimide in Eudragit L100 NP (Table 4).

In case of Eudragit RSPO NP, incomplete release of gliclazide was obtained in pH 1.2, 4.5 as well as in pH 7.4 buffer. Incorporation of surfactants during NP preparation affected release of gliclazide from Eudragit RSPO NP. All the surfactants under study revealed an increase in dissolution rate and also percent cumulative release in pH 1.2, 4.5, and 7.4 buffer. Maximum release was obtained with incorporation of cetrimide in Eudragit RSPO NP. (Table 5). Use of surfactant showed a decrease in the mean particle size and PI in nanoparticles and this led to an increase in release rate as would be expected from the surface area relationship (Pongpaibul et al., 1984; Lemos-Senna et al., 1998).

Release of gliclazide from Eudragit NPs was found to be pH dependent. Maximum release rate was obtained in pH 7.4 buffer as compared to pH 1.2 and pH 4.5 buffer. The release of gliclazide was less in pH 4.5 buffer compared to pH 7.4 and pH 1.2. This is because of the inherent solubility of gliclazide. Gliclazide shows pH dependent solubility. In pH 1.2, gliclazide is more soluble compared to pH 4.5. Further as the pH increased from 5.5, solubility of gliclazide is increased. Further, Eudragit L100 is soluble in pH 6.5 and above pH, which would cause erosion of nanoparticles and hence rapid release of gliclazide.

Eudragit L100 NP with Tween 80 showed lower particle size (522 nm), higher gliclazide loading (66.14%), encapsulation efficiency (90.25%) and sustained gliclazide release as compared to other surfactants and hence was selected. In case of Eudragit RSPO NP, formulation containing cetrimide was chosen for further study as the batch showed lower particle size (121.7 nm), higher gliclazide loading (56.24%), encapsulation efficiency (96.02%), and sustained gliclazide release (Table 5). Freeze-drying of the Eudragit nanoparticles did not reveal any significant change in particle size of the nanoparticles ($p < 0.05$). The batches were selected for further characterization and in vivo study.

Characterization

SEM studies revealed a spherical morphology of the Eudragit L100 NP as well as Eudragit RSPO NP

TABLE 5 Dissolution Profile of Eudragit RSPO NP With and Without Addition of Surfactants in pH 1.2 Buffer, pH 4.5 Buffer and pH 7.4 Buffer

Time	No surfactant			NP-DSS			NP-Cet			NP-CR40			NP-TW		
(h)	pH 1.2	pH 4.5	pH 7.4	pH 1.2	pH 4.5	pH 7.4	pH 1.2	pH 4.5	pH 7.4	pH 1.2	pH 4.5	pH 7.4	pH 1.2	pH 4.5	pH 7.4
1	39.46	24.08	50.26	45.12	40.01	35.26	45.47	40.33	69.26	50.64	45.55	70.25	27.90	30.02	57.84
2	45.19	26.00	55.66	61.41	42.55	40.26	65.98	55.64	72.66	62.30	48.22	75.99	40.02	40.47	72.79
4	50.45	29.66	69.05	75.61	48.66	76.66	80.98	70.76	88.69	63.55	46.25	78.99	76.63	48.99	68.82
8	58.04	31.33	76.22	89.12	55.98	100.11	103.68	75.02	100.26	76.26	48.26	77.04	97.80	52.97	81.07
12	63.92	32.66	76.85	94.22	61.68	–	–	80.25	–	76.22	47.88	74.97	100.12	58.99	100.44
24	65.09	31.04	76.55	94.78	61.02	–	–	84.02	–	76.33	50.11	79.99	–	59.77	–

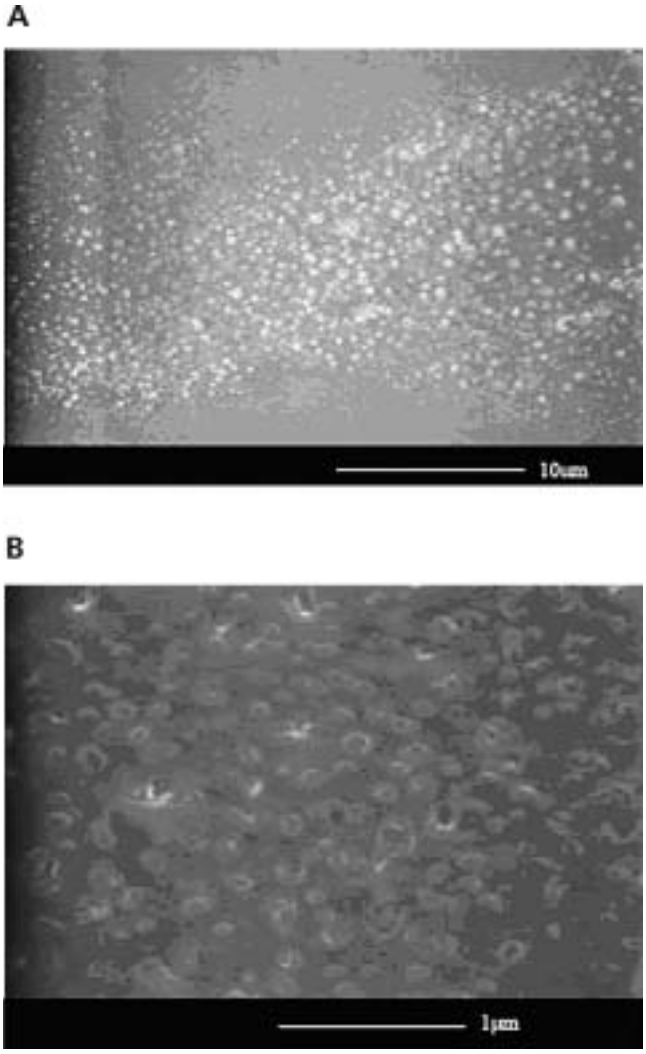

FIGURE 3 **Scanning Electron Microscopy of (a) ELNP and (b) ERSNP.**

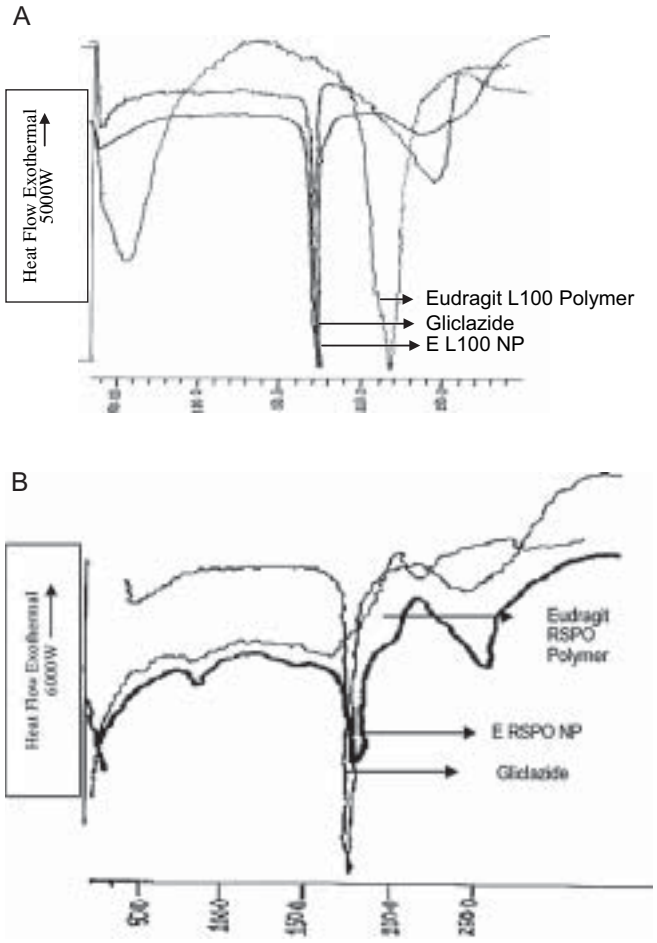

FIGURE 4 **Differential Scanning Thermogram of (a) Eudragit L100 Polymer, Gliclazide and Gliclazide-Loaded ELNP (B) Eudragit RSPO Polymer, Gliclazide and Gliclazide-Loaded ERSNP.**

(Fig. 3A and B). FT-IR of gliclazide showed peaks of –NH stretching (3274 cm^{-1}), =CH stretching (3113 cm^{-1}), O=C (1705 cm^{-1}), C=C aromatic (1596 cm^{-1}) C–H deformation (1467–1430 cm^{-1}) SO_2–NH (1352 cm^{-1}). Similar peaks were seen in gliclazide-loaded Eudragit L100 NP and Eudragit RSPO NP. This observation further confirmed by DSC studies. Gliclazide showed endotherm at 176°C which was identical with gliclazide-loaded ELNP and ERSNP (Fig. 4A and B). Hence, the study confirmed no interaction between drug, polymer, and excipients in drug-loaded nanoparticles.

Stability study

Eudragit L100 NP containing Tween 80 and Eudragit RSPO NP containing cetrimide was taken for stability study. Stability studies revealed that Eudragit (L100 and RS) NPs were stable at the end of 6 months in all the test conditions. No significant changes in particle size, PI, drug loading, and dissolution profile (t_{50}% values) were observed in Eudragit L100 NP as well as in Eudragit RSPO NP ($p < 0.05$).

In Vivo Study

Streptozotocin-Induced Diabetic Rat Model

The normal as well as diabetic control rats did not show any significant changes in serum glucose level (SGL) over a period of experiment. Although as the time progressed, slight decrease in SGL observed in both groups that is, control as well as diabetic control. The decrease may be attributed to the fasting effect on serum glucose level. Gliclazide treated rats showed t_{min} at 8 hr with C_{min} 55.34% decrease in SGL of basal level. Eudragit RSPO NP treated rats also showed t_{min}

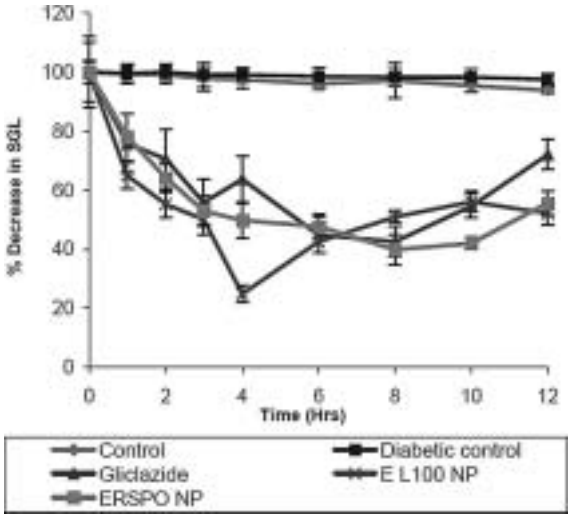

FIGURE 5 **Comparative Effect of Formulations on SGL in STZ-Induced Diabetic Rats.**

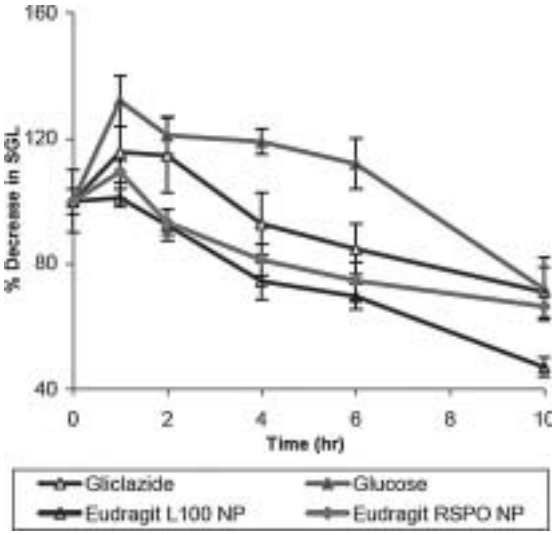

FIGURE 6 **Comparative Effect of Formulations on SGL in Glucose-Loaded Diabetic Rats.**

at 8 hr and C_{min} of 60.15% decrease in SGL of basal level, compared with plain gliclazide. Gliclazide-loaded Eudragit L100 NP treated rats showed early t_{min} of 4 hr and significantly high C_{min} of 75.04% decrease in SGL of basal level respectively compared to plain gliclazide treated rats (Fig. 5). Importantly, Eudragit L100 NP treated rats showed decrease in t_{min} from 8 to 4 hr. Eudragit L100 NP showed significant % decrease in SGL at 2 and 4 hr ($p < 0.05$). In case of Eudragit RSPO NP treated rats, t_{min} was not altered as compared to gliclazide treated rats but sustained activity was observed in Eudragit RSPO NP treated rats (Table 6).

Glucose-Loaded Diabetic Rat Model (Oral Glucose Tolerance Test)

The effect of developed Eudragit NPs was further evaluated in oral glucose tolerance test in STZ-treated diabetic rat. Glucose treated diabetic control rats showed significant increase in SGL up to 6 hr ($p < 0.05$). Gliclazide treated rats showed significant decrease in SGL at 1,2,4, and 6 hr as compared to glucose treated animals ($p < 0.05$) (Fig. 6). Eudragit L100 NP showed significant decrease in SGL at 1,2,4, 6, and 8 hr, respectively whereas Eudragit RSPO NP showed significant decrease in SGL at 1,2,4, and 6 hr as compared to gliclazide treated rats. ($p < 0.05$) (Table 7). Significant difference in AOC was observed in Eudragit L100 NP and Eudragit RSPO NP treated rats with respect to gliclazide treated animals. Eudragit L100 as well as Eudragit RSPO NP showed superior activity over gliclazide, moreover, Eudragit L100 showed rapid onset of activity as compared to Eudragit RSPO NP. This could be related to higher drug loading achieved with Eudragit L100 NP as compared to Eudragit RSPO NP. Both the NPs showed sustained decrease in SGL in rats.

TABLE 6 **Comparative Effect of Formulations on SGL in STZ-Induced Diabetic Rats**

Formulations	t_{min} (hr)	C_{min} (% decrease in SGL)	AOC
Gliclazide	8 ± 0.8	55.34 ± 5.4	29747.10 ± 1256.1
ELNP	4 ± 0.6*	75.04 ± 2.7*	35972.4 ± 987.16*
ERSNP	8 ± 0.9	60.15 ± 4.1	33878.76 ± 485.98*

*$p < 0.05$, Analyzed by ANOVA followed by Dunnett's test, comparec with gliclazide treated group.

TABLE 7 **Comparative Effect of Formulations on SGL in Glucose-Loaded Diabetic Rats**

Formulations	t_{min} (h)	C_{min} (% decrease in SGL)	AOC
Gliclazide	6 ± 1.2	29.99 ± 2.5	41338.80 ± 1956.1
ELNP	8 ± 0.8	52.92 ± 3.2*	36130.13 ± 1056.1*
ERSNP	8 ± 0.7	33.42 ± 2.8	37664.33 ± 958.14*

*$p < 0.05$, analyzed by ANOVA followed by Dunnett's test, compared with gliclazide treated group.

CONCLUSION

Gliclazide-loaded ELNP and ERSNP were prepared by controlled nanoprecipitation and solvent evaporation method respectively. Drug:Polymer ratio and stirring speed were found to be important for obtaining lower particle size with high drug loading and encapsulation. Interestingly, surfactants affected particle size, encapsulation efficiency, and loading of ELNP as well as ERSNP. In vitro release study revealed sustained release of gliclazide from both type of NPs. Incorporation of surfactant revealed an increase in release rate of gliclazide from ELNP as well as in ERSNP. Stability studies revealed that NP formulations were stable at the end of 6 months. The FT-IR and DSC studies revealed no interaction between drug, polymer, and excipients. In vivo study of the developed ELNP and ERSNP in diabetic rat model revealed a better activity as compared to plain gliclazide and in addition activity was found to be sustained. Sustained activity with decreased t_{min} (ELNP) and enhanced bioavailability (ELNP and ERSNP) could reduce dose frequency, decrease side effects, and improve patient compliance.

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