Research Article

Formulation Development, Optimization and *In-vitro* Evaluation of Glimepiride Lipospheres

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ABSTRACT

The present study was aimed to formulate, optimize and evaluate glimepiride loaded lipospheres. Formulation of glimepiride lipospheres was carried out using compritol® 888 ATO as main lipid. Surfactants used in formulation of glimepiride lipospheres were poloxamer 188, polyvinyl alcohol and phospholipon 90G (P 90G). Glimepiride liposphere formulations were prepared by using melt dispersion technique and optimized by using 3^2 full factorial design. Glimepiride optimized formulation was evaluated for entrapment efficiency, drug content, particle size analysis, surface morphology, percentage drug release and stability study. For formulation GLS 4, drug content 85.13 ± 2.35 %, entrapment efficiency 85.37 ± 2.50 % and particle size 25.68 ± 0.18 µm was observed with spherical shaped free flowing particles. Percentage drug release was carried out using USP type II dissolution apparatus in 0.1N HCl medium and drug release of glimepiride lipospheres within 8 hrs was found to be 81.19 ± 3.91 % for GLS 4 batch. Formulation was able to sustain the drug release. Drug release follows non-fickian super case II type of transport and Korsmeyer- Peppas was the best suited model for drug release. Stability study of optimized glimepiride lipospheres formulation revealed that the formulation was stable at $5^{o}C \pm 3^{o}C$ for two months.

Keywords: Glimepiride, Lipospheres, Compritol® 888 ATO, Melt dispersion technique, Factorial design, Stability studies.

INTRODUCTION

Lipospheres are aqueous micro dispersion of solid water insoluble spherical microparticles of particle size between 0.02 to 100 µm in diameter. Lipospheres are composed of a solid hydrophobic lipid core (triglycerides), stabilized by a layer of phospholipid molecules embedded on their surface. The internal core contains the bioactive compound dissolved or dispersed in the solid fat matrix[1]. Around 40 to 70 % of chemical entities are poor water soluble. Several techniques like lyophilisation, drug micronization and microencapsulation used to enhance the dissolution profile of water insoluble drugs[2]. Lipospheres are the most promising drug delivery for the water insoluble drugs. Lipids are used as common excipient and base in creams, ointments and flow modifier. Lipospheres drug delivery technique is suitable for oral, parenteral and topical drug delivery of bioactive compounds and used for the formulation of anti-inflammatory compounds, local anesthetics, antibiotics and anticancer agents, as well as carriers of vaccines and adjuvants[3]. Lipospheres have various advantages like enhanced physical stability due to avoidance of coalescence, extended release of entrapped drug, high entrapment of hydrophobic drug, controlled particle size, ease of preparation, easy availability of excipients and low cost. Lipospheres are formulated by various techniques like melt dispersion, solvent evaporation, hot and cold homogenization, solvent extraction, sonication method, multiple microemulsions and rotoevaporation technique[4].

Glimepiride is one of the drug used to treat type 2 diabetes mellitus. Type 2 diabetes mellitus results from progressive insulin secretory defects on the background of insulin resistance and beta-cell failure and has a heritable basis. Glimepiride is from class second generation sulfonylurea which is insulin secretagogues that act primarily by stimulating release of insulin from functional pancreatic beta cells. Glimepiride is oral hypoglycemic agent belongs to BCS class II drug having low aqueous solubility. The main goals of antidiabetic treatment are to prevent imminent mortality and alleviating symptoms. Glimepiride is absorbed from gastrointestinal tract, having 80 to 100 % oral bioavailability and half-life is around 5 hrs[5,6]. Glimepiride lipospheres are prepared by melt dispersion technique which is one of the most used and best technique for liposphere preparation because it gives high entrapment efficiency with controlled particle size[4].

MATERIALS AND METHODS

Materials

Glimepiride was obtained as gift sample from Sava Healthcare Ltd, Pune, India. Compritol® 888 ATO was purchased from Gattefosse India Pvt. Ltd, Mumbai, India. Poloxamer 188 was purchased from BASF, Mumbai, India. Polyvinyl alcohol (PVA) was purchased from Loba chemie Pvt. Ltd, Mumbai, India.

Phospholipon 90G (P 90G) was purchased from Lipoid, Germany and All the other reagents and chemicals used were of analytical grade.

Preparation Method

Melt dispersion technique was used for formulation of glimepiride lipospheres. Compritol® 888 ATO was used as

lipid matrix and poloxamer 188, PVA and P 90G were used as surfactants. Aqueous phase was prepared by adding poloxamer 188, PVA and P 90G in hot distilled water at 80°C and dissolved. Compritol® 888 ATO lipid matrix was melted below the temperature of aqueous phase at 70°C in separate serological water bath and then glimepiride was added to lipid melt. Aqueous phase was then added in lipid melt with maintained temperature at 80°C and formed emulsion was then homogenized for 3 min by using Ultra Turrax high speed homogeniser (IKA® T-25, Germany). After homogenization liposphere dispersion was cooled to about 10°C by placing the formulation into an ice bath with continuous stirring. The obtained lipospheres was then washed with water and filtered through a 42 number whatman filter paper[7].

Optimization of formulation of glimepiride lipospheres

3^2 full factorial design and Response surface analysis

3^2 full factorial design was used for optimization of glimepiride lipospheres. Design Expert software (Version 8.0.4.1 Stat-Ease Inc., USA) was used for factorial design. Factorial design consists of two variables, independent variables such as lipid concentration and surfactant concentration and dependent variables are entrapment efficiency and particle size. Analysis of variance (ANOVA) was applied to estimate the significance of the model. 3D response surface plots were plotted by using models generated by regression. Effect of the lipid concentration and surfactant concentration on entrapment efficiency and particle size was checked[8,9].

Evaluation of lipospheres batches

Organoleptic Evaluations

Glimepiride liposphere batches were evaluated for organoleptic properties such as colour, odour and shape by visual observations.

Determination of drug content

The drug content of glimepiride lipospheres was determined by dissolving accurately weighed 10 mg dried lipospheres in a mixture of 1 ml chloroform and 9 ml methanol. Solution was sonicated for 5 to 10 min using sonicator (Biomedica, BMI-599). Solution was filtered and analyzed for drug concentration by using UV visible spectrophotometer (Shimadzu, model UV-1800) at wavelength of 225 nm. The drug content was calculated through straight line equation[9].

Determination of Entrapment Efficiency

Entrapment efficiency of glimepiride lipospheres was determined by lysis of the lipospheres with chloroform. A 10 ml of glimepiride dispersion was centrifuged for 45 min. Settled lipospheres were diluted in mixture of 1 ml chloroform and 9 ml methanol and sonicated (Biomedica, BMI-599) for 5 min to obtain a clear solution. Entrapment efficiency was checked by using UV visible spectrophotometer (Shimadzu, model UV-1800) at wavelength of 225 nm after appropriate dilution and calculated through the following formula[9],

Percentage entrapment efficiency = [Amount of drug in lipospheres/ Initial amount of drug incorporated in formulation] × 100

Differential scanning calorimetry (DSC) analysis

DSC measurements were performed on a differential scanning calorimeter equipped with an intra-cooler (DSC Mettler STAR SW 12.10, Switzerland). Analysis was performed on standard aluminium pan with 1 mg glimepiride optimized liposphere formulation sealed in crucibles. Temperature range of 20°C to 220°C and at a heating range of 10 K/min under inert atmosphere was maintained by purging nitrogen gas. An empty aluminum pan was used as reference[10].

Particle size analysis

Particle size analysis of the glimepiride lipospheres was performed on motic digital microscope (Motic Inco Pvt. Ltd, B1- 223ASC). Sample of 10 mg of glimepiride dried solid liposphere formulations were diluted in 10 ml distilled water. Glass slides were mounted and analyzed for particle size at various objective lenses[11].

Surface morphology (SEM)

JEOL Scanning Electron Microscope (Model: JSM 5200, Japan) was used to characterize surface morphology of optimized glimepiride lipospheres. The samples were prepared by sprinkling the lipospheres powder on double size adhesive tape which stucked to aluminium stab and gold coated under vacuum using a sputter coater. Samples were exposed to vacuum for 5-10 min. at 40 mA and investigated at accelerating voltage of 15 kV and 10 kV[12].

X-ray Powder diffraction (XRD) study

X-Ray diffraction measurements were performed on Philips Expertpro MPD diffractometer (PW3710 PAN analytical Inc, Germany) with higher resolution over a frequency range of 5° to 40° of 2θ with 30 mA current and voltage of 40 kV. The samples was sprinkled on glass slide and radiated using a copper target tube[13].

In-vitro drug release study and kinetics

The drug release study of glimepiride lipospheres was studied using USP type I Basket dissolution apparatus in acidic buffer 0.1 N HCl (pH-1.2). Capsules were filled by lipospheres equivalent to that of 10 mg drug. The capsules were maintained at 37°C ± 0.5°C, under stirring at 100 rpm in dissolution apparatus.

Samples were withdrawn periodically (0 min, 1 hrs. upto 8 hr.) and the same volume was replaced immediately by fresh medium. Sample solutions were filtered, diluted appropriately and analyzed by measuring absorbance at 225 nm on UV- Visible spectrophotometer[14]. Capsules were of creamy white colored and size no. 3 was used[15].

Stability testing of batches

Stability study of glimepiride lipospheres optimized batch was carried out according to ICH and WHO guidelines. Optimized formulation was packed in aluminium foil and exposed this to different thermal conditions i.e., 5°C ± 3°C, 25 ± 2°C/ 60 ± 5% RH and 40 ± 2°C/ 75 ± 5% RH for a period of two months[16].

RESULTS AND DISCUSSION

Optimization of formulation of glimepiride lipospheres

3^2 full factorial design and response surface analysis

Glimepiride lipospheres batches were optimized for preparation of lipospheres by using 3^2 full factorial design shown in table 1. From prepared nine batches, GLS 4 was found to be optimized batch having 85.37 ± 2.50 % entrapment efficiency and 25.68 ± 0.18 µm particle size. The linear models generated by the design are given below. The final equations obtained in terms of coded factors for glimepiride lipospheres represented as follows:

Entrapment efficiency $Y_1 = +85.09 - 0.96\ X_1 + 1.22\ X_2 - 5.31\ X_1 X_2 - 4.69\ X_1^2 - 3.57\ X_2^2$

Particle size $Y_2 = +16.03 - 0.050\ X_1 - 1.78\ X_2 – 3.40\ X_1 X_2 + 6.95\ X_1^2 X_2^2$

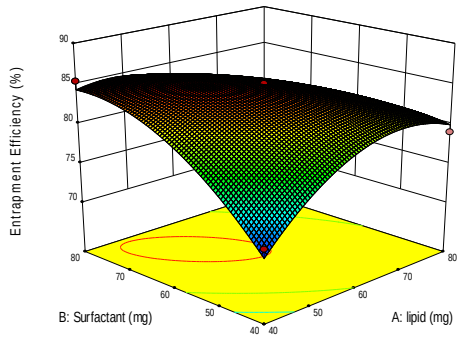
Response surface analysis was checked by plotting 3D response plots by models generated by regression analysis shown in figure 1. Response parameter Y was represented by a curvature surface as a function of X for glimepiride lipospheres batches. From the 3D plots it was observed that the entrapment efficiency and particle size values were affected more by the levels of lipid and surfactants.

Table 1: Glimepiride lipospheres composition from randomized runs in 3^2 full factorial design

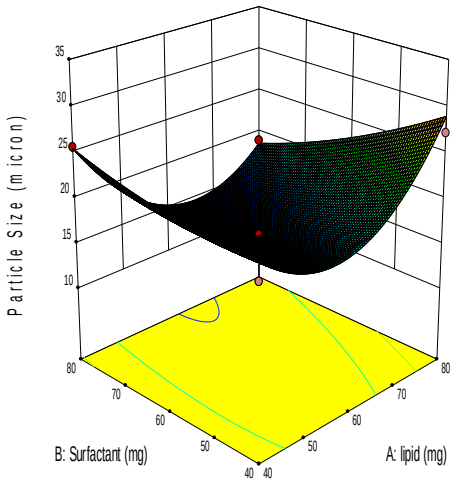
Batch code	Drug (mg) Glimepiride	Lipid (mg)	Surfactants				Aqueous phase (water) (ml)	Stirring speed (rpm)
			Poloxamer 188 (mg)	PVA (mg)	P 90G (mg)	Total (mg)		
GLS1	20	40	10	20	10	40	20	9000
GLS2	20	40	15	30	15	60	20	9000
GLS3	20	40	20	40	20	80	20	9000
GLS4	20	60	10	20	10	40	20	9000
GLS5	20	60	15	30	15	60	20	9000
GLS6	20	60	20	40	20	80	20	9000
GLS7	20	80	10	20	10	40	20	9000
GLS8	20	80	15	30	15	60	20	9000
GLS9	20	80	20	40	20	80	20	9000

ANOVA

Using factorial design Analysis of Variance (ANOVA) was calculated. ANOVA Results shows an *F*-value of 15.03 and P Value (Prob> F) is 0.0247 and equation represented as Y_1 for entrapment efficiency. Equation Y_2 with an *F*-value of 11.36 and P Value (Prob> F) is 0.0364 which is for particle size and was also linear.



(A)



(B)

Figure 1: Response surface plots of glimepiride lipospheres for parameters **(A)** Entrapment efficiency and **(B)** Particle size

Evaluation of Lipospheres batches

Organoleptic Evaluations

Glimepiride lipospheres batches were evaluated for organoleptic properties like colour, odour and shape. All GLS 1 to GLS 9 formulation batches were off-white in color and faint odour with spherical shaped free flowing particles.

Determination of percentage drug content

The role of formulated lipospheres is to deliver intact drug without degradation, drug content is mostly depends on encapsulation of drug in lipid, surfactants used and method of preparation.

Percentage drug content of glimepiride lipospheres were varied in the range of 66.51 ± 1.91 % for GLS 1 to 91.53 ± 2.62 % of GLS 6 batch.

Percentage drug content of optimized glimepiride lipospheres batch GLS 4 was found to be 85.13 ± 2.35 %. Lipid concentration affects the entrapment and for drug to lipid ratio were 1:2 the entrapment was high compared to 1:1 and 1:3 drug to lipid ratio in GLS 1 to GLS 9 glimepiride liposphere formulations.

Determination of entrapment efficiency

Ability of lipospheres to entrap drug at high level is important property. It is expressed as percentage entrapment efficiency.

Entrapment efficiency of glimepiride lipospheres batches GLS 1 to GLS 9 was evaluated.

From results, entrapment efficiency was varied in the range of 70.85 ± 1.67 % for GLS 8 batch to 85.37 ± 2.50 % for GLS 4. Batch GLS 4 was the optimized batch among all nine glimepiride lipospheres batches.

High entrapment of drug was due to its lipophilic nature and compatibility with lipid.

Formulation technique was also responsible for entrapment of drug. Melt dispersion technique was mostly used method for preparation of lipospheres.

Differential Scanning Calorimetry

Differential scanning calorimetry characterizes the melting behavior of compound. DSC thermogram shown in figure 2, endothermic peak at 212.97°C was corresponding to pure glimepiride was observed.

Another endothermic peak was observed at 75.5°C which is of glimepiride loaded lipospheres.

Disappearance of the drug peak and decrease in melting point is due to entrapment of drug in lipid.

Decreased melting point observed is of lipid, which clearly indicated entrapment of drug in lipid and in intact lipospheres, drug is in core which is embedded by lipid compritol® 888 ATO.

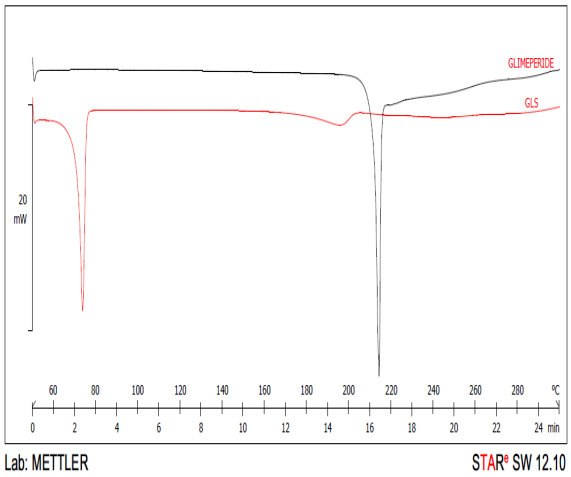


Figure 2: DSC thermograms overlay of glimepiride pure drug with glimepiride lipospheres optimized batch (GLS 4)

Particle size distribution

Glimepiride lipospheres shows particle size distribution in the range of 14.62 ± 0.31 µm for GLS 2 batch to 30.96 ± 0.26 µm for GLS 9 batch. Optimized batch of glimepiride lipospheres GLS 4 shows particle size distribution 25.68 ± 0.18 µm measured by motic digital microscope. Small particles have less entrapment of drug. Particle size was dependent on stirring speed and aqueous phase volume. Formulation was prepared using 20 ml aqueous phase and 9000 rpm stirring speed, gives particle size in expected range.

SEM study for surface morphology

Glimepiride liposphere optimized batch GLS 4 was evaluated for surface morphology. SEM study of glimepiride loaded lipospheres revealed that the lipospheres formed were spherical in shape with smooth surfaced particles for dry solid glimepiride lipospheres as shown in figure 3. It indicates entrapment of drug in spherical shaped lipospheres.

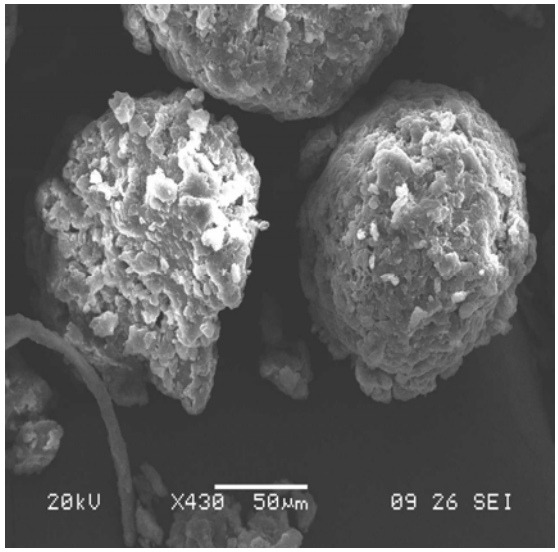
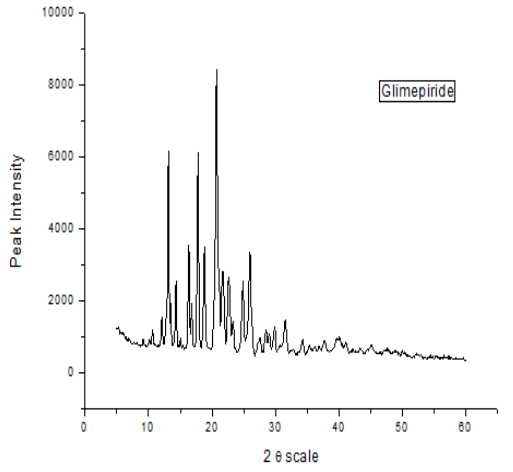


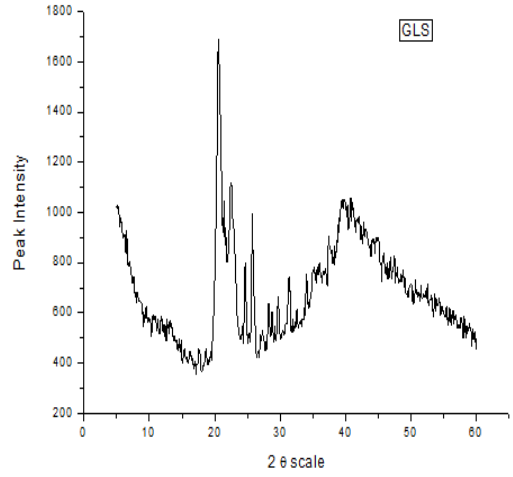
Figure 3: Scanning electron micrograph of dry solid Glimepiride liposphere formulation GLS 4

X-ray diffraction studies

Diffractograms from XRD studies are shown in figure 4. XRD spectrum showed in figure 4 (A) for pure glimepiride drug shows numerous sharp peaks with high relative intensity at 2Θ values 13.57°, 18.68°, 20.95°, 26.84° and (B) Glimepiride drug loaded lipospheres showed peaks at 2Θ values 20.53°, 22.48°, 24.64°, 25.77°. The XRD pattern shows that the sharp peaks of drug reveals the crystalline nature and glimepiride loaded lipospheres were also of crystalline in nature.



(A)



(B)

Figure 4: Powder X-Ray Diffraction pattern of **(A)** Pure Glimepiride and **(B)** Glimepiride lipospheres optimized batch GLS 4

In-vitro drug release study

All batches of glimepiride lipospheres, GLS 1 to GLS 9, showed a sustained drug release profile. The in-vitro drug release profiles of glimepiride lipospheres was carried out using USP type I basket dissolution apparatus using hard gelatin capsule. All of the drug release could last to 8 hrs. As showed in figure 5, release of glimepiride from lipospheres varied in the range of 62.61 ± 3.38 % for GLS 9 batch to 101.73 ± 3.45 % for GLS batch 7. Optimized batch of glimepiride lipospheres GLS 4 showed 81.19 ± 3.91 % drug release.

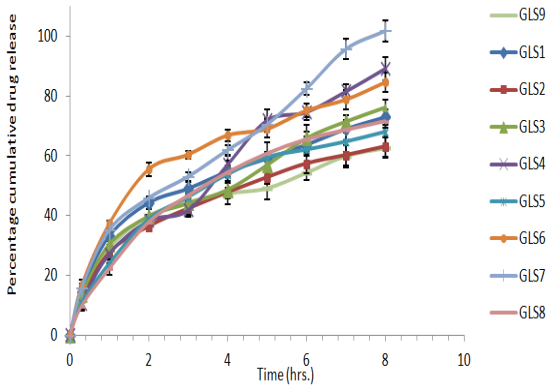


Figure 5: Percentage cumulative drug release of glimepiride lipospheres batches (GLS 1- GLS 9)

Drug release kinetics

Drug release kinetics of glimepiride lipospheres optimized batch GLS 4 was predicted and found with coefficient of correlation (R^2) 0.982 and n value 1.09. GLS 4 batch follows Non-Fickian super case II type of transport and Korsmeyer-Peppas model was best suited for this formulation as the value of n is 1.09 which is >1.0.

Stability testing of batches

Optimized batch of glimepiride loaded lipospheres GLS 4 was subjected for stability study according to ICH and WHO guidelines. GLS 4 formulation batch was packed in aluminium foil and kept in stability chamber. Stability study was carried out for two months and results revealed that the glimepiride lipospheres were stable at 5°C ± 3°C after evaluated for entrapment efficiency and particle size. Entrapment efficiency was found to be 84.15 ± 2.76 % and particle size was 26.42 ± 0.62 µm after two months.

CONCLUSION

Glimepiride drug loaded lipospheres were prepared successfully by melt dispersion technique. 3^2 factorial design was found to be useful for optimization of batches. GLS 4, the optimized batch, was the best among all batches and found potential for further development.

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