

STUDY ON DESIGN, DEVELOPMENT AND CHARACTERIZATION OF DRUG GLIPTIN

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ABSTRACT:

Gliptin has significant inter- and intra-individual variability and has a log P of 2.6. The micro emulsion process was used to generate the solid lipid nanoparticles for GLIPTIN, which were then sonicated using a probe. Using the Box-Behnkan design, the impact of the formulation factors Smix:lipid ratio, drug loading concentration in the lipid phase, and Phospholipon 90 H concentration in the lipid phase on Gliptin SLN was investigated. Creating solid lipid nanoparticles (SLN) for gliptins was the goal of the current investigation. An oral antihyperglycemic medication called gliptins is used to treat non-insulin-dependent diabetic mellitus (NIDDM). The effectiveness of entrapment, drug content, particle size, zeta potential, in vitro drug release, and in vitro permeation into rat duodenum were assessed for GLIPTIN SLN. Additionally, scanning electron microscopy, differential scanning calorimetry, and X-ray diffraction were used to characterise the lyophilized SLN formulation. The optimised formulation, which had a Smix:lipid ratio of 3:1, 15% drug loading, and 20% phospholipid concentration, produced particles with an average size of 116.8 nm, entrapment efficiencies of 92.2%, and a GLIPTIN release rate of 61.3% after 8 hours of dissolution. Phospholipon 90 H increased the physical stability of the formulation while simultaneously reducing the particle size. The in vitro results of GLIPTIN SLN absorption through the rat duodenum showed more drug penetration than pure GLIPTIN. In conclusion, solid lipid nanoparticles of GLIPTIN with greater drug entrapment were effectively developed and show promise as a delivery system for insoluble drugs.

Keywords: Gliptins, solid lipid nanoparticles

1. Introduction

A dangerous metabolic illness called diabetes mellitus type 2 is characterized by low levels of insulin and insulin

resistance, which raise blood sugar levels [1]. Numerous health issues caused by diabetes mellitus lead to greater rates of morbidity and mortality. More than 62 million instances of diabetes have been reported in India as of this writing, underlining a possible future healthcare burden that diabetes may impose [2]. A second-generation antihyperglycemic medication called gliptins (GLIPTIN) is used to treat non-insulin-dependent diabetic mellitus (NIDDM). It is an insulin secretagogue that belongs to the sulfonylurea class and works by raising basal insulin secretion and peripheral glucose uptake. Additionally, GLIPTIN makes insulin receptors more sensitive and reduces hepatic gluconeogenesis [3]. The main problem with the therapeutic use and effectiveness of GLIPTIN as an oral dosage form is its extremely low water solubility, which has a log P of 2.69 and causes variable drug dissolution and absorption in the body. Alterations in the drug's bioavailability as a result of the gastro-intestinal tract [4]. GLIPTIN is mostly sold as oral formulations on the market. Although GLIPTIN is quickly and effectively absorbed, there is significant inter- and intra-individual variation in bioavailability [5]. Therefore, it is necessary to create a GLIPTIN formulation that would lessen inter- and intra-individual variation in absorption, enhancing its bioavailability when taken orally.

Using lipid-based drug delivery systems is one of the most common ways to increase the oral bioavailability of hydrophobic therapeutic compounds. An extensive body of research has shown that lipid-based formulations have the potential to increase drug bioavailability and therapeutic efficacy of the drug through a variety of mechanisms, including increasing luminal solubilization of the drug, bypassing first pass metabolism of the drug by its transportation via lymphatic system, inhibiting Cytochrome P450-4A, an enzyme responsible for intestinal drug metabolism, and inhibiting P-glycoprotein, an efflux transporter [6].

Lipid-based nanoparticle systems have become more significant recently. The colloidal particles known as solid lipid nanoparticles, which range in size from 10 to 1000 nm, are comprised of lipids and surfactants. Due to their small size, the nanoparticles deliver the medicine in the GIT as a molecule dispersion, which improves its solubilization [7]. Additionally, their larger surface area offers a larger interfacial surface for the lipolysis process (an interfacial phenomenon), which speeds up the process and improves the drug's absorption and bioavailability [8]. The use of nanoparticles in drug delivery has several benefits, including increased aqueous solubility, increased residence time in the body, increased half-life for clearance, increased specificity for target receptors, and the ability to target the drug to particular parts of the body [9]. This enables the safe delivery of hazardous therapeutic medications and the preservation of non-target tissues and cells from serious side effects, resulting in concurrent reductions in the quantity of the drug required and probable toxicity [10].

Low drug loading capacity is a major drawback of solid lipid nanoparticles, particularly for medications with

intermediate or low log P values and decreased lipid solubility. Long chain triglycerides can be combined with phospholipids or self-emulsifying waxes to create nanoparticles that have a higher drug loading [11]. We looked examined Gelucire 44/14's impact on glyceryl monostearate nanoparticles in one of our earlier studies [12]. In the current investigation, phospholipon was added to glyceryl monostearate in an effort to create and optimize Gliptin solid lipid nanoparticles. The effect of phospholipon on entrapment and drug release from SLN was investigated. The nanoparticles were made using various concentrations of phospholipon 90 H (P90H) in glyceryl monostearate (GMS). The resultant SLN were assessed for drug release in vitro and in vitro penetration through rat duodenum, as well as for entrapment efficiency, particle size, zeta potential, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray diffraction (XRD).

Materials and Methods

Gliptin was obtained as gift sample from IPCA Laboratories Pvt Ltd, India, Phospholipon 90 H was provided as gift sample by Lipoid, India. Solutol HS 15 was obtained as gift sample from BASF, Delhi. Tween 80, Span 20 were purchased from Lobachemie, Mumbai. All the solvents and reagents used for study were of analytical grade.

Preparation of Gliptin solid lipid nanoparticles

Gliptin were prepared by O/W microemulsion technique followed by probe sonication [13, 14]. GMS and P90H were blended together and melted above their melting point (at 80°C). Gliptin was dissolved in the molten lipid by continuous stirring followed by addition of span 20 as lipophilic surfactant. The aqueous phase with Tween 20 as hydrophilic surfactant was also maintained at 80°C. The aqueous phase was poured slowly to the hot oil phase, along with Solutol HS 15 as a coemulsifier. The resultant dispersion was continuously stirred at 80°C until clear microemulsion obtained. The hot O/W microemulsion was then poured into cold water 2°C in a fixed ratio (1:25) and stirred with homogenizer (Remi, RQ/127A, India) at 8000 rpm for 2hrs in order to form solid lipid nanoparticles. The obtained dispersion was then sonicated using a probe sonicator (PCI analytics, PKS-250F, India) for 20 min.

1.1.1. Preliminary optimization of process and formulation variables for GLIPTIN loaded solid lipid nanoparticles

The studies were performed in order to optimize the process and formulation variables, viz. type of surfactant and cosurfactant, ratio of surfactant: cosurfactant (s:cos ratio) in mixture (S_{mix}) and sonication time (Table 1). The effect of these variables on entrapment efficiency of SLN was studied.

1.1.2. Optimization of GLIPTIN SLN by Box-Behnken design

Randomized response surface Box-Behnken design (33) was used with 15 trial runs to study the impact of three

factors on the two key response variables [15]. The Smix:lipid ratio (X1), drug loading concentration in lipid phase (X2) and P90H concentration in lipid phase (X3) were selected as independent variables and entrapment efficiency (Y1) was selected as dependent variable or response (Table 2). The resulting data was fitted into Design Expert software (Version 9.0) and analyzed statistically using analysis of variance (ANOVA). The data was also subjected to response surface methodology to determine the influence of concentration of Smix:lipid ratio, drug loading concentration in lipid phase, P90H concentration in lipid phase on dependent variable. Table 2 indicates the trial runs prepared using Box-Behnken design.

1.1.3. Lyophilization of GLIPTIN solid lipid nanoparticles

The optimized formulation of GLIPTIN SLN was lyophilized using a freeze dryer (Labconco, FreeZone 2.5, USA). Trehalose (3% w/v) was added as a cryoprotectant to the dispersion before freeze drying. The freezing was done at -20°C and freeze drying was initiated after 24 h of refrigeration [16].

1.2. Evaluation of GLIPTIN solid lipid nanoparticles

All the GLIPTIN SLN formulations were evaluated for the following parameters. The lyophilized solid lipid nanoparticle formulation was reconstituted with water (1:25 dilution), filtered through whatman filter paper and was also evaluated similarly.

1.2.1. Particle Size

The particle size analysis of formulations was performed using Photon correlation Spectroscopy (Sympatec GmbH, Nanophox, Germany). An aliquot of SLN was diluted in deionised water prior to measurements. All the measurements were carried out in triplicate at a temperature of 25.0°C and at a fixed angle of 90° to the incident laser beam. Data was analysed by Windox software (Version 5.7.1.0) and values of mean particle size and particle size distribution curve were recorded [17].

1.2.2. Zeta potential measurement

For measurement of zeta potential, Zetasizer (Beckmen Coulter, Delsa Nano-C, USA) was used. SLN sample (1ml) was taken and dispersed in double distilled water. To prevent the agglomeration, the dispersed SLN was placed for 5 minutes in an ultra sonicator bath. The sample was taken in the glass cuvette and zeta potential was measured [18].

1.2.3. Entrapment Efficiency

For determination of drug entrapment, the amount of drug encapsulated per unit weight of SLN was determined after separation of the free drug and solid lipid from aqueous medium. In the present study, separation was achieved by dialysis bag method [12]. For this, the formulation was first taken into the dialysis bag which allows dialyzing the free drug until the complete release of untrapped drug. The bags were soaked in receiving phase (methanol:distilled water, 30:70) for 12 h before use. SLN dispersion (5 ml) was poured into the bag with the two ends tied. The bags were placed in a beaker and 100 ml receiving phase was added to the later. The beaker was placed on magnetic stirrer at 37°C at a rate of 100 rpm. At 1 h after test, 5ml of medium from beaker was removed by filtration for analysis and fresh medium was added to maintain sink conditions. Untrapped GLIPTIN was analyzed spectrophotometrically (Shimadzu, UV-1700, Japan) at 226.5 nm.

$$EE (\%) = \frac{\text{Total amount of GZ added} - \text{Untrapped (free) GZ}}{\text{Total amount of GZ added}} \times 100$$

1.1.1. Differential Scanning Calorimetry

The physical state of GLIPTIN in SLN was characterized by the differential scanning calorimetry analysis (Perkin Elmer, DSC 4000, USA). Sample analysis was performed in a heating range 30-3000C and rate of heating as 100C/min. DSC was performed in atmosphere of nitrogen with purging rate 20 ml/min [19].

1.1.2. X- Ray Diffraction

X- ray powder diffraction study was performed by X-ray diffractometer (Bruker, D 8 Advanced, USA) using Cu K 2α rays with a voltage of 40 kV and a current of 25 mA. Samples were scanned for 2θ from 10 to 60°. Diffraction pattern for pure GLIPTIN, physical mixture and solid SLN were obtained [20].

1.1.3. Drug release studies

In vitro drug release study from GLIPTIN SLN was performed in the phosphate buffer pH 7.4, using the dialysis bag

method with modified USP dissolution apparatus type I (Veego, DT60, India) [21, 22]. The dialysis bag (Mol. wt. cutoff-11KDa) was soaked in dissolution medium for 12 h before use. An accurate volume (5 ml) of the dispersion containing 5 mg of GLIPTIN was placed inside the dialysis bag, tied at both the ends to basket rod. The bags were placed into dissolution medium containing 250 ml of phosphate buffer pH 7.4 at 37±0.5°C at a

stirring rate of 100 rpm. An aliquot of the dissolution medium (5 ml) were withdrawn at each time interval and the same volume of fresh dissolution medium was then added to maintain a sink condition. GLIPTIN in the sample solution was analyzed by the UV- spectrophotometer at 226 nm. All the operations were carried out in triplicate.

The cumulative amount of drug released from optimized formulation of GLIPTIN SLN at different time intervals was fitted to zero order, first order, Higuchi matrix model and Korsmeyer-Peppas model to find out the mechanism of drug release. The correlation coefficient between the time and cumulative amount of drug release were calculated to find out the fit to appropriate kinetics.

1.2.4. Ex vivo absorption study of solid lipid nanoparticles

Ex vivo absorption study of optimized SLN formulation and drug suspension was carried out through everted rat intestine using an in-house fabricated perfusion apparatus [23]. The apparatus consisted of a U shaped glass tube with volume capacity of 25 ml and internal diameter of 1 cm. The tube consisted of a cannulated cut of 8 cm to one side of the tube where everted intestine was tied. The tube was also connected from upper end through a glass tube joint. The study was conducted with prior approval from institutional animal ethical committee as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, guidelines. The rat was sacrificed humanely by cervical dislocation. After opening the abdomen, an intestinal segment of length 9 to 10 cm was removed, washed carefully and everted with the help of a metal rod. The everted segment was mounted on the glass apparatus and 25 ml of tyroid solution was filled inside the tube. After mounting of the intestine, the glass tube assembly was kept in a beaker containing 250 ml of phosphate buffer pH 6.8 containing 10 ml of SLN formulation and supplied with aeration. The internal part of the tube served as serosal side whereas buffer solution in beaker would serve as mucosal side. The assembly was kept on a magnetic stirrer and temperature of buffer was maintained to 37±0.5°C. The samples from serosal side were collected at an interval of 1 h and analyzed for the percent permeation of drug by UV spectrophotometer at 226 nm. An equivalent volume of fresh tyroid solution was added to compensate for the loss due to sampling.

Table 1: Preliminary optimization of variables for Gliptin SLN

Batch code*	Lipid (mg)		Surfactant (mg)			Co surfactant (mg)			S:cos ratio	sonication time (min)	Entrapment efficiency (%)**
	GMS	P90H	ween20	pan 20	poid S	Solutol	butanol	Transcutol P			
P1	334	166	407.4	-	343	750	-	-	1:1	20	85.5±0.2

P2	334	166	407.4	343	-	750	-	-	1:1	20	85.6±0.8
P3	334	166	407.4	343	-	-	750	-	1:1	20	80.7±1.1
P4	334	166	407.4	343	-	-	-	750	1:1	20	78.1±0.5
P5	334	166	543.2	456.8	-	500	-	-	2:1	20	82.0±0.3
P6	334	166	271.6	228.4	-	1000	-	-	1:2	20	69.7±1.6

*Each formulation contained 50 mg of Gliptins and ratio of lipid to water phase was 1:25 **Mean ±SD (n=3)

Table 2: Optimization of Gliptins SLN using Box-Behnken design

Batch code*	Code value			Y ₁ Entrapment Efficiency** (%)
	X ₁ Smix:lipid ratio	X ₂ Drug loading concentration in lipid phase (%)	X ₃ P90H concentration in lipid phase (%)	
F1	2:1	10	20	85.7±0.7
F2	4:1	10	20	96.6±0.3
F3	2:1	20	20	95.5±0.5
F4	4:1	20	20	98.4±0.1
F5	2:1	15	10	92.9±0.9
F6	4:1	15	10	71.9±1.2
F7	2:1	15	30	87.1±0.8
F8	4:1	15	30	62.2±1.5
F9	3:1	10	10	81.1±0.7
F10	3:1	20	10	79.7±1.4
F11	3:1	10	30	85.0±1.1
F12	3:1	20	30	84.8±0.6
F13	3:1	15	20	87.0±0.2
F14	3:1	15	20	94.1±0.2
F15	3:1	15	20	92.2±0.4

*Each formulation contained 50 mg of Gliptins and ratio of lipid to water phase was 1:25
**Mean ±SD (n=3)

Result and discussion

Formulation of Gliptins solid lipidnanoparticles

Gliptins SLN were prepared by microemulsion method. As the composition of microemulsion is critical factor in

the preparation of SLN, various lipids and surfactants were screened in the preliminary study for obtaining a stable system. Gliptins showed highest solubility in the blend of GMS and P 90 H and was selected as the lipid phase for nanoparticles. The selection of surfactants was done based on HLB system. Instead of using a single surfactant, the blend of one hydrophilic and one lipophilic surfactant was used. Tween 20 was selected as hydrophilic surfactant. In preliminary experiments, two lipophilic surfactants, Lipoid S and span 20 were used in combination with tween 20. Solid lipid nanoparticles prepared with lipoid S (P1) and tween 20 were not physically stable and resulted in lower drug entrapment whereas formulations with span 20 (P2) and tween 20 were found to be stable with high entrapment efficiency. The concentration of tween 20 and span 20 was adjusted to required HLB value of the lipid phase (RHLB 13 for GMS) to make a clear o/w microemulsion. The effect of various cosurfactants, viz. solutol HS 15, butanol and transcutool P on Gliptins SLN was also studied. SLN with butanol (P3) and transcutool P (P4) were not stable for more than 48 h whereas SLN dispersion with solutol HS 15 (P2) were found to be stable even after 7 days of storage with high entrapment efficiency (85.6±0.8%) with no drug precipitation in formulation. Hence, solutol HS 15 was selected as cosolvent for further study.

The formulations of solid lipid nanoparticles were optimized for different s:cos ratios, 1:2, 1:1, 2:1. The drug entrapment was found to be decreased for s:cos ratio, 2:1 (P5) and 1:2 (P6). Nanodispersion with increased amount of cosurfactant (1:2) was found to be unstable and resulted in precipitation during storage. Gliptins entrapment was maximum (85.6±0.8 %) at s:cos ratio 1:1 (P2) and this ratio was selected.

1.1. Optimization by Box-Behnken design

The effects of independent variables on response, entrapment efficiency (%), were analyzed using Design- Expert software. The results of S:mix:lipid ratio (X₁), Drug loading concentration in lipid phase (X₂) and P90H concentration in lipid phase (X₃) on entrapment efficiency (Y₁) are depicted in table 2.

Polynomial equation 1 was obtained from regression analysis of the data.

$$\text{Entrapment efficiency (Y}_1\text{)} = 89.48 - 2.76X_1 + 1.25X_2 + 0.44X_3 - 2.00X_1X_2 + 1.53X_1X_3 + 0.30X_2X_3 + 1.48X_1^2 + 3.10X_2^2 - 9.92X_3^2 \dots\dots\dots 1$$

The statistical validation of the equation was established by analysis of variance (ANOVA). The significance of regression coefficient was carried out by applying student t-test. The response surface plots and contour plots (Fig 1, Fig 2, Fig 3) were generated to study the effect of independent variables on entrapment efficiency of

nanoparticles.

Fig 1 indicates influence of Smix:lipid ratio and drug loading concentration in lipid phase on the entrapment efficiency of nanoparticles. At lower levels of Smix:lipid ratio, when drug concentration was increased, entrapment efficiency was found to be slightly increased whereas at higher ratio of Smix:Lipid, with increase in drug concentration, there was decrease in drug entrapment. This might be due to increase in the partitioning of drug in aqueous phase with increase in amount of surfactant mixture, thus decreasing the entrapment in lipid.

At lower levels of drug concentration, there was no significant effect of Smix:lipid ratio on drug entrapment whereas at higher levels, with increase in Smix:lipid ratio, there was sudden decrease in entrapment efficiency due to increased partitioning of drug in aqueous phase.

Fig 2 indicates influence of drug loading concentration in lipid phase (X_2) and P90H concentration in lipid phase (X_3) on entrapment efficiency. At lower and higher levels of Phospholipon concentration, when drug concentration was increased, there was no significant change in drug entrapment. At all the levels of drug concentration, when Phospholipon concentration was increased from 10 to 20%, there was increase in the GLIPTIN entrapment. With the further increase in Phospholipon concentration from 20 to 30%, there was decrease in entrapment efficiency.

Fig 3. indicates effect of Smix:lipid ratio (X_1) and P90H concentration in lipid phase (X_3) on entrapment efficiency. At lower and higher levels of Phospholipon concentration, when Smix:lipid was increased, there was no change in drug entrapment. At all the levels of Smix: lipid ratio, when Phospholipon concentration was increased from 10 to 20%, there was increase in the entrapment efficiency of SLN. When the Phospholipon concentration was further increased from 20 to 30%, there was decrease in drug entrapment.

1.3. Optimization and validation

The criteria for selection of optimized batch were primarily based upon maximum entrapment efficiency and physical stability of formulation. Formulation with 15 % of drug loading, 3:1 as Smix:lipid ratio and 20% as phospholipon concentration was selected and a new batch of the same composition with predicted response was prepared in order to confirm the validity of optimized formulation. The experimental results of optimized formulation were found to be in good agreement of predicted value.

1.4. Physicochemical characterization of SLN Table 3 indicates data of evaluation parameters for optimized formulation before and after lyophilization.

1.4.1. Particle size and zeta potential

Particle size distribution is important to predict the stability of colloidal system. Higher particle size with wide distribution may lead to physical instabilities of nanoparticles. The particle size of optimized formulation was

found to be 116 nm with polydispersity index of 0.29. Polydispersity index indicates size distribution range and the value near to 0.1 indicates narrow size distribution. The particle size of lyophilized formulation was found to be increased to 190 nm with polydispersity index of 0.32. This might be due to the presence of cryoprotectant. The cryoprotectant effect of the trehalose, as observed here as an increase in size, may be the result of the formation of a protective capping layer around the SLN.

Zeta potential is a key factor to evaluate the stability of colloidal dispersions. The colloidal system is considered as stable with zeta potential value of more than 30 mV. The lower zeta potential value of optimized formulation is due to nonionic surfactants, span and tween 20 that form a stable coat around the particle-water interface resulting in steric stabilization but minimizing electrostatic repulsion between the particles.

Table 3: Data of evaluation parameters of optimized formulation

Formulation Code	Smix:lipid	Drug loading concentration in lipid phase (%)	Phospholipon concentration in lipid phase (%)	EE (%)	Particle size (nm)	PDI	Zeta potential (mV)
F15 (Before lyophilization)	3:1	15	20	92.2±0.4	116nm	0.29	-14.1
F15 (After lyophilization)				88.3±1.1	190nm	0.32	-15.2

X-Ray powder diffraction

From X-Ray powder diffractograms (Fig 5), the internal physical state of GLIPTIN in the SLN was verified. The GLIPTIN showed sharp intense peaks representing crystalline structure of drug. Due to addition of Trehalose additional sharp peaks appeared in the physical mixture but lyophilized formulation did not show any peaks characteristics of GLIPTIN. This further confirms solubilization of drug in lipid.

Ex vivo absorption study

The results of ex vivo absorption study of Gliptins SLN formulations through everted rat intestine are shown in fig

7. It is evident that the drug absorption from SLN formulation was enhanced as compared to pure GLIPTIN. The drug permeation from pure GLIPTIN is limited to only 35.6% in

8 h of study. This could be attributed to poor drug dissolution. The enhanced drug permeation of GLIPTIN from SLN could be attributed to presence of lipids and phospholipon that are reported to enhance the drug absorption by various mechanisms [6, 24].

Conclusion:

The optimized SLN formulation consisting of glycerylmonostearate and Phospholipon 90 H revealed high drug entrapment, lower particle size and sustained drug release behaviour. The drug release pattern was biphasic and release mechanism was identified as Fickian diffusion. Ex-Vivo absorption study indicated higher drug absorption through SLN formulation than pure drug through everted rat intestine. Conclusively, developed formulation may serve as a potential delivery system for Gliptins by oral route.

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