COMPARATIVE STUDY OF SOLID LIPID NANOPARTICLE AND NANOSTRUCTURED LIPID CARRIER OF CARVEDILOL

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ABSTRACT

The objective of the present investigation was to develop solid lipid nanoparticles (SLN) & Nanostructured lipid carrier (NLC) of an antihypertensive drug carvedilol by high shear homogenization coupled with ultrasonication method. Lipid nanoparticles (LNP) were prepared by Glyceryl monostearate as solid lipid and Oleic acid as liquid lipid at various concentrations and tween 80 as surfactant at various concentrations as an attempt for improving its gastrointestinal uptake and oral bioavailability and also to sustain its release. The developed LNP were characterized for particle size, polydispersity index, entrapment efficiency of carvedilol and In vitro release. Optimization was done using 3² factorial design using Design Expert® 8.0.6 software. The mean particle size was found to be in the range of 40-260 nm with a narrow range of polydispersity index. The entrapment efficiency (EE %) of LNPs was more than 70% and increased as the concentration of lipid was increased and decreased as the concentration of surfactant was increased. In vitro release of carvedilol from NLC dispersion was found better as compared to SLN dispersion.

KEYWORDS: Solid lipid nanoparticles; Nanostructured lipid carrier; carvedilol; Glyceryl monostearate; Oleic acid; Tween 80.

INTRODUCTION

Carvedilol (CAR) is a non-selective β-receptor blocking agent and a vasodilatation drug with antioxidant activity. It is used in the treatment of mild to moderate hypertension, angina pectoris, congestive heart failure. It is water insoluble drug with the pKa of 7.9, molecular...
weight of 406.5 and partition coefficient of 3.8. Its absolute bioavailability is 25 to 35 %, thus it belongs to BCS class II drug. It has a relatively short elimination half-life of 4 to 6 hours. It requires twice or even more daily dosing in large number of patients, which often lead to non-compliance.\cite{1}

Solid lipid nanoparticles and nanostructured lipid carriers (SLNs and NLCs) are nanosize lipidic carriers in size range between 50 - 1000 nm, prepared with lipids and surfactants generally recognized as safe. Lipids being biodegradable, SLNs/ 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. Some advantages offered by SLN are good physical stability, high drug payload, controlled drug release, specific targeting, scalability and feasibility of delivering both lipophilic and hydrophilic drugs. These colloidal carriers have shown to improve bioavailability of poorly soluble drugs. SLNs by virtue of their lipophilic nature and low particle size are widely explored as a delivery system to enhance uptake throughout gastrointestinal tract.\cite{2}

SLNs are produced by replacing the oil of an o/w emulsion by a solid lipid or a blend of solid lipids, i.e. the lipid particle matrix being solid at both room and body temperatures. SLN are composed of 0.1 to 30 % w/w solid lipid dispersed in an aqueous medium and if necessary stabilized with preferably 0.5 to 5 % w/w surfactant.\cite{3} NLCs are produced using blends of solid lipids and liquid lipids (oils). To obtain the blends for the particles matrix, solid lipids are mixed with liquid lipids, preferably in a ratio of 70/30 up to a ratio of 99.9/0.1. Because of the oil presence in these mixtures, a melting point depression compared to the pure solid lipid is observed, but the blends obtained are also solid at room and body temperatures.\cite{4} They also have a significantly higher loading capacity for active ingredients.\cite{5}

Thus, there is a clinical need for a dosage form that will deliver CAR in a controlled manner to a patient needing this therapy, thereby resulting in a better patient compliance. The conventional dosage forms exhibit low bioavailability due to extensive first pass metabolism and non-targeted delivery results in numerous side effects. To avoid these disadvantages, an attempt was made to develop SLNs/NLCs based sustained release formulation for oral delivery of CAR.

In the present study, we have formulated CAR SLNs /NLCs by using Glyceryl monostearate (0.5 -1.5 %) as solid lipid base, Oleic acid as liquid lipid (oil) and Tween 80 (1–2 %) as
surfactant by high shear homogenization coupled with ultrasound method as an attempt for improving its gastrointestinal uptake and oral bioavailability and also to sustain its release.

MATERIALS AND METHODS

Materials
Carvedilol was obtained as a gift sample from Panacea Biotech Pvt. Ltd., Navi Mumbai. Glyceryl monostearate (IMWITOR 900K) was generously supplied by Sasol, Germany. Tween 80 and Oleic acid was obtained from Sigma Aldrich, Mumbai. Dialysis membrane was purchased from Hi Media, Mumbai. All other chemicals used were of analytical grade.

Preparation of LNP Dispersion
The preparation of LNPs dispersion was based on the principle of ‘HSH Coupled with Ultrasound’ method. Lipid (GMS 900K) was heated at about 75°C (10°C above the melting point of lipid) and drug (CAR) was added to obtain a clear melting solution. An aqueous phase was prepared by dissolving surfactant (Tween 80) in distilled water and heated to same temperature of oil phase.[6] Hot oil phase was added to the aqueous phase and homogenization was carried out (at 10,000 rpm and temperature 75°C) for 5 minutes by using Ultra Turrax T25 digital (IKA, Germany). Hot oil in water emulsion so obtained was ultrasonicated at ultrasonication power of 20 Watt (W) using a SONAPROS PR-250M ultrasonicator (Oscar Ultrasonics, Mumbai) for 5 minutes. Carvedilol-LNPs were obtained by allowing hot nanoemulsion to cool at 4–8°C under magnetic stirring for 10 to 15 minutes. After cooling, it was stored in refrigerator in cool condition as the shelf life of LNPs dispersion is more at cool condition as compared to room temperature. In case of NLC dispersion, ratio of solid lipid (Glyceryl monostearate) to oil (Oleic acid) is kept as 3:1.[7,8]

Optimization
The SLNs and NLCs formulation were optimized using $3^2$ full factorial designs by Design Expert 8.0.6 software. In this design 2 factors were evaluated, each at 3 levels and experimental trials were performed in all 9 possible combinations as shown in table 1. The amount of Lipid ($X_1$) and the amount of surfactant ($X_2$) were selected as independent variables. In case of NLC formulation, the ratio of solid lipid to liquid lipid is kept constant as 3:1. The % EE and particle size were selected as dependent variables in a $3^2$ randomized full factorial design to evaluate the responses.
Table 1: Optimization of SLN and NLC dispersion using $3^2$ factorial designs

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Weight of drug (mg)</th>
<th>Weight of total lipid (mg) $X_1$</th>
<th>Weight of surfactant (mg) $X_2$</th>
<th>DW (q. s.) in ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>25</td>
<td>100</td>
<td>200</td>
<td>20</td>
</tr>
<tr>
<td>F2</td>
<td>25</td>
<td>100</td>
<td>300</td>
<td>20</td>
</tr>
<tr>
<td>F3</td>
<td>25</td>
<td>100</td>
<td>400</td>
<td>20</td>
</tr>
<tr>
<td>F4</td>
<td>25</td>
<td>200</td>
<td>200</td>
<td>20</td>
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<tr>
<td>F5</td>
<td>25</td>
<td>200</td>
<td>300</td>
<td>20</td>
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<tr>
<td>F6</td>
<td>25</td>
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<td>F7</td>
<td>25</td>
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<td>F8</td>
<td>25</td>
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<td>20</td>
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<tr>
<td>F9</td>
<td>25</td>
<td>300</td>
<td>400</td>
<td>20</td>
</tr>
</tbody>
</table>

Entrapment efficiency

EE corresponds to the percentage of drug encapsulated within and adsorbed onto the Nanoparticles. Aqueous solution of Tetrahydrofuran (30 % w/w) was used for aggregation of nanolipid carrier. After centrifugation (Remi instruments, India) of resultant dispersion, the amount of free drug in the supernatant was estimated by using UV/VIS spectrophotometer at wavelength of 242 nm. The amount of incorporated drug was determined as the difference between the initial drug content and free drug in the supernatant. EE for each batch was calculated in terms of percent entrapment as per the following formula.$^9$

$$\% \text{ Entrapment efficiency (EE)} = \frac{\text{Amount of drug added} - \text{Unentrapped drug}}{\text{Amount of drug added}} \times 100$$

Particle size analysis

The particle size analysis was done by using NANOPHOX particle size analyzer (Sympatec GmbH, Germany). The principle behind the particle size measurement is cross correlation laser diffraction. Dispersions were diluted with doubled distilled water to ensure that the light scattering intensity was within the instruments sensitivity range. Measurements were carried out at a scattering angle of 90° at 25°C to the incident light and data were collected over a period of 2 minutes. The PDI measured the size distribution of the NPs population. The mean diameter, correlation function diagram, distribution diagram and PDI of each batch were recorded.

In Vitro drug release study

Drug release of CAR from optimized SLN and NLC dispersion batches and aqueous suspension of CAR was studied in pH 6.8 phosphate buffer using USP 35 NF 30 dissolution testing apparatus type I with rotating basket at 50 rpm and temperature was maintained at 37°.
± 0.5°C. The dispersion was filled in dialysis membrane 70 which was soaked previously in double distilled water for 12 hours and tied it properly at both the ends and kept inside the basket. 5ml samples were withdrawn at different time intervals of 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours from dissolution medium and replaced with 5 ml of fresh buffer maintained at same temperature in order to maintain constant volume. The withdrawn samples were filtered through Whatmann filter paper. The filtrate was injected into the column and assayed for CAR content using validated HPLC method. By determining the amount of CAR released at various time intervals, the % release versus time graphs were plotted for optimized SLN and NLC dispersion batches and aqueous suspension of CAR.¹⁰

RESULTS AND DISCUSSION

Preparation of CAR loaded SLN and NLC

CAR loaded SLN and NLC were successfully developed using high shear homogenization coupled with ultrasound method. An o/w nanoemulsion was obtained as a clear solution after addition of heated aqueous phase to oily phase, maintained at temperature of 75°C. The SLNs and NLCs were obtained immediately by cooling hot nanoemulsion under magnetic stirring in cold water. The cold water facilitates rapid lipid crystallization and prevents lipid aggregation.

Optimization

From optimization study, it was found that NLC have more percentage entrapment efficiency and low particle size as compared to SLN shown in table 2. The software gives optimized formula for SLN and NLC dispersion. The optimized formula of SLN contains 127.5 mg of GMS 900K and 205 mg of Tween 80 whereas optimized formula of NLC contains 200 mg of total lipid (150 mg of GMS 900K and 50 mg of Oleic acid) and 200 mg of surfactant. The overlay plot of SLN & NLC dispersion obtained from software has shown in figure 1 & 2 respectively.

Table 2: Responses of various batches of SLN and NLC dispersion

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>SLN Dispersion</th>
<th>NLC Dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% EE</td>
<td>Particle size (nm)</td>
</tr>
<tr>
<td>F1</td>
<td>78.63</td>
<td>110.63</td>
</tr>
<tr>
<td>F2</td>
<td>76.11</td>
<td>43.00</td>
</tr>
<tr>
<td>F3</td>
<td>69.77</td>
<td>36.39</td>
</tr>
<tr>
<td>F4</td>
<td>81.54</td>
<td>235.06</td>
</tr>
<tr>
<td>F5</td>
<td>77.80</td>
<td>163.11</td>
</tr>
<tr>
<td>F6</td>
<td>72.59</td>
<td>146.36</td>
</tr>
</tbody>
</table>
Entrapment efficiency

The independent parameters such as lipid concentration and surfactant concentration affect the dependent variables such as EE. The % EE for various factor level combinations was found for SLN and NLC in the range of 69.77 % to 84.72 % and 72.15 to 87.69 % respectively. The % EE increases as the concentration of total lipid increased from 0.5 to
1.5% due to the solubility of CAR in lipid. The % EE of CAR significantly decreased on increasing the concentration of Tween 80 from 1 to 2%.

As the amount of emulsifier increased at a constant amount of lipid, the surface of formed LNPs is too small to adsorb all surfactant molecules, which will result in the formation of micellar solution of the CAR. Hence, the solubility of the CAR in the water phase will be increased. Therefore, CAR could partition from the LNPs into the formed micelles in the water phase during HSH, thus reducing the entrapment of CAR in LNPs.[11]

**Particle size**

Particle size distribution is one of the most important characteristics for the evaluation of the stability of the colloidal systems. The predicted particle size of optimized SLN and NLC formulation were 150.58 nm and 160.45 nm respectively but in actual particle size found to be 143.64 nm and 152.74 nm respectively. The PDI gives information about the homogeneity of particle size distribution in the system. A small value of PDI is an indication of narrow size distribution in the system whereas large value indicates wide size distribution in the system. The PDI of optimized SLNs and NLCs batch were found to be 0.297 and 0.0894. The mean diameter of dispersion confirmed that the SLN produced are submicron colloidal carriers, suitable for enabling gastrointestinal absorption by M-cells on PP.[7]

**In vitro drug release study**

In vitro drug release was performed for SLNs formulation revealing a biphasic release pattern of CAR i.e. a burst release in initial stage followed by a sustained release. About 21% of loaded CAR was released within first 2 hours, followed by the release of 73.98% within 24 hours of assay. The occurrence of burst release clearly indicates the location of certain amount of CAR onto the surface of SLN, whereas the sustain release profile suggests the release of CAR from the core of lipid matrix to the release medium. While in case of NLC dispersion, the release of drug was in sustained manner from initially or little burst release and % drug release at the end of 24 hour was found to be 83.96%. The % drug released at the end of 24 hour from CAR suspension was found to be 20.02% as shown in figure 3.[12]
Figure 3: Comparison of In-Vitro drug release profile of SLN and NLC dispersions with Conventional CAR Suspension.

CONCLUSION
High shear homogenization coupled with Ultrasound method was used for production of SLNs and NLCs dispersion. GMS 900K was used as solid lipid, Oleic acid as oil and Tween 80 as surfactant for formulation and development studies. Optimization technique ($3^2$ factorial design) was applied in the preparation of CAR loaded SLN and NLC. It was found that as concentration of lipid increases, both dependent parameter i.e. % EE and particle size increases and as concentration of surfactant increases, both % EE and particle size decreases. The EE of NLC batches was found to be more as compared to SLN batch. In-vitro drug release shows that there was better sustain and more release of drug from NLC as compared to SLN. Due to incorporation oil in the formulation of NLC, the structure of solid lipid gets disorganized hence there is more loading of drug and less drug expulsion over the period of time. Therefore, it can be concluded that NLC are better carrier system as compared to SLN.

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REFERENCES


