Formulation, Characterization and Pharmacokinetic Studies of Carvedilol Nanoemulsions

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Abstract

The present study involves the formulation and evaluation of oral o/w nanoemulsions (NE) with two simple edible oils, avoiding the large quantities of surfactants and co-surfactants which were prepared by high energy emulsification technique. The particle size, polydispersity index (PDI), and zeta potential of prepared nanoemulsions were determined by using zeta sizer and were found to be in the range of 33.4±3.9 nm, to 183.56±1.78nm, 0.051±0.04 to 0.38±0.06 and -2.87±0.65 to -14.2±0.72 mv respectively. Centrifugation, freeze-thaw cycling, storage at 4°C for 60 days, X-ray diffraction (XRD) and Transmission electron microscopy (TEM) studies revealed the physical and chemical stability of the NEs. Entrapment efficiency and in-vitro release studies showed successful incorporation of carvedilol into NE with high drug loading efficiency and good stability. Comparative pharmacokinetic studies of NE and marketed dosage form in male SD rats revealed a significant increase in oral bioavailability in NEs.

Key words: Nano emulsions, Sesame oil, Olive oil, Carvedilol, Sonication, LC-MS/MS

Introduction

Nano technology has emerged as one of the very promising fields of biomedical research in the last few decades. Nano technology based health care products will expected to reach 2 trillion dollars target by the year 2015(1). Nano emulsion technology is one of the segments of nano technology market and can be defined as thermodynamically stable, transparent or translucent dispersions of oil and water having a size range of 50-200 nm (2).

Different types of oils and components can be used for the preparation of NEs to impart specific properties such as crossing different types of biological barriers like blood brain barrier upon administration (3). NEs can also be used as excellent vehicles in pharmaceutical field for the parenteral, oral or ocular or transdermal delivery of poorly permeable lipophilic drugs. Because of these unique properties, nano emulsions are gaining more importance as potential vehicles for the efficient delivery of lipophilic drug molecules (3, 4, 5).

Cardiovascular diseases including hypertension are one of the major causes of mortality in the world. Delivery of therapeutic agents to cardiac organelles has the potential to increase the efficiency of treatment protocols for heart failure. However, cardiac cells present special problems to the delivery of drugs, and the number of papers reported in this area is also a very few (6). Carvedilol was selected for the present study. It is a non selective beta adrenergic...
blocking agent and widely used in the treatment of mild to moderate hypertension and angina pectoris. It has antioxidant and anti proliferative properties which makes it suitable to combat the deleterious effects of sympathetic nervous system activation in heart failure. It undergoes extensive first pass metabolism and its systemic bioavailability is only 25 to 35% (7). As per Biopharmaceutical classification it belongs to class II compound and therefore it is insoluble in aqueous medium (8). Certain strategies such as fast dissolving tablets (9), muco adhesive drug delivery systems (10), self emulsifying drug delivery systems (11) and other controlled release systems (12, 13) were reported to improve the aqueous solubility, to overcome first pass metabolism and thereby increase the systemic bioavailability. But these technologies require unique production processes and also have certain disadvantages like limited formulation flexibility and complexity in manufacturing, high production costs, low stability, low drug loading and few choices of dosage forms.

The objectives of present study are to investigate the feasibility of development of o/w NEs loaded with carvedilol using sesame oil or olive oil by high energy homogenization technique, to study the effect of oil phase volume on the drug loading efficiency, particle size and zeta potential, to study the effect of concentration and type of surfactants on the globule size, zeta potential and in-vitro drug release characteristics and to carry out the comparative pharmacokinetic study of optimized nano emulsion with marketed tablet dosage-form in rats.

Materials and Methods

Materials: Carvedilol was a gift sample from Orchid Pharmaceuticals, India. Brij-97, Tween-80, dialysis tubing cellulose membrane (size: 43 mm × 27 mm) were purchased from Sigma Aldrich, USA. Sesame oil was purchased from Thiagarajan Agro Products Pvt Ltd., Chennai, India. Olive oil was purchased from Figaro, Sri Roda Foods Pvt Ltd., New Delhi India. All other chemicals, water and solvents are of HPLC grade and purchased from S.D. Fine Chemicals, India.

Preparation of carvedilol nano emulsion:
Various Carvedilol NEs were prepared by using high energy emulsification technique. Prior to emulsification the oil phase and aqueous phase were prepared separately.

A specified quantity of oil was taken in a beaker and 6.3 mg of drug was added and the total weight of the oil phase was determined. To this 200 µl of chloroform was added to dissolve the drug in oil. Then nitrogen gas was purged to evaporate the chloroform. This was confirmed by reweighing oil phase.

Aqueous phase was prepared by dissolving the non-ionic surfactant in HPLC grade water pH- 6.8±0.2. A volume of this surfactant solution was added and a coarse emulsion was prepared by using a high shear stirrer (RQ-127A, Remi Motors, India) for 25 min at 6000 rpm. Then the coarse emulsion was subjected to high energy emulsification using a probe sonicator (Bandelin Sonoplus, Heinrichstrab 3-4 D-12207, Berlin, Germany) in a continuous mode at 37 to 40 HZ.

NEs were prepared using 100 µl of sesame oil and non-ionic surfactants Brij-97 or tween-80. Then various formulation parameters such as oil phase volume (100, 150 and 200 µl) and concentration of each surfactant (1.25, 1.5 and 1.75) were optimized for particle size, zeta potential and drug loading efficiency. The procedure was repeated for the preparation and optimization of carvedilol loaded olive oil nanoemulsions.
Preparation of Carvedilol tablet suspension: The various pharmaco kinetic parameters of carvedilol NE formulation were compared with carvedilol extended release oral tablet (Cardivas, Sun Pharma, India) suspension in order to compare the oral bioavailability of carvedilol by NE formulation with tablet suspension. One tablet containing 10mg of carvedilol was taken and crushed into powder in a mortar and pestle. To this 19.2 ml of 1 % tween-80 solution was added and triturated to prepare a fine suspension with strength of about 0.52mg/ml.

Characterization of Nanoemulsions

Measurement of particle size and zeta potential: The average particle size, poly dispersity index (PDI), and Zeta potential were measured by photon correlation spectroscopy (PCI) using a Malvern zeta sizer (Nano ZS Malvern Instruments Ltd., UK). The PDI represents the uniformity of the globule size and size distribution of the NE. The prepared formulations were diluted with HPLC grade water pH-6.8±0.2. The diluted NEs were kept in the cuvette with an attached dip cell. The cuvette was placed inside the instrument and the observations were recorded at 90° light scattering angle and temperature was maintained at 25 °C. During the measurement, average particle count rate was maintained between 50 to 500kcps. The zeta potential was also measured by using the same instrument with inbuilt software based on the electrophoretic mobility of globules and the Helmholtz-Smoluchowski equation (14, 15, and 16).

Helmholtz-Smoluchowski equation (Zeta potential (Zp) = 6πυηεχ) Where Zp is in volts, υ = migration velocity cm/sec, η = viscosity of the medium in poise, ε = dielectric constant of the external medium, and χ = potential gradient in volts. Average size, poly dispersity index (PDI) and zeta potential were measured for all samples using particle sizer Nano ZS (Malvern Instruments, UK).

HPLC analysis: A simple HPLC method was developed in the laboratory with a Phenomenex P/N0-00G-4274-EO C-18, Luna 5µ, Size column on liquid chromatograph (Shimadzu-10ATVP) and a UV/visible detector (SPD-10ATVP). The mobile phase was Acetonitrile:0.01M phosphate buffer (pH-5.2±0.02) in the ratio of 69:31, at a flow rate of 1ml/min and the effluent was monitored at 242nm.

Solubility studies: Solubility studies of carvedilol were carried out in water, phosphate buffer saline pH 7.4 and Phosphate buffer saline containing 1% tween 80 in order to select the diffusion medium to perform in-vitro release studies. A volume of water or phosphate buffer saline pH 7.4 or Phosphate buffer saline containing 1% tween 80 was taken in to a conical flask. Excess quantity of carvedilol was added and shaken for 2 hrs on a mechanical shaker and kept aside for overnight. Then the solution was centrifuged. The supernatant liquid was taken, filtered, sonicated and appropriate dilutions were made and 20µl quantities were injected into HPLC.

Determination of carvedilol content in the formulations: A volume of formulation was taken from the bulk and made up to 5ml with methanol. From this appropriate dilutions were made with phosphate buffer saline pH 7.4. Then filtered, degassed and 20 µl was injected in to HPLC. The analysis was carried out by the above described HPLC method. The total drug content was calculated from the calibration curve y = 83.106+0.9715(R² = 0.9993).

In vitro drug release studies: The in vitro drug release studies of carvedilol were carried out using dialysis bag diffusion technique (43mm x 27mm size, mol. wt. cutoff 12000 or greater, Sigma-Aldrich, USA). The bag containing 2ml of
NE was placed and immersed in a 50 ml beaker containing 25 ml of phosphate buffer saline pH 7.4 containing 1% tween 80. The entire system was kept at 37°C ± 0.5°C with continuous magnetic stirring. The samples were withdrawn at periodical time intervals (0 min, 15 min, 30 min, 1, 2, 4, 6, 8 and 24 hrs) and replaced with equal volume of fresh medium to maintain sink conditions. The samples were filtered through 0.22 µ membrane filter, degassed in a bath sonicator (Spincotech, India) and injected into HPLC column. HPLC analysis was carried out by the above mentioned method. All experiments were performed in triplicate (17).

**X-ray diffraction:** X-ray diffraction studies were carried out for the formulations that were stable in thermodynamic stability studies (C4, C7, C14 and C17) by using Siemens D-5000 (Germany). XRD studies performed on the samples by exposing them to CuKα radiation (40Kv, 30mA) and scanned from 10° to 80°, 2θ at a speed of 2° per minute.

**Transmission electron microscopy (TEM):** Transmission electron microscopic studies were conducted for the formulation C14. The globule size and morphology were observed with TEM analysis. The samples were placed on Formvar-coated copper grid. Then the samples were negatively stained with 50 µl of 2% phosphotungstic acid for one minute and air dried. Excess liquid was blotted with whatman filter paper. Then the samples were observed under Philips TECNAI – FE12 Transmission Electron Microscope (120 kV) (18).

**In-vivo Pharmacokinetic analysis:** Based on physicochemical properties (Table 2) and *in-vitro* release studies (Fig 2A and 2B) C14 was selected and comparative pharmacokinetic studies were carried out with marketed carvedilol tablet (Cardivas, Sun Pharma, India). The experimental protocol was approved by Institutional Animal Ethics Committee (Vimta Labs, Hyderabad). Study number: VLL/0510/NG/D017. Male Sprague Dawly rats of approximately 6-8 weeks of age weighing between 200 and 240 gms were purchased from, National Institution of Nutrition (NIN), Hyderabad, India. The animals were acclimatized for a period of 5 days. All the rats had free access to reverse osmosis generated potable water and standard animal diet. Throughout the study period, room temperature and relative humidity were maintained at 20°C ± 2°C and 30% to 70% RH respectively. Illumination was controlled to give 12 hrs dark cycles during the 24hrs period.

Overnight fasted rats were used for the study. Prior to the initiation of the study rats were weighed for the body weights. Twelve rats were randomized based on their body weights and distributed equally into 2 groups. One group of rats received carvedilol tablet suspension and another group of rats received carvedilol nanoemulsion. Both the formulations were administered by oral gavage at the dose equivalent to 2.5 mg/Kg of carvedilol. The dose volume administered was 4.8 ml/kg body weight. Following oral administration approximately 0.3 ml of blood samples were collected after anaesthetizing with isoflurane from a group of 3 animals per time point from respective group at respective time intervals that is pre-dose (0), 5 min, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hrs post dosing from retro-orbital plexus in pre-labelled eppendorf tubes containing 20 µl of 10% K2EDTA. The blood samples collected as pre-dose at (0) time were taken as control.

**Analysis of blood samples:** A volume of study sample and calibration curve samples and quality control samples were transferred to the pre-labeled ria vials, add 10 µl of ISTD (4 µg/ml felodipine) and vortex, 2.5 ml of Tertiary Butyl Methyl Ether (TBME) was added to all the samples. These vials were placed on a shaker and
for 10 minutes and centrifuged for 10 minutes at 4000 rpm at 20°C and supernatant was transferred in to pre-labeled ria vial and evaporated under a stream of nitrogen at 35°C until dryness, reconstituted the dried residue with 400µl of mobile phase and vortexed. Samples were loaded in to pre-labeled auto-injector vials and 10 µl of samples were injected onto LC-MS/MS system containing HPLC (AGILENT 1200 series (VLS-UTL/HPLC/01) and Mass spectrophotometer (AB MDS Sciex 4000,VLS-UTL/MASS/01) with a Column of Hypurity Advance, 100 X 4.6mm, 5µ. The column oven temperature was maintained at 40°C and the mobile phase was 0.1% Formic acid: Acetonitrile (25:75 v/v) with a flow rate of 0.6ml/min and an injection volume of 10µl. The separation was conducted under isocratic conditions, and the total run time was within 4minutes. The electron spray ionization was performed in the selected ion monitoring mode. The detection ions were at mass-to-charge ratios m/z of 407.3 amu (parent) to 222.1 amu (product) and 384.1 amu (parent) to 338.1 amu (product) for carvedilol and internal standard felodipine respectively. The chromatograms were evaluated by analyst 1.4.2 version software and the concentration of carvedilol was calculated. Then the pharmaco kinetic parameters were calculated by non-compartmental analysis by winn online (R) 5.2 soft ware.

**Optimization of oil phase volume and surfactant concentration:** Based on literature survey and oil water partition coefficient studies sesame oil and olive oil were selected as sole lipid phase. To determine the optimum content of oil for the preparation of stable and high drug loaded NEs, different volumes of (100,150 and 200 µl) sesame or olive oil with 6.3 mg of carvedilol were taken. Brij 97 and Tween 80 were used as surfactants. In order to determine the optimum surfactant type and concentration NEs were prepared with 1, 1.25, 1.5 and 1.75% w/v of each surfactant. Globule size, ZP and PDI were measured for all the NEs as described above. The composition of all the NEs was given in the Table 1.

**Characterization of carvedilol nano emulsions:** The physical properties such as globule size, PDI and zeta potential are essential parameters in predicting the physical stability of nano emulsions. The mean globule sizes of nano emulsions were in the range of 33.4±3.9 to 183.56±1.78nm and the PDI of nano emulsions were in the range of 0.07±0.08 to 0.35±0.026 which shows a narrow globule size range and size distribution in all formulations. All the carvedilol loaded nano emulsion formulations had zeta potentials between -2.87±0.65 to -14.18±0.72mv. These results were lower than the reported value of above 30 mV in stable parenteral emulsions, which suggests that the prepared formulations were more stable. All the characterization parameters were shown in the Table 2 and the effect of oil phase volume was shown in Fig.1.

**Determination of drug content and In-vitro drug release studies:** Drug content was determined in the formulations which showed narrow particle size and polydispersity and found to be in the range of 43.81±2.68 to 98.93±1.31. *In - vitro* drug release studies were carried out in phosphate buffer saline pH 7.4 (PBS) containing 400µl of mobile phase and vortexed. Samples were loaded in to pre-labeled auto-injector vials and 10 µl of samples were injected onto LC-MS/MS system containing HPLC (AGILENT 1200 series (VLS-UTL/HPLC/01) and Mass spectrophotometer (AB MDS Sciex 4000,VLS-UTL/MASS/01) with a Column of Hypurity Advance, 100 X 4.6mm, 5µ. The column oven temperature was maintained at 40°C and the mobile phase was 0.1% Formic acid: Acetonitrile (25:75 v/v) with a flow rate of 0.6ml/min and an injection volume of 10µl. The separation was conducted under isocratic conditions, and the total run time was within 4minutes. The electron spray ionization was performed in the selected ion monitoring mode. The detection ions were at mass-to-charge ratios m/z of 407.3 amu (parent) to 222.1 amu (product) and 384.1 amu (parent) to 338.1 amu (product) for carvedilol and internal standard felodipine respectively. The chromatograms were evaluated by analyst 1.4.2 version software and the concentration of carvedilol was calculated. Then the pharmaco kinetic parameters were calculated by non-compartmental analysis by winn online (R) 5.2 soft ware.

**Statistical analysis:** The pharmacokinetic parameters of the olive oil nano emulsion and marketed tablet suspension were compared by the student t-test. A p-value of less than 0.05 was considered as statistically significant.

**Results**

Nano emulsions loaded with carvedilol were successfully prepared by using high energy emulsification technique.
1% tween 80 for the formulations (C14, C4, C7 and C17) that showed maximum drug loading and narrow globule size and narrow PDI. The \textit{in-vitro} drug release profiles for the formulations C14, C4, C7 and C17 were shown in Fig-2A and 2B. The cumulative amount released was 63.68±0.8%, 31.8±3.0%, 27.56±9.2%, and 38.04±5.9% in formulations C14, C4, C7 and C17 respectively. Plots of log % drug remaining against time were linear which indicates that the rate of drug release follows first order kinetics.

\textbf{X-ray diffraction studies:} X-ray diffraction studies were carried out to reveal the crystalline modification of the drug during preparation of nano emulsions.

\begin{table}
\centering
\caption{Composition of Carvedilol loaded nano emulsions.}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Code} & \textbf{Volume of oil phase (ml)} & \textbf{Quantity of surfactant (mg)} & \textbf{Volume of aqueous phase (ml)} \\
\hline
& \textbf{Sesame oil} & \textbf{Olive oil} & \textbf{Brij 97} & \textbf{Tween 80} & \\
\hline
C1 & 100 & - & 150 & - & 10 \\
C2 & 150 & - & 150 & - & 10 \\
C3 & 200 & - & 150 & - & 10 \\
C4 & 150 & - & 125 & - & 10 \\
C5 & 150 & - & 175 & - & 10 \\
C6 & 100 & - & - & 150 & 10 \\
C7 & 150 & - & - & 150 & 10 \\
C8 & 200 & - & - & 150 & 10 \\
C9 & 150 & - & - & 125 & 10 \\
C10 & 150 & - & - & 175 & 10 \\
C11 & - & 100 & 150 & - & 10 \\
C12 & - & 150 & 150 & - & 10 \\
C13 & - & 200 & 150 & - & 10 \\
C14 & - & 150 & 125 & - & 10 \\
C15 & - & 150 & 175 & - & 10 \\
C16 & - & 100 & - & 150 & 10 \\
C17 & - & 150 & - & 150 & 10 \\
C18 & - & 200 & - & 150 & 10 \\
C19 & - & 150 & - & 125 & 10 \\
C20 & - & 150 & - & 175 & 10 \\
\hline
\end{tabular}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{Effect of oil phase volume on particle size of nano emulsions \textsuperscript{a} C, S, O, B and T represents the carvedilol, Sesame oil, Olive oil, Brij 97 and Tween 80 respectively.}
\end{figure}

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Table 2. Particle size, zeta potential, polydispersity index and % drug content measurements of optimized carvedilol nano emulsions.

<table>
<thead>
<tr>
<th>Code</th>
<th>Particle size (nm)</th>
<th>Polydispersity (mv)</th>
<th>Zeta potential (mv)</th>
<th>%drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>33.4±3.9</td>
<td>0.248±0.05</td>
<td>-7.45±0.56</td>
<td>50.07±0.8</td>
</tr>
<tr>
<td>C2</td>
<td>75.74±0.92</td>
<td>0.07±0.08</td>
<td>-6.6±0.54</td>
<td>89.27±0.72</td>
</tr>
<tr>
<td>C3</td>
<td>169.63±1.4</td>
<td>0.285±0.04</td>
<td>-6.8±0.27</td>
<td>91.22±0.46</td>
</tr>
<tr>
<td>C4</td>
<td>97.77±0.9</td>
<td>0.116±0.07</td>
<td>-6.45±0.46</td>
<td>91.92±0.9</td>
</tr>
<tr>
<td>C5</td>
<td>67.75±1.58</td>
<td>0.37±0.02</td>
<td>-6.67±0.68</td>
<td>85.89±1.32</td>
</tr>
<tr>
<td>C6</td>
<td>63.09±1.19</td>
<td>0.176±0.02</td>
<td>-2.87±0.65</td>
<td>30.19±0.43</td>
</tr>
<tr>
<td>C7</td>
<td>66.58±1.54</td>
<td>0.188±0.06</td>
<td>-4.09±0.78</td>
<td>87.63±1.14</td>
</tr>
<tr>
<td>C8</td>
<td>175.29±6.9</td>
<td>0.31±0.029</td>
<td>-7.04±0.29</td>
<td>87.94±0.68</td>
</tr>
<tr>
<td>C9</td>
<td>69.45±9.42</td>
<td>0.28±0.063</td>
<td>-6.04±0.42</td>
<td>89.16±0.74</td>
</tr>
<tr>
<td>C10</td>
<td>75.54±1.07</td>
<td>0.05±0.04</td>
<td>-9.04±0.16</td>
<td>87.72±1.7</td>
</tr>
<tr>
<td>C11</td>
<td>34.45±1.167</td>
<td>0.31±0.01</td>
<td>-4.53±0.3</td>
<td>51.1±1.56</td>
</tr>
<tr>
<td>C12</td>
<td>45.49±0.73</td>
<td>0.326±0.04</td>
<td>-6.84±0.41</td>
<td>77.91±1.58</td>
</tr>
<tr>
<td>C13</td>
<td>153.76±2.72</td>
<td>0.33±0.02</td>
<td>-10.37±1.69</td>
<td>88.69±2.04</td>
</tr>
<tr>
<td>C14</td>
<td>54.18±0.37</td>
<td>0.12±0.04</td>
<td>-14.18±0.72</td>
<td>98.93±1.31</td>
</tr>
<tr>
<td>C15</td>
<td>38.81±1.32</td>
<td>0.36±0.03</td>
<td>-13.26±0.34</td>
<td>98.20±0.6</td>
</tr>
<tr>
<td>C16</td>
<td>47.38±1.61</td>
<td>0.26±0.02</td>
<td>-6.35±0.6</td>
<td>43.81±2.68</td>
</tr>
<tr>
<td>C17</td>
<td>75.21±1.8</td>
<td>0.38±0.06</td>
<td>-5.7±0.36</td>
<td>89.47±1.15</td>
</tr>
<tr>
<td>C18</td>
<td>183.56±1.78</td>
<td>0.35±0.026</td>
<td>-7.78±0.44</td>
<td>89.94±1.38</td>
</tr>
<tr>
<td>C19</td>
<td>61.47±4.21</td>
<td>0.31±0.025</td>
<td>-4.49±1.62</td>
<td>87.25±1.69</td>
</tr>
<tr>
<td>C20</td>
<td>60.73±0.54</td>
<td>0.251±0.04</td>
<td>-5.69±1.62</td>
<td>88.47±0.57</td>
</tr>
</tbody>
</table>

Data represents mean ± standard deviation (n=3)

Fig. 2A and 2B. In-vitro drug release profiles

Formulation, characterization and Pharmacokinetic studies
emulsions. The formulations C4, C14 and C17 did not show any kind of crystallinity which suggests that the drug molecules were in amorphous state. But in case of formulation C7 two peaks appeared in right angles. This may be due to the re-crystallization of the drug. The results were shown in Fig.3.

**Transmission electron microscopy:**
Transmission electron microscopic studies were carried out for the formulation C14 to observe the physical properties of NE droplets. Transmission electron micrographs revealed that the droplets were spherical, homogeneous and no signs of precipitation. The droplet size was correlated with the results from particle size analysis using zeta sizer. All these results were presented in Fig.4.

**Pharmacokinetic studies:** The plasma concentration vs time profiles of the carvedilol NE and carvedilol tablet suspension after administration of a single dose of 2.5mg/kg body weight were shown in Fig.5. The pharmacokinetic parameters were computed by non compartmental analysis using winN online software and the results were summarized in the Table 3. The NE was more effective in enhancing oral absorption and availability of carvedilol in the plasma. The average plasma concentrations of carvedilol were 70.07±8.90 and 39.59±5.78 after 1 hour following oral administration of NE and tablet suspension respectively. Plasma concentration of carvedilol when administered with the NE remained higher than with the tablet suspension for up to 6hrs. Significantly higher C$_{\text{max}}$, AUC and AUMC values were observed in case of NE compared to tablet suspension. MRT, T$_{\text{max}}$ and t$_{1/2}$ values of carvedilol with NE were comparable with that of oral tablet suspension. Mean T$_{\text{max}}$ values were 0.5 hours in case of NE and 0.67±0.29 hours in case of tablet. There was no significant difference in t$_{1/2}$. All these results revealed that the extent of oral absorption and bioavailability of carvedilol was significantly increased from oral nano emulsion compared with the tablets.

**Discussion**
Several drugs and dosage forms available for the treatment of hypertension, the mortality
rate is high in the world and over one million people per year suffer adverse reactions from doctor prescribed drugs. Carvedilol is a $\alpha_1$, $\beta_1$ and $\beta_2$ adrenergic receptor antagonist. It is indicated for the management of mild to moderate essential hypertension. In addition carvedilol is proven, with chronic treatment, to reduce cardiovascular mortality and improve survival in patients with systolic dysfunction after myocardial infarction. However, because of its poor aqueous solubility and extensive first pass metabolism many of the current dosage forms do not provide adequate drug concentration in the blood. As such there is a critical need to develop a novel drug delivery system of carvedilol to enhance solubility, permeability and oral bioavailability. Lymphatic delivery is an alternate choice to avoid first pass metabolism in drug delivery and improves bioavailability, because intestinal lymph vessels drain directly into thoracic duct, further in to the venous blood, thus by passing the portal circulation. Main function of lymphatic system is to facilitate absorption of long chain fatty acids via chylomicron formation. Lipid based formulations such as nano emulsion may enhance oral drug absorption by lymphatic transport via transcellular path way, by increasing gastrointestinal membrane permeability or transit time or by modifying the metabolism of drug. In this study the potential of oral carvedilol administration using oil in water nanoemulsion systems was examined.

![Fig. 5. Plasma concentration of carvedilol after oral administration of nano emulsion and oral tablet suspension (n=3).](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nano emulsion</th>
<th>Oral tablet suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>Mean±SEM 90.99±17.06</td>
<td>Mean±SEM 58.57±5.59</td>
</tr>
<tr>
<td>$T_{max}$ (hrs)</td>
<td>0.5±0.00</td>
<td>0.67±0.17</td>
</tr>
<tr>
<td>$K_e1$ (hrs)</td>
<td>0.17±0.02</td>
<td>0.11±0.05</td>
</tr>
<tr>
<td>$T1/2$ (hrs)</td>
<td>4.2±0.54</td>
<td>4.46±0</td>
</tr>
<tr>
<td>$AUC0-t$ (ng*hr/ml)</td>
<td>291.76±18.99</td>
<td>190.13±9.07</td>
</tr>
<tr>
<td>AUMC</td>
<td>907.48±36.50</td>
<td>649.61±76.31</td>
</tr>
<tr>
<td>MRT</td>
<td>3.12±0.08</td>
<td>3.4±0.26</td>
</tr>
</tbody>
</table>

Each value represents mean±SD, n=3

Table 3. Pharmacokinetic parameters after oral administration of carvedilol nano emulsion (Formulation C14) and carvedilol oral tablet suspension

Formulation, characterization and Pharmacokinetic studies
In the present study the influence of oil type, quantity of oil, type and concentration of surfactant on particle size, zeta potential and loading efficiency were studied by using photon correlation spectroscopy, HPLC with UV detector. An essential component of the emulsion is the internal lipid core which constitutes the drug dissolved in oil and is surrounded by a thin layer of non-ionic surfactant. Sesame and olive oils are of vegetarian origin, bio-compatible and being used as edible oils from ancient times (19, 20). Moreover they are easily available and to our knowledge carvedilol loaded nano emulsions were not reported with these oils. Both the oils were stable at room temperature and they do not become rancid like other oils due to the presence of natural antioxidants. All these reasons motivate to select the sesame and olive oils for the present study. They have blood pressure reducing properties therefore their quantity was limited to 200µl (19).

In all the formulations with 100µl of either sesame oil or olive oil the drug was deposited at the bottom of the beaker and the drug content was found to be less than 50%. This indicated that the volume of the oil was not sufficient to hold the entire drug inside. Hence the volume of oil was increased to 150 µl. In this case no drug was seen at the bottom of beaker and the drug content was drastically increased to 98.93±1.31% which revealed that 150µl of oil was sufficient to hold 6.3mg of carvedilol. With increasing oil phase volume from 100µl to 200µl, a significant increase in globule size was observed in both the oils and the results were shown in Fig.1.

Surfactants play a major role in the preparation and stability of NEs. Surfactants form a monomolecular film around the dispersed droplets and there by reduces the interfacial tension and prevent the droplet coalescence. Brij-97 and tween-80 were used as surfactants for the present study. Though they are non-natural surfactants, they do not produce any toxic effects when administered orally. The amount of surfactant is also important to form rigid film around dispersed globules. However use of excess amount of emulsifier can cause decrease in entrapment efficiency, burst release and formation of other colloidal species like liposomes and micelles and may even cause toxic effects (20).

From the table it was evident that with increasing concentration of surfactant the globule size was decreased but the drug content was decreased and the optimum concentration of surfactant was found to be 1.25% in case of brij 97 and 1.5% in case of tween 80.

All the nanoemulsion compositions posses’ negative zeta potential, this may be due to the negative charge of fatty acids. The zeta potential is a measure of the surface charge and is important in keeping the droplets in dispersed state. The particle interactions are also controlled by the magnitude of the apparent surface charge of the dispersed globules. Torrey et al (2006) reported that, the ZP of particle formulations is a value of greater than +30mv or lower than -30mv for ensuring electrostatic stability. However, this suggested zeta potential cut off point is an experience based value and cannot be reliably used to predict the stability of NEs because a wide range of absolute zeta potential values (i.e., 1.5, 12.5, 45.5mv) have been reported for SNEDS and NEs (21,22). The zeta potential depends on the pH of the nanoemulsion. At lower PH values the zeta potential will be positive and at higher pH values it will be negative. The pH of all NEs was in the range of 6.5±0.03 to 6.8±0.03 and was also responsible for negative zeta potentials of NEs.

Carvedilol was a poorly soluble and poorly permeable drug and its Pka at pH 7.4 was 8.8. At this pH the degree of ionization will be almost 100% and it is assumed that the rate of solubility and hence the rate of drug release will be
Regeneration in *Parthenium argentatum*

enhanced. In-order to evaluate this parameter
Phosphate buffer saline pH 7.4 was selected as
the diffusion medium to carry out *in-vitro* drug
release characteristics. The rate of drug release
also depends on the solubility and partitioning
characteristics of the drug in diffusion medium
(23). The solubility studies of carvedilol revealed
that the presence of tween 80 in PBS enhance
its solubility in PBS. Therefore PBS containing
1% tween 80 was used as the diffusion medium.
The *in-vitro* drug release follows first order
kinetics. The rate and cumulative amount of drug
release was highest in formulations C14 which
contains olive oil as the lipid phase. This may be
due to its high drug content, low surfactant
concentration and narrow globule size. Drug
release from the nano emulsions is also related to
the partition coefficient of the drug in oil/ water
system. More specifically for efficient transport
of the drug from the formulation in to the systemic
circulation, the drug must first pass from the lipid
phase to the aqueous phase and then in to the
gastro intestinal tract (GIT) lumen. The oil water
partition coefficient studies of carvedilol revealed
that the logP values of carvedilol in olive oil was
higher than that in the sesame oil (23). This
property of olive oil may cause the more amount
of drug release from the nano emulsions (C14) in
which olive oil was used as the oil phase. In X-
ray diffraction studies formulation C7 showed two
peaks at right angles. This may be due to the re-
crystallization of the drug (13). Transmission
electron micrographs revealed that the dispersed
globules were spherical and show no signs of
coalescence of the droplets and precipitation of
the drug in oil phase or in continuous phase.

The *in-vivo* pharmacokinetic studies
were performed for the formulation C14 which
showed maximum *in-vitro* drug release.
Pharmacokinetic parameters revealed that the
extent of oral absorption and hence oral
bioavailability of carvedilol was enhanced with
NE compared with that of marketed tablet
suspension. AUC is expected as an indicator of
the extent of absorption, where as Cmax and Tmax
are considered as estimates of the absorption rate
(24).This may be explained by the fact that the
presence of omega-6 and omega-3 PUFA (poly
unsaturated fatty acids) in olive oil which are
essential fatty acids and are not produced by the
human body may enhance the rate of oral
absorption (25). The small droplet size, and hence
the large surface area, lymphatic transport through
the transcellular pathway (26) may also contribute
to the increased bio availability.

**Conclusion**

Carvedilol loaded Nano emulsions were
successfully prepared by high energy
emulsification method using sesame oil or olive
oil and non ionic-surfactants Brij 97 and and
tween-80. The resultant Nano emulsions possess
high drug loading efficiency. The *in-vitro* rate of
drug release followed first order kinetics. *In-vivo*
pharmaco kinetic studies revealed that the rate
and extent of absorption of carvedilol was higher
with oral NE compared with that of oral tablet
suspension. Brij 97 was found to be effective non
ionic surfactant for the preparation of stable NEs
and co-surfactants are not required along with
this.

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