FORMULATION, CHARACTERIZATION & EVALUATION OF SOLID LIPID NANOPARTICLES OF CARVEDILOL

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ABSTRACT

Aims: The objective of the present research study was to formulate and characterize a lipid based delivery system for Carvedilol, to develop a controlled release formulation, to increase the bioavailability of Carvedilol by avoiding its first-pass metabolism, to increase the biological half-life of Carvedilol achieved successfully. Study Design: Design and development of formulation includes short listing of ingredients for the formulation, effect of various processes as well as formulation parameters of hot homogenization followed by ultrasonication method, and effect of drug concentration. Characterization of SLNs, which includes, entrapment efficiency (%), Drug loading (%), In-vitro drug release using dialysis bag diffusion technique, Particle size analysis, transmission electron microscopy, and charge determination.

KEYWORDS: SLN, Carvedilol, Nanoparticles.

INTRODUCTION

Solid lipid nanoparticles
Advantages of SLN technology: SLN is site specific drug delivery system due to their small size and narrow size distribution, controlled and sustained release of active drug can be achieved. Drug incorporated is protected from the onslaughts of biochemical degradation. It is sterilized by autoclaving or gamma irradiation. Toxic metabolites are not generated. It is relatively cheap and stable. Surface modification can be easily performed. Ease of industrial scale production by hot dispersion technique.11

Method
Preparation of Solid Lipid Nanoparticles
For the preparation of SLNs Hot homogenization followed by ultrasonication method was used. In this method, the solid lipid was weighed and melted at 10°C above its melting point. To that melted lipid, the drug was added and a clear solution was formed by adding PG as a co-solvent. The formed solution was added to hot aqueous surfactant solution (having the same temperature as that of the lipid phase), which was then homogenized and ultrasonicated at the same temperature. Cooling of the resultant solution to room temperature produced SLNs.8,9,10

Qualitative and Quantitative Evaluation
UV Method of Analysis For Carvedilol
Preparation of standard curve of carvedilol in distilled water, preparation of standard curve of carvedilol in pH 7.4, preparation of standard curve of carvedilol in methanol. Carvedilol was also analysed by using entrapment efficiency (EE%), Drug loading (DL%), Transmission electron microscopy, Zeta potential, Particle size analysis by using Zeta sizer.
Zeta Potential\textsuperscript{[11]}  
Zeta Potential is a crucial factor to evaluate the stability of colloidal dispersion. Surface charge on the Carvedilol-SLN F was determined using Malvern Zetasizer. 1 ml of sample of Carvedilol-SLN F suspension was filled in clear disposable zeta cell, ensured there was no air bubble within the sample, and the system was set at 25°C temperature, 5 minute sample run time for one measurement with 20 zeta runs, measurement position was set at 2.00 mm, and count rate was 71.3 kcps. Water was taken as dispersant with values such as 1.330 RI, 0.8868 cP viscosity, 78.5 dielectric constant. The zeta potential reported was 0.397 mV.

Transmission Electron Microscopy  
TEM is the invaluable tool for materials scientist and can provide valuable information on particle size, shape and structure as well as on the presence of different types of colloidal structures within the dispersion.\textsuperscript{(100)} The particle size, shape of Carvedilol-SLN F was examined by TEM (Hitachi, H7500, Japan). Optimised batch of SLNs loaded with Carvedilol was examined by TEM for confirming shape and size of the particles, 1 ml of SLN suspension was taken and diluted to 10 ml with double distilled water. No staining of the sample was done. The sample was passively adsorbed onto the copper grid and then dried prior to observation.

Particle Size Analysis: Zeta Sizer  
Zetasizer technique was used to determine the Particle size of SLNs. It was done by Zetasizer ZS+MPT-Z-Autotitrator, Malvern Instruments. The parameters like temperature was set at 25°C, water was taken as dispersant with RI 1.330, count rate was set at 73.7 kcps and duration used was 20 s.

Cell cleaned with the help of methanol two times was filled with distilled water and background was measured. SLNs suspension was added to the cell and particle size was analysed thrice for each sample. Zeta potential is a key factor to evaluate the stability of a colloidal dispersion. Zeta potential of one of the drug compound, reported in the literature and SLNs suspension was measured by the electrophoretic mobility of the nanoparticles in a U-type tube at 20°C.

Drug Loading (DL\%)  
20 ml of Carvedilol-SLN suspension was centrifuged at 20,000 rpm for 1 hour. The supernatant was removed and SLNs were completely dried at 40°C. 25 ml methanol was added to it and shaken for 1 hour in rotary shaker. Resultant solution was centrifuged at 15000 rpm for 30 minutes and filtered through 0.22 \( \mu \)m membrane filter. 5 ml of the filtrate was taken and diluted with fresh methanol and analysed for drug content in a double beam UV-Spectrophotometer at 241 nm.

Entrapment Efficiency (EE\%)  
The EE\% of carvedilol in SLNs of the system was determined by measuring the concentration of free drug in the dispersion medium by centrifugation followed by filtration method. Briefly, 5 ml of freshly prepared SLNs dispersion was centrifuged at 20,000 rpm in a refrigerated centrifuge (Eltek, elektrocraft [India] Pvt. ltd., Mumbai, India) for 1 hr. Supernatant was taken and filtered through a syringe filter (Millipore Millex-GN Nylon, Ambala, India) of pore size 0.22 \( \mu \)m which was pre-saturated with the supernatant. Drug concentration in various SLNs formulations was determined using UV-Spectrophotometer from the standard curve of the drug prepared in distilled water. EE\% was determined as a result of the initial drug minus the free drug divided by initial drug multiplied by 100.

In Vitro Dissolution Studies  
Percentage Carvedilol release from the Compritol 888 ATO nanoparticles was determined by dialysis bag diffusion method with PBS pH 7.4 as a dissolution medium at 37±0.5°C. Dialysis bag was made from dialysis membrane 70 had molecular weight cut off between 12000 to 14000 Da and pore size of 2.4 nm. USP dissolution type II was used for \textit{in-vitro} drug release study.

Accelerated Stability Study  
Freeze dried formulation of SLNs was kept at accelerated stability conditions i.e. 40°C temperature and 75% relative humidity. Three lyophilized Carvedilol SLNs sample (equivalent 100 mg) was kept in well closed container for the accelerated stability study. According to ICH guidelines sampling time point in accelerated stability study are 0, 3 and 6 months, but here in this study the sample was only sampled at 3 months. Particle size and \textit{in-vitro} drug release are the two parameters on which the results of study depend. Stable formulation should not show any change in particle size and drug release.

RESULTS AND DISCUSSION  
1. Three formulations of Carvedilol-SLN F, namely F1, F2 and F3 were prepared and particle size of all three was determined with zeta-sizer, which was observed in range of 50 nm to 400 nm.
2. Zeta potential study showed that, the formulation was low positive charged; that is, 0.397 mV. This value is not sufficient to produce a stable SLN preperation.
3. TEM images showed that, SLNs were spherical in shape and its size was identical to that obtained from zeta-sizer (Fig.11).
4. Carvedilol-SLN F, which contained Carvedilol, Compritol 888 ATO, Tween 80, Poloxamer 407 and Polypropylene glycol in the ratio of 0.45%, 2%, 3.5%, 3.5% and 1% respectively, because it showed desirable particle size range of 50 nm to 400 nm, maximum entrapment efficiency of 86.00% (Fig.4), maximum drug loading 6.74% (Fig.5) and controlled release upto 50.09% after 24 hrs.
5. Sterilization of immediately prepared SLNs was done in autoclave at 121°C for 15 minutes. Increase
in particle size was observed after sterilization (Fig 8,9).

6. *In-vitro* drug release study of stability SLNs sample showed that, more controlled release of Carvedilol (only 30.02%) (Fig. 7) was observed as compared to drug release from freshly prepared SLNs. This may be because of decreased surface area exposed to dissolution media (due to increased particle size) or it may be due to conversion of lipid to its more stable form.

UV Method of Analysis for Carvedilol

![Graph](image1)

**Figure 1: Standard curve of Carvedilol in distilled water**

![Graph](image2)

**Figure 2: Standard curve of Carvedilol in PBS pH 7.4**

![Graph](image3)

**Figure 3: Standard curve of Carvedilol in methanol**
Entrapment Efficiency (EE%).

Figure 4: Entrapment Efficiency of all Carvedilol-SLN formulations.

Drug loading (DL%)

Figure 5: % Carvedilol loading in various Carvedilol-SLN formulations

Comparison of Drug Loading and Entrapment Efficiency between Compritol and Stearic acid slns[12]

Figure 6: % Drug loading and % Entrapment efficiency of all Carvedilol-SLN formulations
**In-vitro Drug Release**

![% Carvedilol Release from Carvedilol-SLNs formulation C, F and L](image)

Figure 7: % Carvedilol Release From Freshly Prepared Carvedilol-SLns C, F And L Using Pbs (Ph 7.4 At 37.0±0.5 C) As Dissolution Medium

**Particle Size Analysis**

![Particle size distribution of Carvedilol-SLNs formulation F1, F2 and F3](image)

Figure 8: Particle size distribution of Carvedilol-SLNs formulation F1, F2 and F3

![Average Particle size of Carvedilol-SLNs formulation F](image)

Figure 9: Average Particle size of Carvedilol-SLNs formulation F
Zeta Potential

Figure 10: Zeta Potential of Carvedilol-SLNs formulation F

Transmission Electron Microscopy

Figure 11: TEM image of Carvedilol SLNs formulation F

Accelerated Stability Study

Figure 12: Comparison of Particle size before and after Accelerated Stability Studies
Figure 13: Comparison of % Carvedilol Release from fresh and Stability study sample of Carvedilol-SLNs formulation F in PBS pH 7.4 medium at 37±0.5°C.

REFERENCES