International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2349-2819
Email: editor@ijarets.org Volume-10 Issue-1 January-2023 www.ijarets.org

STUDY FOR DESIGN AND DEVELOPMENT OF GLIPTINS NANOPARTICLES

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ABSTRACT:

The optimised formulation, which had a S mix: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. 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). 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. 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 calorimetric, and X-ray diffraction were used to characterize the lyophilized SLN formulation. 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

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 [1]. 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 [2]. 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 [3]. A dangerous metabolic illness called diabetes mellitus type 2 is characterised by low levels of insulin and insulin resistance, which raise blood sugar levels [9]. 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 [7]. 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 solublization 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].

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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 glycerylmonoste a rate nanoparticles in one of our earlier studies [12]. In the current investigation, phospholipid was added to glycerylmonoste a rate 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 glycerylmonostearte (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 P90Hwereblendedtogetherandmeltedabovetheirmeltingpoint (at 80ºC). Gliptin was dissolved in the molten lipid by continuous stirring followed by addition of span 20aslipophilic surfactant. The aqueous phase with Tween 20as hydrophilic surfactant was also maintained at 80ºC. The aqueous phase was poured slowly to the hot oil phase, along with Solutol HS 15 as a coemulsifier. The result ant dispersion was continuously stirred at 80ºC until clear microemulsion obtained. The hot O/W microemulsion was then poured into cold water 2ºC in a fixed ratio (1:25) andstirred with homogenizer (Remi, RQ/127A, India) at 8000rpm for 2hrs in order to form solid lipid nanoparticles. The obtained dispersion was then sonicated using aprobesonicator(PCIanalytics,PKS-250F, India)for20 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 co surfactant, ratio of surfactant: co surfactant (s:cosratio)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 GLIPTINSLN 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(Table2).There sulting data was fitted into Design Expert software(Version9.0)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,P90Hconcentration in lipid phase on dependent variable. Table 2 indicate 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,FreeZone2.5,USA).Trehalose (3% w/v) was added as a cry protectant to the dispersion be fore freeze drying.The freezing was done at-20°C and freeze drying was in tilted after24h of refrigeration**[16]**.

1.2. Evaluation of GLIPTIN solid lipid nanoparticles

All the GLIPTINSLN 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 deionized water prior to measurements. All the measurements were carried out in triplicateata temperature of 25.0°Can data fixed angle of 90° to the incident laser beam. Data was analysed by Windo x software (Version 5.7.1.0) and values of mean particle size and particle size distribute on 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 takenanddispersedindoubledistilledwater.Topreventtheagglomeration,thedispersedSLNwasplaced 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 unentrapped 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 receiving phase was added to the later. The beaker was placed on magnetic stirrer at 37°C at a rate of 100 rpm. At1 h after test, 5ml of medium from beaker was removed by filtration for analysis and fresh medium was added to maintain sink conditions. Unentrapped GLIPTIN was analyzed spectrophotometric ally(Shimadzu,UV-1700,Japan)at 226.5nm.

$$EE\ (\%) = \frac{\text{Total amount of GZ added} - \text{Unentrapped (free) GZ} \quad X100}{\text{Total amount of GZ added}}$$

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 dynamo sphere 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 diffract meter (Bruker, D 8 Advanced, USA) using Cu K2α 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±0.5ºC 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 off rash dissolution medium was then added to maintain 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 Korsemayer-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 fitto appropriate kinetics.

Table1: Preliminary optimization of variables for Gliptin SLN

Batch code*	Lipid(mg)		Surfactant(mg)			Cosurfactant(mg)			S:cos ratio	sonication time(min)	Entrapment efficiency (%)**
	GMS	P90H	Tween 20	Span 20	Lipoid S	Solutol	butanol	TranscutolP			
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 was1:25**Mean±SD(n=3)											

Table2: Optimization of Gliptins SLN using Box-Behnken design

Batch code*	Codevalue			Y_1 Entrapment Efficiency** (%)
	X_1 S mix:lipid ratio	X_2 Drug loading concentration in lipid phase(%)	X_3 P 90 H 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 50mg of Gliptins and ratio of lipid to water phase was1:25 ***Mean±SD (n=3)*				

Result and discussion

Formulation of Gliptins solid lipid nanoparticles

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 as table system. Gliptins showed highest solubility in the blend of GMS and P 90 H and was selected as the lipidphase 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,LipoidSandspan20 were used in combination with tween20.Solid lipid nanoparticles prepared with lipoidS(P1)and tween20 were not physically stable and resulted in lower drug entrapment where as formulations with span20(P2)and tween20 were found to be stable with high entrapment efficiency.The concentration of tween20 and span 20 was adjusted to required HLB value of the lipid phase (RHLB 13 for GMS) to make a clearo/wmicroemulsion.The effect of various surfactants, viz. solutol HS15, butanol and transcutol P on Gliptins SLN was also studied. SLN with butanol (P3) and transcutol P(P4) were not stable for more than 48 h whereas SLN dispersion with solutol Hs 15 (P2) were found to best ableevenafter7daysofstoragewithhighentrapmentefficiency(85.6±0.8%)withnodrugprecipitationinformulation. Hence,solutol HS15 was selected as co solvent 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: cosratio,2:1(P5)and1:2(P6).Nano dispersion with increased amount of co surfactant (1:2) was found tobeunstableandresultedinprecipitationduringstorage.Gliptinsentrapmentwasmaximum(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 indepedant variables on response, entrapment efficiency (%), were analyzed using Design-Expert software. The results of Smix:lipid ratio (X_1), Drug loading concentration in lipid phase(X_2)andP90Hconcentration in lipid phase (X_3) on entrapment efficiency(Y_1) are depicted intable2. Polynomialequation1wasobtainedfromregressionanalysisofthedata.

Entrapment efficiency(Y1)= 89.48-2.76X1+ 1.25X2+0.44X3-2.00X1X2+1.53X1X3+0.30X2X3+1.48X12+ 3.10X22-9.92X32 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-test. The response surface plots and contour plots (Fig 1,Fig 2,Fig 3) were generated to study the effect to find ependent variables on entrapment efficiency of nanoparticles.

Fig1 indicates influence of S mix: 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 within crease 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) andP90Hconcentrationinlipid 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 10to20%, there was increase in the GLIPTIN entrapment. With the further increase in Phospholipon concentration from 20 to30%,there was decrease in entrapment efficiency.

Fig 3.indicates effect of Smix:lipid ratio (X1) and P 90 H concentration in lipid phase(X3)on entrapment efficiency. At lower and higher levels of Phospholipon concentration, when Smix: lipid was increased, there wasno change in drug entrapment. At all the levels of Smix: lipid ratio, when Phospholipon concentration was increased from10to20%,therewasincreaseintheentrapmentefficiency 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:l ipidr atio 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 being agreement of predicted value.

1.4. Physicochemical characterization of SLN Table3 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 in stability nanoparticles. The particle size of optimized formulation was found to be 116nm with polydispersity index of 0.29.Polydispersity index indicates size distribution range and the valu enearto0.1indicates narrow size distribution. The particle size of lyophilized formulation was found to be increased to190 nm with polydispersity index of 0.32.This might be due to the presence of cry protectant. The cry protectant effect of the observed here as increase in size,may be the result of the formation of 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 nonionic surfactants, span and tween 20 that form as table coat around the particle- water interface resulting in steric stabilization but minimizing electrostatic repulsion between the particles.

Table3:Data of evaluation parameters of optimized formulation

Formulation Code	Smix:lipid	Drug loadingconcentrationinlipidphase (%)	Phospholiponconcentrationinlipidphase (%)	EE (%)	Particle size(n)	PDI	Zetapotential(V)
F15 (Beforelyophilization)	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 diffract grams (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 peak sappeared in the physica lmixture but lyophilized formulation did not show

any peaks characteristics of GLIPTIN. This further confirms solublization of drug in lipid.

Ex vivo absorption study

The results of ex vivo absorption study of Gliptins SLN formulations through evertedratin test in eared shown in fig 7.It is evident that the drug absorption from SLN formulation were enhanced as compared to pure GLIPTIN. The drug permeation from pure GLIPTIN is limited to only35.6%in 8 h of study.This could be attributed to poor drug dissolution. The enhanced drug permeation of GLIPTIN from SLNcould be attributed to presence of lipid sand phospholip on that are reported to enhance the drug absorption by various mechanisms [6,24].

Conclusion:

Ex-Vivo absorption study indicated higher drug absorption through SLN formulation than pure drug through ever tetra intestine. Conclusively, developed formulation may serve as a potential delivery system for Gliptins by oral route. The optimized SLN formulation consisting of glycerylmonostea rate 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 Ficki and iffusion.

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