FORMULATION AND EVALUATION OF NANOSTRUCTURED LIPID CARRIERS CONTAINING GLIPIZIDE

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ABSTRACT

A new and improved generation of lipid nanoparticles named as Nanostructured lipid carriers (NLCs) has been developed to overcome the limitations of traditional lipid nanoparticles. NLCs are designed by controlled mixing of solid lipids with spatially incompatible liquid lipids leading to special nanostructures with improved drug loading, drug release profile and stability. The purpose of present study was to develop and evaluate NLCs based oral formulation of Glipizide. Glipizide, an oral hypoglycemic agent which is most commonly used in the treatment of noninsulin dependent diabetes mellitus. It has poor solubility in water and the half life of drug is 2-4 h. Hence by formulating Glipizide loaded NLCs (GPZ-NLCs) the dosing frequency will be decreased and also absorption variations will be eliminated. GPZ-NLCs were prepared by high shear homogenization coupled with ultrasonication method. Entrapment efficiency and mean particle size of GPZ-NLCs dispersion was found to be 86.73% and 99.83 nm respectively. Free flowing GPZ-NLCs powder was produced from dispersion after lyophilization using trehalose as a cryoprotectant, which was then used to develop GPZ-NLCs based capsule. In vitro drug release of GPZ-NLCs based capsule was found to be 86.79% with initial burst release followed by sustained release as compared marketed SR tablet which showed a slow and sustained release of 78.72% at the end of 24 hrs. Based on these results, it is concluded that the GPZ-NLCs based capsule may be proposed as sustained release formulation for oral delivery of Glipizide.
KEYWORDS: Glipizide; Nanostructured lipid carriers; Noninsulin dependent diabetes mellitus; Sustained release; Oral delivery.

INTRODUCTION

Recently, several approaches have been investigated to develop nanosized drug delivery system. These systems can generally be divided into two groups: polymeric and lipidic systems. Lack of safe polymers and solvents with regulatory approval and their high cost have limited the applications of polymeric nanoparticles.\(^1\) In order to overcome these problems, lipids have been put forward as an alternative carrier. These lipid nanoparticles are known as Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) which are attracting wide attention of formulators worldwide. SLNs and NLCs are nanosized lipidic carriers in size range between 50 - 1000 nm, prepared with lipids and surfactants generally recognized as safe. Lipids being biodegradable, SLNs and NLCs have excellent biocompatibility. They have combined advantages of liposome, polymeric nanoparticles and microemulsions and avoid the drawback of several colloidal carrier of its class. They provide a controlled drug release and an increase in chemical stability of the incorporated drugs. Moreover, they are safe carriers which can be produced easily on large scale. These lipid nanoparticles are thus preferred for oral delivery of drugs with poor water solubility. Oral route is the most convenient and preferred route of administration. Popularity of oral route is attributed to its greater convenience, non-invasive nature, high patient compliance, ease of administration, reduced risk of cross-infection and an advantage in drug absorption due to large surface area of gastrointestinal tract (GIT).\(^2,3,4\)

Glipizide, 1-cyclohexyl -3- [4- [2- [(5-methylpyrazin-2-yl) carbonyl] amino] ethyl] -phenyl] sulphonyl] urea, is a potential second generation oral hypoglycemic agent widely used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) by stimulating insulin release from pancreatic \(\beta\)-cells.\(^5\) Glipizide is a class II drug, which has low solubility and high permeability. Glipizide is reported to have short biological half life of 2-4 h necessitating it to be administered in 2 to 3 doses of 2.5-10 mg per day. Thus it is a potential candidate for the development of sustained release (SR) formulations. SR formulations releases drug slowly for maximum period of time and maintain plasma level of drug for 12-24 h may improve the therapeutic effect and also reduce dosing frequency which is sufficient for once a day dosing of glipizide. SR formulations are needed for glipizide to prolong its duration of action and to improve patient compliance.\(^6\)
NLCs is a new generation of lipid nanoparticles which proves to be a good solution for problems like poor drug loading and drug expulsion as experienced with solid lipid nanoparticles (SLN). They are solid lipid particles with varying contents of liquid lipid (oils). This blend is used to produce the lipid particles that are still solid at room temperature as well as body temperature. Due to many imperfections in NLCs, drug-loading capacity is enhanced and drug expulsion during storage is minimized. NLCs by virtue of their lipophilic nature and low particle size are widely explored as a delivery system to enhance uptake throughout gastrointestinal tract.[7]

This present study is carried out to incorporate Glipizide into NLCs for oral delivery. Glipizide loaded NLCs formulations were prepared by using Precirol ATO 5, Oleic acid and Tween 20 as formulation components using high shear homogenization coupled ultrasonication method. This nanostructure improves drug loading and firmly retains the drug during storage. Glipizide loaded NLCs were then lyophilized using trehalose as suitable cryoprotectant to obtained free flowing powder, which was then filled in suitable size of capsule. From formulation point of view NLCs lyophilized powder will give a stable formulation. The prepared systems were characterized for particle size, entrapment efficiency and drug release. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies also carried out to check for crystallinity, polymorphic changes in Glipizide loaded NLCs.

MATERIALS AND METHODS

MATERIALS

GPZ was kindly gifted by Cipla Pharmaceuticals Ltd. Mumbai, India. Solid lipids such as Precirol ATO5 (Glyceryl Palmitostearate); Compritol ATO888 (Glyceryl Behenate) were obtained as gift samples from Gattefosse India Pvt. Ltd., Imwitor 491 and 900K, Glyceryl tripalmitate and Glyceryl tristearate were obtained from Sasol GmbH, Germany. Oleic acid and Tween 80 were purchased from Sigma Aldrich, Mumbai, India. Tween 20 was purchased from Loba chemie Pvt. Ltd. Mumbai, India. Dialysis bag (molecular weight cut off 12–14 kDa; pore size 2.4 nm) was supplied by Hi Media, Mumbai, India. All solvents and reagents used were of analytical reagent grade.
METHODS

Screening of lipids and oils
The solubility of GPZ in different solid lipids was determined by semi quantitative method. An accurately weighed fixed quantity of drug was taken in a series of test tubes and solid lipids were added in increments until drug is completely solubilised. The temperature of test tubes was controlled at 10°C above the melting point of respective lipids. The test tubes were intermittently vortexed using cyclone mixture and observed for any drug residues. The amount of lipid (mg) required to completely solubilised the drug in molten state was determined.\[8\]

For determining solubility in different oils, an excess amount of GPZ was added to 2 ml of oil in a vial and mixed using cyclone mixture. The mixture was agitated on mechanical shaker for 72 h at room temperature for equilibration. After equilibrium, each sample was centrifuged at 10,000 rpm for 30 min using a centrifuge (Remi instruments, India) to separate the undissolved drug. Supernatant thus obtained was removed, filtered through 0.45 µ membrane filter. The filtrate was diluted suitably with methanol and saturation solubility of glipizide (mg/ml) in oil was determined by recording absorbance using ultraviolet (UV) spectroscopy at 274nm.\[9\]

The solubility of glipizide in various surfactants was determined by adding excess amount of drug to a 3% w/v surfactant solution to each respective vial. Vials containing this mixture were mixed using cyclone mixture and agitated on mechanical shaker for 72 h at room temperature to attain equilibrium. The mixture was centrifuged at 10,000 RPM for 30 minutes to separate the un-dissolved drug. The supernatant thus obtained was then filtered through membrane filter (0.45µ) and diluted suitably with methanol. Solubility of glipizide (mg/ml) in surfactant was determined by recording absorbance at 274 nm using UV spectroscopy.\[10\]

Selection of solid lipid to liquid lipid ratio
In order to estimate the relative effectiveness of various concentration of solid lipid to liquid lipid, various formulations were prepared to achieve good entrapment efficiency (% EE) and desired particle size. Three formulations having different solid lipid to liquid lipid ratio 3:1, 3:2, 3:3 (w/w) were designed.
Preparations of NLCs

High shear homogenization coupled with ultrasonication method was used to prepare NLCs. An accurately weighed solid lipid (Precirol ATO5) and liquid lipid (Oleic acid) were heated at 5-10°C above the melting point of lipid mixture on a water bath. To this lipid mixture drug was added to obtained clear melting solution. An aqueous phase was prepared by dissolved surfactant (Tween 20) in distilled water and heated to same temperature as that of the oil phase. Then this hot aqueous surfactant solution was poured into drug lipid mixture and homogenised using Ultra Turrax® T25 digital (IKA, Germany) at 9000 rpm for 10 minutes. The coarse emulsion thus formed was then sonicated for 10 minutes using Sonapros PR-250 M (Oscar ultrasonics, Mumbai). The resulting nanoemulsion was cooled under magnetic stirring at 2-4°C to obtained NLCs dispersion.[11]

The optimized NLCs dispersions were freeze dried with the selected cryoprotectant (Trehalose) to convert the nanoparticles in the form of dry powder and were directly filled in hard gelatin capsules of suitable size.

Evaluation and Characterization of NLCs

Particle size analysis

The particle size analysis of formulations was performed using NANOPHOX® (NX0073) particle size analyzer (Sympatec GmbH, Germany). Before measurement, NLCs dispersion was diluted suitably with ultra-pure water. All the measurements were done in triplicate, at a fixed angle of 90° to the incident laser beam and at a temperature 25 ± 2°C. Data was analyzed by Windox software and values of mean particle size, ploydispersity index (PDI) and particle size distribution curve were recorded.[12]

Entrapment efficiency

Entrapment efficiency corresponds to the percentage of drug encapsulated within and adsorbed onto the nanoparticles. The entrapment efficiency (% EE) was determined by measuring the concentration of unentrapped drug in the lipidic dispersions. NLCs dispersion was aggregated by diluting appropriately with saturated sodium chloride solution to facilitate the separation of nanoparticles. Appropriately diluted lipid dispersion was subjected to centrifugation at 10,000 rpm (Remi instruments, India) for 30 minutes. After centrifugation the amount of free drug in supernatant was estimated by using UV/VIS spectrophotometer at wavelength of 274 nm. The amount of incorporated drug was determined as the difference between the initial drug content and free drug in the supernatant.[13]
X-ray diffraction (XRD) study
X-ray scattering measurements were carried out with Pan-analytical Xpert PRO MPD X-ray diffractometer. X-ray diffraction measurements was carried out on pure glipizide, Lmix, glipizide loaded NLCs based freeze dried powder and blank NLCs based freeze dried powder.

Differential scanning calorimetric (DSC) study
DSC analysis was performed to check the drug-lipid interaction in nanoparticulate formulations and crystallinity of drug. Samples were analyzed on SII Nanotechnology EXSTAR DSC 6220 in scanning range of 30-300°C at a heating rate of 10°C/min. DSC scans of plain drug, Drug-lipid physical mixture and freeze dried NLCs formulation were recorded and compared.

Comparison of in vitro release profiles of GPZ loaded NLCs based capsule and marketed SR tablet
The marketed sustained release (SR) tablet of glipizide (Glynase® XL 5mg) was used for the study. In vitro drug release from both the formulations was carried out in pH 6.8 phosphate buffer (900 ml) using USP XXIII dissolution testing apparatus type I with rotating basket at 50 rpm and temperature was maintained at 37 ± 0.5°C. 5 ml aliquots were withdrawn at different time intervals of 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h from dissolution media and replaced with 5 ml of fresh buffer maintained at same temperature in order to maintain sink condition. The aliquots withdrawn were filtered through 0.45 μ filter and analyzed using validated HPLC method. The % cumulative release versus time graphs was plotted and compared.\[14\]

The HPLC analysis was carried out using an Agilent Technologies 1200 series system, Based on quaternary pump plus autosampler, UV Variable wavelength detector and an EZ chrome software. The column Hypersil C-18 (250 x 4.6 mm, 5 mm i.d. and 5 µm particle size) was maintained at 25°C. Acetonitrile: 50 mM Potassium dihydrogen phosphate buffer (pH 4.5) (60:40% v/v) was used as a mobile phase with the flow rate of 0.6 ml/min and detection was carried out at wavelength of 274 nm.
RESULTS AND DISCUSSION

Screening of lipids and oils

Lipid nanoparticles have been reported as useful tools in development of oral, parenteral, topical dosage forms for poorly water soluble drugs. The components selected to produce biodegradable and biocompatible lipid nanoparticles are of Generally Recognized as Safe (GRAS) status.[15] Lipid solubility study carried out to determine the maximum solubility of drug in particular lipid with the aim to select a lipid based on the affinity of drug for that lipid matrix type. This also helps to choose lipid which can show high drug loading capacity as well as entrapment efficiency. Highest drug solubility in lipid also reduces chances of drug expulsion from lipid particles. Among various solid lipids screened, Precirol ATO 5 was able to solubilise glipizide to maximum extent (295.10 ± 1.18 for 10 mg of drug) as compared to other solid lipids. Hence, it was selected as a model lipid for formulation and development studies. Table 1 shows the comparative solubility of drug in different lipids. Precirol ATO 5, being a mixture of fatty acid esters, is suspected of having more space that is more imperfections in its lipid matrix to accommodate drug.[16]

Table 1: Amount of solid lipid required to solubilised 10 mg of Glipizide.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Lipids</th>
<th>Amount of lipid (mg) required to solubilised glipizide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Compritol 888 ATO</td>
<td>369.27 ± 0.92</td>
</tr>
<tr>
<td>2</td>
<td>Precirol ATO 5</td>
<td>295.10 ± 1.18</td>
</tr>
<tr>
<td>3</td>
<td>Imwitor 900 K</td>
<td>535.10 ± 1.26</td>
</tr>
<tr>
<td>4</td>
<td>Imwitor 491</td>
<td>413.77 ± 1.41</td>
</tr>
<tr>
<td>5</td>
<td>Dynasan 114</td>
<td>401.44 ± 1.13</td>
</tr>
<tr>
<td>6</td>
<td>Glyceryl tripalmitate</td>
<td>More than 1 gm</td>
</tr>
<tr>
<td>7</td>
<td>Glyceryl tristearate</td>
<td>More than 1 gm</td>
</tr>
</tbody>
</table>

Among various liquid lipids screened, it was clear that glipizide has maximum solubility in oleic acid (9.31 mg/ml of oil) as it accommodate maximum amount of drug which is greater as compared to other oils as shown in table 2. Main role of oleic acid in NLCs formation is to disorganize the structure of lipid matrix and also additionally improves drug incorporation ability and drug loading.

Table 2: Solubility of different Glipizide in different oils.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oils</th>
<th>Saturation solubility (mg of glipizide/ml of oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Capryol 90</td>
<td>1.17</td>
</tr>
<tr>
<td>2</td>
<td>Oleic acid</td>
<td>9.31</td>
</tr>
<tr>
<td>3</td>
<td>Capmul MCM</td>
<td>1.57</td>
</tr>
<tr>
<td>4</td>
<td>Isopropyl Myristate (IPM)</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Solubility of glipizide was screened in various surfactants. Generally, surfactant exhibiting poor drug solubility will materialize the drug moieties in the lipid core and impart firm association of drug with the lipid matrix. So, surfactants exhibiting least solubility for glipizide were to be selected. Glipizide exhibited least solubility in Tween20, Poloxamer 188, and Lutrol 400 as compared to other surfactants. The selected surfactants were screened by taking batches and further evaluated for parameters like stability and particle size (table 3.) From observations, it was concluded that Tween 20 as surfactant gives stable batches with desired particle size and sufficient stability to the batch. Hence, Tween 20 was chosen as model surfactant for formulation.

Table 3: Composition of batches for surfactant selection.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Drug (mg)</th>
<th>Total lipid (mg)</th>
<th>Surfactants</th>
<th>Surfactant Concentration (%)</th>
<th>% Entrapment efficiency (%EE)</th>
<th>Particle size (nm)</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>150</td>
<td>Tween 20</td>
<td>1</td>
<td>86.73</td>
<td>99.83</td>
<td>Stable</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>150</td>
<td>Poloxamer 188</td>
<td>1</td>
<td>57.19</td>
<td>310.32</td>
<td>Aggregation of particle</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>150</td>
<td>Lutrol 400</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>Unstable</td>
</tr>
</tbody>
</table>

Selection of solid lipid to liquid lipid ratio

The % EE and particle size of different batches of NLCs having the ratio of solid lipid: liquid lipid from 3:1 to 3:3 is shown in table 4. The optimum concentration of oil is important; as the concentration of liquid lipid plays a crucial role with respect to % EE. It was maximum in batch 2 i.e. 87.75 ± 0.38. As we further go on increasing the concentration of oil; the entrapment was decreased. One probable reason for this could be the increased liquid lipid amount was not accommodated by solid lipid and hence expelled out. Based on these observations, Precirol ATO 5 and oleic acid in the ratio of 3:2 was selected as final working ratio of solid lipid: liquid lipid since at this ratio it exhibited good solubility for GPZ had higher entrapment efficiency and at the same time formed solid matrix on cooling, which is most important for NLCs fabrication.
Table 4: % EE for different solid lipid: liquid lipid ratio.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Precirol ATO 5: Oleic acid</th>
<th>% Entrapment efficiency (%EE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3:3</td>
<td>84.14 ± 0.86</td>
</tr>
<tr>
<td>2</td>
<td>3:2</td>
<td>87.75 ± 0.38</td>
</tr>
<tr>
<td>3</td>
<td>3:1</td>
<td>80.89 ± 0.35</td>
</tr>
</tbody>
</table>

Evaluation and Characterization of NLCs

Particle size analysis

Particle size distribution is one of the most important characteristics for the evaluation of the stability of colloidal systems. The value of mean particle size of GPZ-NLCs containing 150 mg of total lipid and 1% surfactant concentration was found to be 99.83 nm as shown in table 3. The PDI gives information about the homogeneity of particle size distribution in the system. A small value of PDI is indication of narrow size distribution in the system whereas large value indicates wide size distribution in the system. The PDI of formulation was found to be 0.38 which indicates that there is narrow particle size distribution and hence stable for longer duration of time.

Entrapment efficiency

The % EE increased with increase in concentration of total lipid content of formulations. This is probably due to increase in viscosity of medium resulting in faster solidification of nanoparticles. This would further prevent the drug diffusion to external phase of medium. This could also be attributed to the effect of liquid lipid i.e. oleic acid. This oleic acid can lead to imperfections in the solid lipid structure by incorporating itself inside the matrix of the solid lipid. The value of % EE of GPZ-NLCs containing 150 mg of total lipid and 1% surfactant concentration was found to be 86.73 as shown in table 3.

X-ray diffraction (XRD) study

Fig 1 shows the characteristic XRD peaks of Glipizide, Lmix and blank as well as drug loaded freeze dried NLCs respectively. In this study, the characteristics peaks for GPZ and Lmix were not seen in the diffraction pattern of the optimized NLCs freeze dried powder. From this we can conclude that GPZ was completely incorporated into NLC freeze dried powder and remains in the amorphous form.
Differential scanning calorimetric (DSC) study

DSC studies were performed to investigate the physical state of the drug in the Nanoparticles, because this aspect could influence the in vitro and in vivo release of the drug from the system. Different combinations of drug/lipid may co-exist in the lipidic carriers, such as: (i) amorphous drug either in an amorphous or a crystalline lipid and (ii) crystalline drug in either an amorphous or a crystalline lipid. Moreover, a drug may be present either as a solid solution or solid dispersion in an amorphous or crystalline lipid. Glipizide showed a sharp endothermic peak at 211.26˚c corresponding to the melting as shown in Fig 2 A. The melting peak of pure drug was totally disappeared in the thermogram of freeze dried NLCs as shown in Fig 2 C evidencing the absence of crystalline drug in the formulation. The absence of drug melting peaks in the DSC thermogram are usually signs of dissolved, amorphous or molecularly dispersed drug within the lipid or interactions between the drug and the lipid (a plasticizing effect of the drug) or a polymorph change of the drug which can be detected as peak shifts in the DSC thermogram. Therefore, it could be concluded that glipizide in the LNPs was either in an amorphous, molecular dispersion or a solid solution state in the lipid matrix after the production as there is complete disappearance of the endothermic peak.

Fig 1. X-Ray diffraction pattern of A. Glipizide B. Lipid Mixture C. Blank freeze dried NLCs D. Drug loaded freeze dried NLCs.
Fig 2. DSC thermogram of A. Glipizide B. Drug lipid physical mixture C. Freeze dried NLCs.

Comparison of *in vitro* release profiles of GPZ loaded NLCs based capsule and marketed SR tablet

Fig 3 shows comparative release of GPZ from NLC based capsule and marketed sustained release tablet. The drug release of from NLC based capsule and marketed SR tablet at the end of 24 h was found to be 83.09% and 78.72% respectively. A biphasic release pattern was observed for glipizide loaded NLCs based capsule i.e. a burst release in initial stage followed by a sustained release. The occurrence of burst release clearly indicates the location of certain amount of glipizide onto the surface of NLCs, whereas the sustain release profile suggests the release of glipizide from the core of lipid matrix to the release medium. While in case of marketed SR tablet, the drug was released in a sustained manner right from initially. The initial fast release of drug from the NLC based capsule could be desirable for oral hypoglycemic agents in order to prevent postprandial plasma glucose levels in diabetic patients.
Fig 3. Comparison of \textit{in vitro} release profiles of GPZ loaded NLCs based capsule and marketed SR tablet.

CONCLUSION
Nanostructured lipid carriers have been used as one of the drug carrier system for increasing solubility and dissolution rate poorly water soluble drugs, which intern increase its oral bioavailability. The current study attained the successful formulation and evaluation of GPZ loaded NLCs by high shear homogenization coupled with ultrasonication method which was converted into dry powder by lyophilization. Formulation developed using Precirol ATO 5, Oleic acid and Tween 20 showed high entrapment efficiency and satisfactory particle size distribution. XRD and DSC studies proved the favorable crystalline behavior of NLCs for protection and entrapment of GPZ. \textit{In vitro} release studies of GPZ loaded NLCs based capsule was comparable to marketed SR tablet with NLCs formulation showing better release characteristics. Thus GPZ loaded NLCs can be proposed as a promising drug delivery system for reducing the dosing frequencies and improving release kinetics of drug.

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