# Formulation *in vitro* and *in vivo* characterization of carvedilol loaded solid lipid nanoparticles.

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## Abstract

**Introduction: The main aim of the present work was formulation *in vitro* and *in vivo* characterization of Carvedilol SLN's and improve Carvedilol bioavailability. Due to high first pass effect carvedilol a beta blocker having low systemic availability (25%).**

**Materials and Methods: Solvent injection method was used to prepare SLN's using glycerylmonostearate (GMS) and Oleic acid as lipids, Tween 80 as Surfactant and Poloxamer 407 as Co-surfactant were used. The developed SLN's were characterized for Particle size, Polydispersity index, Entrapment efficiency of carvedilol and TEM.**

**Discussion: The physicochemical interaction between Carvedilol and lipid was investigated by Fourier transform infrared (FTIR) Spectroscopy and Differential Scanning Calorimeter (DSC). The release exponents showed that the value of 'n' was 0.993, indicating that it fallows follows zero order kinetics and Super case-II.A Comparative Pharmacokinetic studies were performed for Carvedilol Solid lipid nanoparticles and Carvedilol Suspension.**

**Results: The results indicate that half – life 4.83 ± 1.32 to 12.52 ± 0.5 hrs and MRT5.32 ± 1.42 to 18.19 ± 0.123 hrs provide the sustained and prolonged release and the residence time of SLNS in blood streamwhen compared with Carvedilol Suspension and enhanced its oral bioavailability when delivered in SLN's.**

**Keywords:** Carvedilol, Solid lipid nanoparticles, Solvent injection method Tween 80, Poloxamer 407, Oleic acid.

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## Introduction

Carvedilol is a nonselective beta-adrenergic antagonist used in the management of hypertension and angina pectoris. It is significantly metabolized in the liver, that results in nearly 25% reduced bioavailability although it is well absorbed in the gi tract. Consequently, for improving the therapeutic effectiveness of carvedilol, it is necessary to increase its bioavailability [1].

Among the present colloidal carrier systems, one is solidlipid nanoparticles that is considered as alternate to polymers that for parenteral nutrition is similar to oil in water emulsion, but solid lipid has been used in place of emulsion's liquid lipid. They consists of various benefits like low toxicity, good biocompatibility as well as solid lipid nanoparticles provides better delivery of lipophilic drugs and physically stable system. Also, cost effectiveness and biodegradability advantages are included by lipid excipients. Furthermore, for poorly water soluble, hydrophilic and lipophilic drugs, lipid nanoparticles are appropriate within the lipid matrix when used in significant amount [2].

Furthermore, researchers has discovered that when drugs are encapsulated into SLNs, where over lymphatic system, drug uptake occurs it results in increasing the oral bioavailability. Carvedilol properties like low dose and high first pass metabolism and due to its necessity for repetitive dosing and long-term treatment, this drug is considered as a significant option for oral administration in which there exists a controlled drug release as well as for enhancing overall bioavailability, first pass metabolism is avoided where carvedilol loaded SLNs' lymphatic uptake is promoted. Practically, carvedilol (log P= 4.2) is insoluble in intestinal fluids (pH 7.5), gastric fluids (pH 1.1) and water. Even though, after conventional tablets' ingestion, the gastrointestinal tract entirely absorbs the carvedilol, because of liver metabolism, it has nearly 25% of systemic availability [3].

Depending on these facts, present work's main aim was Carvedilol Solid Lipid N anoparticles' (SLN's) formulation and characterization by utilizing Poloxamer 407 in the Co-surfactant, Tween 80 as Surfactant and Oleic acid and glycerylmonostearate (GMS) as lipids for improving Carvedilol's dissolution rate through which biological activities can be increased. As SLN's are considered as alternate for colloidal carrier (transport) system, and are capable of enhancing the lipophilic drugs' permeability/solubility, thus, drug absorption can also be enhanced [4].

## Materials and Methods

Carvedilol was obtained as a gift sample. Glyceryl Monosterate (GMS) and Poloxamer 407 were procured from the Qualigens fine chemicals Pvt. Ltd. Oleic acid was purchased from Thermo Fischer scientific Pvt. Ltd. Tween 80 and Ethanol were supplied from Merck chemicals. Deionized water was purchased from the Biochemical's Pvt. Ltd [5].

### *Preformulation studies*

**Fourier transform infrared spectrophotometer:** FTIR spectroscopy is used for studying the interaction of drug polymers. Fourier Transform Infrared (FTIR) spectrometer is used to record the drug as well as drug loaded solid lipid nanoparticles' IR spectra [6]. (FTIR–ALPHA Bruker with KBr pellets. The range of scanning is noticed as 400-4000 cm –1.

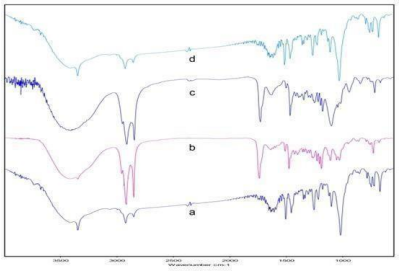


***Figure 1.*** *FTIR Spectra of a) Carvedilol b) Carvedilol and glyceryl monosterate c) Carvedilol glyceryl monosterate and Pol0xamer 407 d) Carvedilol solid lipid nanoparticles.*

#### *Differential scanning calorimetry*

Thermal characterization of Solid lipid nanoparticles was performed with a (Perkin-Elmer Wellesley, MA). About 10 mg of the sample were weighed as well as stored in closed aluminum pans [7,8]. Indium was used for calibration of equipment. From 50ºC to 450ºC at 20ºC/ min, scanning of samples are done.

#### *Preparation of carvedilol solid lipid nanoparticles*

Solvent injection method is used for preparing the SLN (Solid Lipid Nanoparticles) [9]. This approach involves dissolving drug and solid lipid in the water miscible solvents like ethanol (organic phase) and dissolve surfactant and co-surfactant in deionised water in another beaker (aqueous phase) and both phases are heated up to 60˚C and then the lipid mixture was to be injected in to the aqueous phase with magnetic stirring at 1200 rpm for 30 minutes at 60˚C and then the resultant dispersion formed was filtered to remove any excess lipid. And the resulted dispersion was diluted (1:20) by deionised water and then probe Sonicated for a period of 15 mins.

### *Characterization of solid lipid nanoparticles*

**Entrapment Efficiency:** Unloaded drug's concentration is measured for determining the entrapment efficiency in the dispersion medium. The prepared SLN's samples were placed in 9 centrifuge tubes. Using a Research centrifuge (REMI) at 10,000 rpm for 30 mins, the nanoparticles were separated from the medium. The supernatant clear liquid was then diluted (if necessary) with deionised water and the CDV concentration in 9 samples was measured by using UV visible spectrophotometer (LAB INDIA, 3000 +) at 255 nm [10].

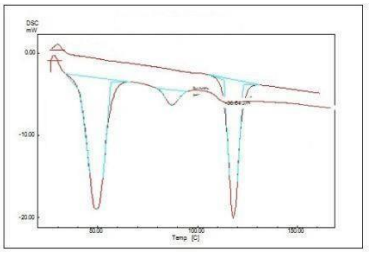

***Figure 2.*** *DSC thermogram of carvedilol and glyceryl monosterate.*

### *Surface morphology by TEM*

**Dip preparations for nanoparticles by floating method:**

A drop of sample is placed on a piece of parafilm, the carbon coated copper grid wait for 5-10 mins, and drain the excess with the help of filter paper, wash with distilled water and stained with 2% uranyl acetate dry observed under transmission electron microscope at various magnifications [11]. (model: Hitachi, h-7500) as per the standard protocol practicing at RUSKA lab, College of veterinary sciences, P.V. Narsimha Rao Telangana Veterinary University Rajendra nagar, Hyderabad.

### *In vivo and pharmacokinetic study on rabbits*

**Subject population:** The male New Zealand White rabbits (2.5 5 ± 0.17 kg) were used to conduct *in vivo* study and administered with Carvedilol dose 1 mg/kg and revise study protocol. One day preceding to study rabbits were fasted and allowed free access to water [12]. The rabbits were administered Carvedilol orally with same dose which were divided in to Two groups (6 per each). Carvedilol Solid lipid nanoparticles dispensed in distilled water (test) was administered and optimized by first group. Carvedilol suspended in distilled water was received by another group. To administer the dose the Carvedilol suspended in distilled water for enough timeby using 0.25% sodium carboxy methyl cellulose. Comparative randomized

single- dose, open labeled, parallel study design were used to conduct study.

**Sample collection and chromatographic analysis:**

Test or reference formulations blood samples of (0.5 ml) at regular intervals of time points (0, 0.5, 1, 2, 4, 6, 8, 12, 24 h) were withdrawn from the marginal ear vein and centrifuge the sample for 5 min at 3500 rpm and the plasma samples were stored at - 20ºC until assay using the previously reported modified HPLC method.

## Results and Discussion

Drug and excipients compatibility studies investigated to check incompatibility between drugs and lipids. FTIR results have shown that there are no considerable interaction among excipients and drugs [13]. The characteristics peak at 3420.15 cm -1 (N-H Stretching) and 1103.81 cm-1 (C-O-C Stretching) indicates the presence of Carvedilol drug, 1732.20 cm-1 (carbonyl) confirms the presence of the Glycerylmonostearate and 945.18 cm-1 (C-C Stretching) indicates the existence of Poloxamer 407 (Table 1).

***Table 1.*** *Formulation of carvedilol sln's (gms) and (oleic acid).*

| Ingredients | ACF1 | ACF2 | ACF3 | ACF4 | ACF5 | ACF6 | BCF1 | BCF2 | BCF3 | BCF4 | BCF5 | BCF6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Carvedilol mg | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Lipid(Oleic acid) mg | - | - | - | - | - | - | 50 | 75 | 100 | 125 | 150 | 200 |
| Surfactant (Tween 80) % | 1 | 1 | 1 | 1 | 1.5 | 2 | 1 | 1 | 1 | 1 | 1.5 | 2 |
| Cosurfactant Poloxamer 407 % | 0.05 | 0.05 | 0.05 | 0.15 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.15 | 0.05 | 0.05 |

The thermo grams were displayed in the results using GMS. Carvedilol drug it showed the onset at 113.33˚C and end set at 123.01˚C and an endothermic peak at 118.22˚C. GMS showed a onset at 54.57˚C and end set at 77.79˚C and an endothermic peak at 69.85˚C. In the lipid matrix, drug incorporation resulted in lipid crystal lattice with increased defects as well as therefore, it results in reduction in lipid's melting point.

GMS has a higher melting point (55-600 C) than Oleic acid (13–140 C) resulting in higher viscosity in addition to presence of long chain hydrocarbons of GMS leads to larger particle compared to oleic acid. There was a decrease in particle size with increasing surfactant concentration.

In the dispersion, degree of repulsion among similarly and close charged particles is indicated by Zeta potential (±). Particle's aggregation is prevented by the repulsion forces. Thus, it is an important parameter for predicting the SLN's dispersions' physical stability. The zeta potential values of the formulations using GMS were found to be in between 12.1 to 20.6 and Oleic acid were found in between 10.4 to 24.3 and it predicts the good particle stability. The repulsive forces prevent aggregation with aging (Table 2).

***Table 2.*** *Results of particle size, zeta potential and poly dispersity index (pdi), in-vitro drug release studies.*

| Formulations | Particle Size (nm) | Zeta Potential (mv) | PolyDispersity Index (PDI | Entrapment efficiency (%) | *In-vitro* drug release (%) |
|---|---|---|---|---|---|
| ACF1 | 1063 | 20.6 | 1 | 50.91 ± 0.25 | 48.48 ± 0.66 |
| ACF2 | 1080 | 12.4 | 0.686 | 62.15 ± 0.16 | 61.94 ± 0.06 |
| ACF3 | 1062 | 12.1 | 1 | 66.30 ± 0.64 | 65.30 ± 0.1 |
| ACF4 | 340.9 | 14.4 | 0.539 | 70.17 ± 0.39 | 69.54 ± 0.06 |
| ACF5 | 653.8 | 11.8 | 0.849 | 74.75 ± 0.78 | 73.27 ± 0.12 |
| ACF6 | 688.2 | 15.9 | 0.811 | 81.10 ± 1.02 | 80.62 ± 0.5 |
| BCF1 | 142.7 | 10.4 | 1 | 55.34 ± 0.17 | 53.92 ± 0.08 |
| BCF2 | 305.7 | 9.71 | 0.355 | 67.54 ± 0.25 | 66.50 ± 0.6 |
| BCF3 | 97.84 | 24.3 | 0.409 | 72.78 ± 0.42 | 71.49 ± 0.01 |
| BCF4 | 29.67 | 18 | 0.66 | 74.74 ± 0.35 | 74.65 ± 0.03 |
| BCF5 | 137.6 | 7.76 | 0.816 | 80.45 ± 0.12 | 79.50 ± 0.04 |
| BCF6 | 97.44 | -19.1 | 0.322 | 88.69 ± 0.65 | 87.73 ± 0.2 |

The SLN'S formulations' EE are given in Table 3 ranges from 50.91 ± 0.25 -81.10 ± 1.02 % for ACF1 to ACF6. The entrapment efficiency increases as the concentration of surfactant increases, Oleic acid for the BCF1 to BCF6 was found to be 55.34 ± 0.17 ± - 88.69 ± 0.65 % The observed high drug Entrapment efficiency % with Oleic acid and might be attributed to long chain fatty acids' high hydrophobicity

associated with triglycerides which leads in enhancing lipophilic drugs accommodation.

The entrapment efficiency of SLN's formulations is shown in Table 2 ranges from 50.91 ± 0.25 -81.10 ± 1.02 % for ACF1 to ACF6. The entrapment efficiency increases as the concentration of surfactant increases, Oleic acid for the BCF1 to BCF6 was found to be 55.34 ± 0.17 ± - 88.69 ± 0.65 % The observed high drug Entrapment efficiency with Oleic acid and might be attributed to the high.

***Table 3.*** *Pharmacokinetic parameters of carvedilol suspension and carvedilol solid lipid nanoparticles F8 Formulation.*

| S.NO. | Pharmacokinetic Parameters | Carvedilol Suspension | Optimized formulation of Carvedilol Solid lipid nanoparticles F8 |
|---|---|---|---|
| 1 | C max (µg/ml) | 28 ± 1.12 | 22 ± 0.23 |
| 2 | t max (h) | 14 ± 0.01 | 24 ± 1.35 |
| 3 | Elimination rate constant | 0.37696 ± 1.32 | 0.213 ± 0.51 |
| 4 | AUC ( 0-24 ) | 67.1 ± 3.66 | 122.6 ± 1.37 |
| 5 | t ½ | 4.83 ± 1.32 | 12.52 ± 0.5 |
| 6 | MRT | 5.32 ± 1.42 | 18.19 ± 0.123 |

The *in-vitro* diffusion profile of the SLN's prepared using GMS and Oleic acid has showed the drug release with an intial burst release followed by sustained drug release at higher concentration levels. Among the ACF1 to ACF6 and BCF4 to BCF6 formulations showed a release of 48.48±0.66 – 80.62 ± 0.66 and 74.65 ± 0.03 – 87.73 ± 0.2 showed more sustained release as it contains the higher concentration ofSurfactant and co-surfactant [14].

Optimized CVD-SLN's formulation and drug in suspension of single dose administered orally the *in vivo* pharmacokinetic evaluations on rabbits were performed. Pharmacokinetic parameters, the time profiles and mean plasma concentration is shown in Table 3. Oral bioavailability is enhanced as well as drug action is prolonged delivered in SLN's. The half life prolonged from 4.83 hrs to 12.52 hrs for SLN's. MRT extended from 5.32 to 18.19 hrs. Prolongation in half life and MRT provide the sustained and extended release and the residence time of SLNS in blood stream. This confirmed the CVD Clearance results decreased for 0.37696 ml/hour to 0.213 ml/hr.

## Conculsion

Based on the outcomes it was concluded that Carvedilol loaded Solid Lipid Nanoparticles as a potential lipid carrier for improving bioavailability. By considering these evaluations, formulation BCF6 (Oleic acid showed a better results and the particle size reduced by increasing concentration of surfactant and co-surfactant when compared with GMS and optimized formulation particles size was in nano range 97.44 nm. And have higher drug entrapment efficiency 88.69 ± 0.65 and drug release at the end of 12 hr was found to be (87.73 ± 0.2) and more controlled drug release. The release exponents showed that the value of 'n' was 0.993, indicating that it fallows zero order kinetics and Super case-II transport Mechanism on drug release behavior. The bioavailability enhanced more than 2 folds when formulated as SLNS for Carvedilol which could be due to low aqueous solubility of Carvedilol which results in the negative effect on bioavailability of a drug in Suspension. Furthermore, they will show the sitespecific drug delivery due to the smaller size of particles and its distribution properties may allow their delivery to target sites.

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