Lacidipine Loaded Solid Lipid Nanoparticles for Oral Delivery: Preparation, Characterization and In vivo Evaluation

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ABSTRACT

The objective of this study was to develop and evaluate ladicipine (LD) loaded solid lipid nanoparticles (LD-SLNs) for improving the oral bioavailability. LD-SLNs were prepared in two steps. First step was hot homogenization and next by ultrasonication method, using triglycerides (tripalmitin and tristearin), monoglyceride and surfactants (Poloxamer 188 and egg lecithin E80). The prepared LD-SLNs were characterized for particle size, PDI, zeta potential, drug content, entrapment efficiency (EE %). In vitro drug release studies using a dialysis bag method in 0.1N HCl and pH 6.8 phosphate buffer were conducted. In addition, long-term physical stability of the optimized SLNs was investigated at refrigerated and room temperature for 60 days. FTIR and DSC studies revealed that no interaction between the drug and lipids. LD-SLNs prepared with Dynasan-116 (F3), having the size of 141.86nm, PDI of 0.293, ZP of -22.3 m with 94.75% of EE was optimized and stable for 60days. Scanning electron microscopic studies showed nearly spherical shaped particles. Further, pharmacokinetic studies were conducted in wistar rats. The relative bioavailability of LD in SLNs was 2.03 times when compared with that of the LD suspension. The results are indicative of SLNs as suitable lipid based carrier system for improving the oral bioavailability of LD.

KEYWORDS: Ladicipine; Solid lipid nanoparticles; Oral bioavailability; Triglycerides; Poloxamer; Pharmacokinetics.

Introduction

Aqueous solubility of the drugs is the major factor responsible for their poor oral bioavailability. Lipid-based formulations can reduce the inherent limitations of slow and incomplete dissolution of poorly water soluble drugs, and facilitate the formation of solubilized phases from which absorption may occur. The attainment of an appropriate pre-absorptive solubilized phase will not necessarily arise directly from the administered lipid, but most likely from the intra luminal processing to which lipids are subjected (Andrew and William, 1997).

Solid lipid nanoparticles (SLNs) were considered as alternative carriers to colloidal delivery systems (Mühlen et al., 1998), for controlled systems and targeted delivery. These are in submicron size range (50-1000nm), are made of biocompatible and biodegradable materials, at room temperature the particles remained in solid state (Schwarz et al., 1994), capable of incorporating lipophilic and hydrophilic drugs. SLNs combine the advantages of different colloidal carriers, for instance, like emulsions and liposomes, these are physiologically acceptable and like polymeric nanoparticles, controlled release of drug from lipid matrix can be anticipated (Müller et al., 2000; Mehnert and Mähler, 2001; Mehnert and Mähler, 2012). The mechanism proposed for enhancement of bioavailability of poorly water soluble drugs by use of oral lipids include; promotion of lymphatic transport, which delivers drug directly to the systemic circulation while avoiding hepatic first-pass metabolism and by increasing gastro intestinal membrane permeability (Dahan and Hoffman, 2008; Weijia et al., 2012).

Lacidipine (LD) is a potent vasodilator with antihypertensive activity, whose effects are related to calcium channel inhibiting properties (Calcium channel blocker). It is a member of the 1,4-dihydropyridine class of calcium channel blockers (McCormack and Wagstaff, 2003; Frank et al., 1992). Currently, LD is available in market as tablets and with brand name of Lacipil®. It undergoes extensive hepatic first-pass metabolism resulting in a low bioavailability of 5%. Ladicipine is completely metabolized in the liver by cytochrome P450 3A4 (CYP3A4) to pharmacologically inactive metabolites. To overcome hepatic first-pass metabolism and to enhance oral bioavailability, lipid–based drug delivery systems like solid lipid nanoparticles can be used. These systems enhance the lymphatic transport of the lipophilic drugs and therefore increase the bioavailability. Previously, nanostructured lipid carriers were reported to enhance the oral bioavailability of LD (Anuradha and Senthil, 2014). But, SLN delivery system was not reported.

The main objective of the present investigation was to incorporate LD into triglycerides and monoglyceride to get solid lipid matrices to improve the oral bioavailability by exploiting the intestinal lymphatic transport. Accordingly, LD-SLNs were prepared using hot
homogenization and subsequent ultrasonication steps. Prepared SLNs were characterized and optimal system was evaluated for pharmacokinetic (PK) effect in comparison to a suspension of LD in wistar rats.

**Material and Methods**

**Materials**

Lacidipine was a kind gift sample from Dr. Reddy’s labs, India. Tripalmitin (Dynasan-116), tristearin (Dynasan-118) and glyceryl mono stearate (Imwitor) were purchased from Sigma-Aldrich Chemicals, Hyderabad, India. Egg Lecithin E-80 was a gift sample from Lipoid, Germany. Poloxamer-188 was gift sample from Aurobindo and egg lecithin were dissolved in 5 mL of 1:1 mixture of chloroform and methanol. Organic solvents were completely removed by using a rota evaporator (Heidolph, Germany). The drug embedded lipid layer was molten by heating to 5°C above melting point of the lipid. Aqueous phase was prepared by dissolving Poloxamer 188 in double distilled water and heated to same temperature (based on lipid melting point) of oil phase. Hot aqueous phase was added to the oil phase, and homogenization was carried out (at 12,000 rpm) using homogenizer (Diax900, Heidolph, Germany) for 4 min. The coarse hot oil in water emulsion so obtained was ultrasonicated using a 12T probe sonicator (Vibracell, Sonics, USA) for 20 min. LD loaded SLNs were obtained by allowing the hot nanoemulsion to cool to room temperature. The composition of various formulations was shown in Table 1.

**Methods**

**Preparation of LD- SLNs**

Hot homogenization and ultrasonication steps were used for the preparation of LD loaded SLNs (Müller et al., 2000; Venkateswarlu and Manjunath, 2004). LD, lipid, and egg lecithin were dissolved in 5 mL of 1:1 mixture of chloroform and methanol. Organic solvents were completely removed by using a rota evaporator (Heidolph, Germany). The drug embedded lipid layer was molten by heating to 5°C above melting point of the lipid. Aqueous phase was prepared by dissolving Poloxamer 188 in double distilled water and heated to same temperature (based on lipid melting point) of oil phase. Hot aqueous phase was added to the oil phase, and homogenization was carried out (at 12,000 rpm) using homogenizer (Diax900, Heidolph, Germany) for 4 min. The coarse hot oil in water emulsion so obtained was ultrasonicated using a 12T probe sonicator (Vibracell, Sonics, USA) for 20 min. LD loaded SLNs were obtained by allowing the hot nanoemulsion to cool to room temperature. The composition of various formulations is shown in Table 1.

<table>
<thead>
<tr>
<th><strong>TABLE 1</strong></th>
<th><strong>Composition of lacidipine loaded SLN formulations and a suspension (F7).</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ORGANIC PHASE</strong></td>
<td><strong>Formulation code</strong></td>
</tr>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Lacidipine (mg)</td>
<td>6</td>
</tr>
<tr>
<td>Dynasan-118 (mg)</td>
<td>100</td>
</tr>
<tr>
<td>Dynasan-116 (mg)</td>
<td>-</td>
</tr>
<tr>
<td>Glyceryl monostearate (Imwitor) (mg)</td>
<td>-</td>
</tr>
<tr>
<td>Egg lecithin (E-80) (mg)</td>
<td>100</td>
</tr>
<tr>
<td>Chloroform : Methanol (1:1) (mL)</td>
<td>10</td>
</tr>
</tbody>
</table>

| **AQUEOUS PHASE** | **Formulation code** |
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
| Lacidipine (mg) | - | - | - | - | - | 10 | |
| Sodium carboxy methyl cellulose (mg) | - | - | - | - | - | 50 | |
| Poloxamer-188 (mg) | 150 | 150 | 150 | 150 | 150 | 150 | - |
| Double distilled water (mL) | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

*F7 - suspension of LD

**Characterization of Solid Lipid Nanoparticles**

**Measurement of Particle size, PDI and Zeta potential of SLN**

The size, polydispersity index and zeta potential (ZP) of the SLNs were measured by using a Zetasizer (Nano ZS90, Malvern, UK). From the prepared SLN dispersion, 100 μL was diluted to 5 mL with double distilled water to get optimum kilo counts per second (Kcps) of 50-200 for measurements.

**Determination of entrapment efficiency**

Entrapment efficiency (EE) was determined by measuring the concentration of free drug (unentrapped) in aqueous medium as reported previously (Manjunath and Venkateswarlu, 2005; Suvarna et al., 2015). The aqueous medium was separated by ultra-filtration using centrifisrt tube (Sartorius, Goettingen, Germany), which had a membrane with molecular weight cut off 20,000 Da at the base of the sample recovery chamber. About 2.5 mL of the formulation was placed in the outer chamber and sample recovery chamber was placed on top of the sample and centrifuged. The SLN along with encapsulated drug was retained in the outer chamber and aqueous phase moved into the sample recovery chamber through filter membrane. The amount of LD in the aqueous phase was estimated by HPLC method (Mannur et al., 2012).

**Determination of total drug content**

About 100 μL of the SLN formulation was dissolved in chloroform and methanol mixture (1:1) and then further dilutions were made with mobile phase. The diluted samples were injected onto the column of HPLC and the amount of LD in formulations was calculated by HPLC method.

**In vitro drug release studies**

**In vitro** release studies were performed using dialysis method. Dialysis membrane (Himedia, Mumbai, India) having a pore size of 2.4 nm and molecular weight cut-off between 12,000-14,000 was used for the release studies (Narendar and Kishan, 2016). Dialysis membrane was soaked overnight in double distilled water prior to the release studies. Hydrochloric acid (0.1N) and phosphate buffer pH 6.8 containing 20% v/v ethanol were used as release media. The experimental unit had donor and receptor compartments. Donor compartment consisted of a boiling tube which was cut open at one end and tied with dialysis membrane at the other end into which 1mL of SLN dispersion was taken for release study. Receptor compartment consisted of a 250 mL beaker which was filled with 100 mL of release medium and the temperature was maintained at 37±0.5°C. At 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h time points, 2 mL samples were withdrawn from receiver compartment and replenished with the same volume of release medium. The collected samples were suitably diluted and analyzed by UV-Visible Spectrophotometer (SL-150, ELICO, Hyderabad, India) at 240 nm (De Filippis et al., 2002).

**Stability studies**

LD loaded solid lipid nanoparticles were stored at room and refrigerated temperatures for two months.
The average size, PDI, ZP, assay and entrapment efficiency were determined periodically after 1st day, 15 days, one month and two months (Vinay Kumar et al., 2012).

**Lyophilization of SLNs**

The optimized LD-SLNs containing 10% w/v lactose were prepared and kept in deep freezer at -40°C (Sanyo, Japan) for overnight. The frozen samples were then transferred into freeze-dryer (Lyodel, Delvac Pumps Pvt. Ltd, India). Vacuum was applied and sample was subjected to various drying phases for about 48hrs to get powdered lyophilized product (Cavalli et al., 1997).

**Solid state Characterization**

**Drug-excipients compatibility study by FTIR**

The Fourier Transform Infrared (FTIR) spectra of samples were obtained using FTIR spectrophotometer (BX I, Perkin Elmer, USA) to study the drug-excipient compatibility. Pure drug, individual lipids, physical mixtures (1:1) and lyophilized formulations were subjected to FTIR study. About 2-3 mg of sample was mixed with dried potassium bromide of equal weight and compressed to form a KBr disk. The samples were scanned from 400 to 4000 cm$^{-1}$.

**Drug-excipient compatibility studies by differential scanning calorimetry (DSC)**

DSC analysis of LD, tripalmitin (TP), physical mixture of tripalmitine and drug (PM in 1:1 ratio), and optimized lyophilized LD-SLNs were performed using Mettler-Toledo (DSC 821e, Columbus, OH, USA). The instrument was calibrated with indium. All the samples (~10 mg) were heated in aluminum pans using dry nitrogen as the effluent gas. The analysis was performed within a heating range of 20-200°C and at a rate of 20°C/min (Narendra and Kishan, 2015).

**Morphology by scanning electron microscopy (SEM)**

The morphology of nanoparticles was studied by Scanning Electron Microscope (SEM, Hitachi, Japan). Freeze dried solid lipid nanoparticles of LD were suitably diluted with double distilled water (1 in 100) and a drop of nanoparticle formulation was placed on sample holder and air dried. Then the sample was observed at accelerating voltage of 15000 volts at various magnifications. Imaging was carried out in high vacuum.

**Bioavailability Study**

**Study design and sampling schedule**

A single dose bioavailability study was designed in male wistar rats under fasting conditions. The oral bioavailabilities of the optimized SLN formulation F3 and suspension F7 were estimated by conducting bioavailability studies in male wistar rats with oral dose of 5mg/kg body weight (Pellegratti et al., 1990). All experimental procedures were reviewed and approved by the institutional animal ethical committee, University College of Pharmaceutical Sciences, Kakatiyya University (Warangal, India). Male wistar rats weighing 200-250 g were taken for study (6 animals per group). Blood samples were withdrawn by retro-orbital venous plexus puncture at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24 and 36 h post dose. About 0.5-1 mL of blood samples were withdrawn in eppendorf tubes and centrifuged at 3000 rpm for 30 min. The serum was transferred to another eppendorf tube and stored at −20°C until analysis.

**HPLC analysis**

The HPLC column, Merck C18, (250x4.6 mm), was equilibrated with an eluent mixture of acetonitrile and water with composition of (80:20 v: v) at a flow rate of 1 mL/min. The peaks were detected at 240 nm wavelength without any interferences from serum.

**Extraction procedure**

To 100 μL of serum, 100 μL mobile phase and 100 μL of internal standard (felodipine, 2 μg/mL) was added and vortexed for 2 min. Then 25 μL of 1 M sodium hydroxide solution (1M NaOH) was added and mixed for 3 min. To this, 5 mL of dichloro methane was added and vortexed for 5 min followed by centrifugation at 5000 rpm for 15 min. Then, organic phase was transferred to another glass tube and evaporated to dryness using vacuum evaporator (Toshiba, India). The residue was reconstituted with 100 μL mobile phase and 20 μL of the reconstituted sample was spiked onto the HPLC system for analysis.

**Estimation of pharmacokinetic (PK) parameters and statistical significance**

The PK parameters such as peak serum concentration ($C_{\text{max}}$), time for peak serum concentration ($t_{\text{max}}$), AUC$_{\text{total}}$, biological half-life ($t_{1/2}$) and mean residence time (MRT) were calculated by using the Kinetic software (version 5.0). The values were expressed as mean±SD. The statistical comparison of data of two samples was done with unpaired student t-test using Graph pad prism software (version v.5.0, 2007) and p<0.05 was considered as statistically significant.

**Results and Discussion**

**Measurement of Particle Size, PDI and Zeta Potential of SLN**

Lacidipine loaded SLNs were prepared in two steps. First hot homogenization and next by ultrasonication step, using two triglycerides (F1 & F2 with Dynasan-118 and F3 & F4 with Dynasan-116) and monoglyceride (F5 & F6 with Inwitor). F1 and F2 formulations showed particle size ranging from 242.0 to 314.06 nm, PDI 0.317 to 0.324 and ZP -20.8 to -21.5 mV at 1% and 2% lipid concentration, respectively (Table 2). In all these formulations, the size of nanoparticles was increased with increasing lipid concentration (at constant surfactant concentration) of Dynasan-118 (tristearate), Dynasan-116 (tripalmitate), and Inwitor (glyceryl monostearate) as the concentration of the surfactant may not be sufficient to cover the particles. The obtained polydispersity indices were not always within the acceptable limits (<0.3) for all the SLN.
formulations and suggested that the particles formed were not of uniform size (Mehnert and Mäder, 2001). The ZP of the LD-SLNs was found to be in between -19.23 ± 1.51 mV to -22.3 ± 2.10 mV for all the formulations. In general, the ZP of -20 mV to -30 mV is required for electrostatic stabilization (Thatipamula et al., 2011).

Drug-Excipients Compatibility Study by FTIR

The FTIR spectrum showed characteristic peaks of lacidipine such as NH stretching (3349cm⁻¹), aliphatic CH stretching (2934cm⁻¹), aromatic -C=C stretching (1560cm⁻¹, 1542cm⁻¹, 1498cm⁻¹, 1450cm⁻¹), ester C=O stretching (1676cm⁻¹), aliphatic CH bending (CH₃1368cm⁻¹), disubstituted ortho benzene stretching (767cm⁻¹), CN stretching (988cm⁻¹) (Amit et al., 2012).

The FTIR spectra of LD and Dynasan-116 mixture and formulation displayed all characteristic bands of drug. In the formulation a peak corresponding to NH stretching was observed, but as a broad absorption band, due to the presence of OH group stretching, which occurred at the same wave number range. The OH group was due to the presence of lactose, surfactants used in the formulation. Hence, suggested that there was compatibility between lacidipine & Dynasan 116 (Figure 1). All the selected lipids were compatible with drug based on FTIR studies.

TABLE 2
Physical characters - size, PDI, ZP, assay and EE of LD-SLNs (meansSD, n=3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>ZP (mV)</th>
<th>Assay (%)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>242.00 ± 10.50</td>
<td>0.317 ± 0.009</td>
<td>-20.8 ± 1.24</td>
<td>98.07 ± 1.00</td>
<td>95.86 ± 0.43</td>
</tr>
<tr>
<td>F2</td>
<td>314.06 ± 12.01</td>
<td>0.324 ± 0.004</td>
<td>-21.5 ± 3.34</td>
<td>95.94 ± 1.49</td>
<td>97.11 ± 0.68</td>
</tr>
<tr>
<td>F3</td>
<td>141.86 ± 6.13</td>
<td>0.293 ± 0.058</td>
<td>-22.3 ± 2.10</td>
<td>97.60 ± 1.61</td>
<td>94.75 ± 0.46</td>
</tr>
<tr>
<td>F4</td>
<td>254.30 ± 14.32</td>
<td>0.354 ± 0.045</td>
<td>-20.46 ± 1.17</td>
<td>96.87 ± 1.53</td>
<td>95.81 ± 1.39</td>
</tr>
<tr>
<td>F5</td>
<td>352.26 ± 16.81</td>
<td>0.343 ± 0.028</td>
<td>-20.66 ± 1.75</td>
<td>92.61 ± 1.82</td>
<td>86.69 ± 1.01</td>
</tr>
<tr>
<td>F6</td>
<td>634.80 ± 27.41</td>
<td>0.551 ± 0.072</td>
<td>-19.23 ± 1.51</td>
<td>94.35 ± 2.36</td>
<td>90.57 ± 1.89</td>
</tr>
</tbody>
</table>

Fig. 1. FTIR Spectra of LD-SLN formulation prepared with: 1a) dynasan-118, b) dynasan-116 and 1c) Imwitor.
In vitro Release Studies

The cumulative drug release from formulations F1, F3, F5, and F7 was 28.65%, 34.05%, 25.39%, and 69.15%, and whereas from the formulations F2, F4, and F6 was 24.75%, 29.28%, and 22.23% respectively in a period of 24 h in 0.1NHCl (pH 1.2). Formulation F3 had shown maximum release of 34.05% when compared to other SLNs and lacidipine suspension (F7) showed more % release (69.15%) than SLNs in 0.1NHCl within 24 h (Figure 2). Similarly, in pH 6.8 phosphate buffer, the cumulative % of release from formulations F1, F3, F5, and F7 was 23.37%, 30.54%, 22.21%, and 55.92%, whereas from the formulations F2, F4, and F6 was 21.96%, 26.33%, and 16.51% respectively for 24 h period. Formulation F3 showed maximum release of 30.54% when compared to other SLNs, and lacidipine suspension (F7) showed more % release i.e., 55.92% than SLNs in pH 6.8 phosphate buffer in 24 h (Figure 3). In general, the cumulative % release of lacidipine from all the formulations was more in 0.1NHCl (pH 1.2) than in pH 6.8 phosphate buffer for a period of 24 h. The drug release of LD was decreased with increasing size of nanoparticles. Formulation F3 showed maximum release and possessed lower particle size than F1 and F5. Of all, F3 formulation with Dynasan-116 showed lowest particle size, better EE and highest drug release with better ZP, when compared to other SLNs prepared of Dynasan-118 and Imwitor and was selected as optimized formulation and used for further studies.

Physical Stability Studies

The optimized LD-SLN formulation (F3) was stored at room and refrigerated temperature for 60 days, and average size, zeta potential and PDI were determined periodically (Table 3). It was observed that, there were slight changes in the size, PDI and ZP of the formulation, but, statistically insignificant (p>0.05), which indicated the susceptibility for stability problems during storage at room temperature and 4°C for prolonged periods.

Lyophilization of SLNs

Lyophilized SLN powder was diluted with (1 in 50) double distilled water and measured the size, PDI and ZP. Size, PDI and ZP were found to be 141.86nm, 0.266, -22.3mV and 348.50nm, 0.597, -20.83mV before and after lyophilization, respectively. This might be due to the aggregation of smaller SLN particles resulting in large particles due to overall freeze-drying process. Such type of aggregations were reported during lyophilization (Narendar and Kishan, 2016). In case of drug release, interestingly, there was no significant difference found in the release of lacidipine from F3 formulation (34.05 ± 2.01% in 0.1N HCl and 30.54 ± 3.06% pH 6.8 phosphate buffer) and lyophilized F3 formulation (36.35 ± 1.27% in 0.1N HCl and 27.94 ± 1.05% in pH 6.8 phosphate buffer) during 24 h.

![Graph](image1.png)

Fig. 2. In vitro release profiles of LD from LD-SLNs in 0.1N HCl (mean ± SD, n = 3).

![Graph](image2.png)

Fig. 3. In vitro release profiles of LD from LD-SLNs in pH 6.8 phosphate buffer (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 4°C</td>
<td>At 25°C</td>
<td>At 4°C</td>
</tr>
<tr>
<td>1</td>
<td>149.06 ± 9.31</td>
<td>149.06 ± 9.31</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>30</td>
<td>156.83 ± 3.56</td>
<td>162.36 ± 5.08</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>60</td>
<td>165.86 ± 7.60</td>
<td>171.90 ± 4.55</td>
<td>0.27 ± 0.05</td>
</tr>
</tbody>
</table>

TABLE 3

Physical Stability studies of the optimized formulation (F3) at room temperature (25°C) and refrigerated temperature (4°C) for a period of 2 months mean ± SD, n=3.
Solid State Characterization – Differential Scanning Calorimetry

The purity of drug and the status of lipids in the SLN formulation were determined by DSC study. DSC studies of drug, pure lipid, physical mixture of drug and lipid (1:1 ratio) and optimized lyophilized formulation (F3) are shown in Figure 4. The DSC analysis of lacidipine, exhibited a sharp endothermic peak at 180.8°C (Amit et al., 2012). The peak was observed at its reported melting point (180-185°C) which indicated the purity of drug. The DSC thermogram of Dynasan-116 showed a sharp endothermic peak at 70.17°C. In case of physical mixture, very broader drug endothermic peak at 179.43°C (slight shift in melting point) and lipid peak at 68.98°C were noticed. The relevant drug peak was not visible in the lyophilized formulation (F3), which might be due to insufficient amount of drug in the lyophilized product. Alternatively, the drug might be uniformly dispersed at molecular level in the lipid matrix loosing the complete crystal structure (Shamsunder et al., 2013).

![Fig. 4. DSC thermograms of A) drug, B) dynasan-116, C) physical mixture of drug and dynasan-116 and D) lyophilized (F3) LD-SLN formulation.](image)

Morphology study by Scanning Electron Microscope (SEM)

The size and morphology of nanoparticles were studied by Scanning Electron Microscope (SEM, Hitachi, Japan). The SEM pictures of F3 formulation and lyophilized F3 formulation are showed in Figure 5a and 5b and the nanoparticles were spherical in shape. SEM picture of F3 formulation showed that nanoparticles had size range of 51-234nm and with an average nanoparticle size of 123.93nm. The SEM image of lyophilized F3 formulation showed that nanoparticles were aggregated and had size range of 115-403 nm with an average size of 245.70 nm.

![Fig. 5. SEM images of LD SLN (F3) formulation before (a) and after lyophilization (b).](image)

In vivo Bioavailability Study

Male wistar rats were used to study the in vivo oral bioavailability of optimized LD-SLN (F3) formulation in comparison to LD suspension (F7). In Figure 6 the serum concentration vs time profiles of suspension and SLN formulation (F3) are shown. Different PK parameters obtained for both suspension and SLN preparation (F3), are given in Table 4. The C_{max} and AUC_{(0-24)} of SLN formulation (F3) (0.596 ± 0.0350 μg/mL and 8.353 ± 0.465 μg/mL h) were higher than that of suspension (0.237 ± 0.028 μg/mL and 4.116 ± 0.370 μg/mL h) and there was statistically significant difference between (P<0.0001) the C_{max} and AUC_{(0-24)} of SLN formulation (F3) and suspension. Although the mean t_{1/2}, MRT values of SLN formulation (F3) (12.73 ± 1.14 h, 18.23 ± 1.22 h) were higher than that of suspension (F7) (11.65 ± 1.29 h, 17.58 ± 1.54 h), there is insignificant (p>0.05) differences in the mean t_{1/2}, MRT values for SLN formulation (F3) and suspension (F7) by statistical analysis. From above results, it was found that C_{max} and AUC_{(0-24)} t_{1/2}, MRT for suspension (F7) was lower than that of the optimized formulation F3.

![Fig. 6. Mean serum concentration vs time profiles of LD-SLN and suspension formulation after oral administration in rats (mean ± SD, n = 6).](image)
TABLE 4
Pharmacokinetic parameters of lacidipine in rats from SLN formulation (F3) and suspension (F7) (mean±SD, n=6).

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>F3</th>
<th>F7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>0.596 ± 0.0350</td>
<td>0.237 ± 0.028</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>2.00 ± 0.00</td>
<td>2.00 ± 0.00</td>
</tr>
<tr>
<td>AUC0-t (µg/mL·h)</td>
<td>8.35 ± 0.46**</td>
<td>4.11 ± 0.37</td>
</tr>
<tr>
<td>AUC∞ (µg/mL)</td>
<td>12.73 ± 1.14</td>
<td>11.65 ± 1.29</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>15.23 ± 1.22</td>
<td>17.58 ± 1.54</td>
</tr>
<tr>
<td>F (%)</td>
<td>203.90</td>
<td>100</td>
</tr>
</tbody>
</table>

** indicates statistical significance at p<0.0001 with lacidipine suspension.

From above studies, lacidipine serum concentration (Cmax) after administration of SLN formulation (F3) was more than 2.5 times that of lacidipine suspension. The relative bioavailability of the SLN formulation F3 was found to be increased 2.03 times than that of suspension F7. This might be due to lymphatic transport of drug from SLN formulations, through bypassing the hepatoclipid metabolism (Dahan and Hoffman, 2008).

Conclusions
Lacidipine is a poorly water soluble drug with oral bioavailability of 5% due to extensive first-pass metabolism. SLNs were prepared by two step method, containing hot homogenization step followed by ultra sonic method. All the six SLNs were prepared with three lipids, each at two different concentrations. Characterization of SLNs was done by measuring particle size, poly dispersity index and ZP by zeta sizer. By formulating the lacidipine as SLNs, an increase in oral bioavailability by 2.03 times than a control suspension was observed. This could be due to overcoming the effect of first-pass metabolism by following lymphatic transport pathway.

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Disclosure
Authors declare no conflict of interest in this study.

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