Exploring the solvent–antisolvent method of nanosuspension for enhanced oral bioavailability of Lovastatin

KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi-590010, Karnataka, India.

ABSTRACT:
Objectives: Lovastatin is an anti-lipidemic drug which belongs to class of statins. It has poor oral bioavailability due to its low solubility and variable dissolution rate. The main aim of this study was to enhance the solubility and dissolution rate of the drug and thereby its oral bioavailability.

Materials and Methods: Lovastatin nanosuspension was formulated using solvent/anti-solvent method using probe sonication technique. Nanosuspension was prepared by using HPMC K15M and Pluronic F68 as stabilizers. The formulated nanosuspensions were characterized for particle size, PDI, zeta potential, surface morphology and in vitro release rate. Further, in vivo bioavailability study and stability studies were also performed.

Results: Optimized formulation showed particle size of 127± 0.01nm and PDI of 0.492±0.001 and zeta potential of -37.9mV which indicates good stability. Morphological study showed that the particles were in nano range. The drug content was found to be in the range of 73% - 87%. In vitro release revealed much faster release of drug in one hour compared to the pure drug and marketed formulation. In vivo bioavailability study was carried out in wistar rats which showed improvement in bioavailability of approximately 2.5 folds compared to marketed formulation. Stability studies indicated that optimized formulation F2 was more stable at 4°C±2°C.

Conclusion: The prepared Lovastatin nanosuspension showed improvement in solubility, dissolution rate and oral bioavailability compared to pure drug and marketed formulation. Hence, Lovastatin nanosuspension could be a potential valuable tool for improving the oral bioavailability of Lovastatin.

Keywords: Lovastatin, oral bioavailability, Solubility, Nanosuspension.

INTRODUCTION:
For administration of drug, oral route is the highly preferred route as it provides high patient compliance.1 Large number of drugs which are available in the market exhibit low oral
bioavailability because of their low aqueous solubility and intrinsic dissolution rate. According to Biopharmaceutical Classification System (BCS), drugs with poor aqueous solubility are classified either as class II or class IV drugs. Poor aqueous solubility of drugs in turn results in low oral bioavailability, varying absorption rate, and inter and intra-subject proportionality. Oral bioavailability of various drugs is also affected by another factor i.e., poor gastrointestinal permeability. According to literature, Various techniques like solubilization, salt formation, micronization, change in physical form, use of prodrug and drug derivatives, addition of surfactants, pH alteration have been utilized for improving the dissolution and bioavailability of drugs having poor aqueous solubility. Nanotechnology has reshaped the field of drug delivery and research. Pharmaceutical nanoparticles are defined as solid sub-micron sized (less than 100 nm in diameter) drug carriers which may or may not be biodegradable. Types of nanoparticles which are applied in drug delivery are nanosuspensions, polymeric nanoparticles, lipid nanoparticles etc. Nanosuspension is the colloidal dispersion of solid drug particles in a liquid phase having particle size below 1 µm with an average particle size of 200 to 600 nm. It consists of pure drug and stabilizers (surfactants or polymers). Their small particle size facilitates effective transportation of drug molecules to cells, with optimum therapeutic effect and reduced adverse effects. The potential benefits of nanosuspension technology for poorly soluble drug delivery are; increased drug dissolution rate, increased rate and extent of absorption, hence the bioavailability of drugs. The selection of suitable stabilizer or surfactant as well as the manufacturing method can offer nanosuspension with highest stability for long-term storage. Nanosuspension can be formulated by bottom-up or top-down approach.

Cardiovascular disease remains the leading cause of morbidity and mortality worldwide and hyperlipidemia is a major factor contributing to its development. Lovastatin, a cholesterol lowering agent that has been isolated from a strain of *Aspergillus ferrus*. It is a very effective and well tolerated by patients with moderate hypercholesterolemia. Lovastatin also manifests pharmacological activities of bone formation and chemoprevention. Due to its rapid metabolism in the gut and liver, Lovastatin exhibits poor oral bioavailability of <5% and shorter half-life of 2-5 hr. According to literature, various attempts have been made to improve the aqueous solubility and bioavailability of Lovastatin by preparing self-emulsifying drug delivery systems, nanostructured lipid carriers, extended release formulation by one step melt granulation method etc. for instance, Ruby Singh et al., Jun Zhou et al., and Gande Suresh et al. prepared stabilized self-emulsifying drug delivery systems in the form of hydrogel, nanostructured lipid carriers and solid-lipid nanoparticles of Lovastatin respectively, with an objective to enhance solubility and bioavailability of Lovastatin but there is no comparative data of their formulation with the already existing marketed product to justify the enhancement in bioavailability of the drug. Hence, in present study, attempts were made to improve solubility and oral bioavailability of Lovastatin by formulating Lovastatin nanosuspension via solvent-anti-solvent method using probe sonication technique. Further, nanosuspensions were evaluated for particle size, PDI, zeta potential, drug content, *In-vitro* release study, *In-vivo* bioavailability study and stability study. MATERIALS AND METHODS:

**Materials:**

Lovastatin was procured as a gift sample from Lupin Pharmaceuticals, Goa. HPMC K15M was purchased from Yarrow Chem Products, Mumbai. Pluronic F68 was purchased from Ozone Pharmaceuticals, Mumbai. Acetone, Chloroform, Methanol, Ethanol were purchased from
Molychem Mumbai. Dialysis membrane having cut-off molecular weight between 12,000 to 14,000 was purchased from HiMedia.

Methods:
Optimization of Process Parameters:
Selection of Suitable Solvent/Antisolvent Ratio:
Prior to proceeding towards formulation of lovastatin nanosuspension, solvent and antisolvent were selected based on solubility studies of drug in different solvents. In solvent/antisolvent method, the selected solvent should be water miscible and capable enough to dissolve the drug to a greater extent so that a clear solution is obtained. On the other hand, the solvent in which the drug is least soluble or is completely insoluble is selected as an antisolvent. As drug exhibited maximum solubility in methanol, it was selected as solvent and water as antisolvent since drug was least soluble in water. Different ratios like 1:1, 1:2, 1:3, 1:4, and 1:5 were tried for formulating nanosuspension. The ratio of solvent: antisolvent which resulted in nanosuspension with best and reproducible particle size and PDI was selected as an optimized ratio.

Optimization of Sonication Time:
Optimization of sonication time was done by sonicating the formulation for 2, 5, 10, 15 and 20 mins. Based on best and reproducible results of particle size and PDI, optimized sonication time was selected.

Selection of Polymer and Surfactant:
Suitable polymer was selected by screening various polymers like HPMC K15M, HPMC K100M and PVPK30. Based on best and reproducible results of particle size, PDI and ability to inhibit the crystal growth, suitable polymer was selected. Various surfactants like Pluronic F68, Pluronic F127, and PEG 6000 were tested to determine the effective surfactant in reducing the particle size of the drug.

Effect of Flow Rate:
Particle size of obtained nanosuspension was measured at varied flow rate of drug solution in to polymer solution at 2-8 ml/min to select the optimum flow rate during formulation of nanosuspension.

Formulation of Nanosuspension by Solvent-Anti Solvent Technique:
Six formulations of (F1-F6) Lovastatin nanosuspensions were prepared by Solvent / Anti-solvent method using probe sonication technique. Briefly, specified amount of drug was completely dissolved in water-miscible solvent (methanol). In another beaker polymer /surfactant was added to the water (antisolvent) and further sonicated till a clear polymeric solution was formed. The prepared drug solution was then added to the polymeric solution at a rate of 2 ml/min maintained in an ice-bath to prevent particle collision till the precipitation occurs. The quantities of ingredients used in the formulation are mentioned in Table 2 and the Solvent/Anti-solvent technique is depicted in Figure 1.

Evaluation of Lovastatin Loaded Nano-suspensions:
Particle Size Analysis:
Particle size of the prepared nanosuspension was determined by using Dynamic Light Scattering (DLS) particle size analyzer. For analysis, the nanosuspension was diluted with millipore water in the ratio of 1:5 and further sonicated for 2 mins. Samples were analyzed in triplicate.

Polydispersity Index:
Polydispersity index is also measured using Dynamic light scattering (DLS) particle size analyzer. The obtained PDI values gives an idea about the particle size distribution of
nanoparticles. Its value ranges from 0.000-1.000 which demonstrates that lower the value, narrower will be the size distribution of nanoparticles and vice-versa.\textsuperscript{18}

**Drug Content:**
Lovastatin nanosuspension equivalent to 1 mg was added to 10 ml of methanol, further diluted with phosphate buffer up to 100 ml and stirred continuously for 2 h. It was subjected to ultracentrifugation at 15,000 rpm for 20 min. Supernatant was collected, suitably diluted and analyzed spectrophotometrically at 238 nm.\textsuperscript{19}

**Zeta potential:**
A physical property exhibited by particles in suspension is called zeta potential. It is used for the optimization of emulsions and suspensions as its knowledge reduce the number of trial formulations. It is also an important parameter to predict the long-term stability.\textsuperscript{20}

**Transmission Electron Microscopy (TEM):**
The external morphology of Lovastatin nanosuspension was studied by using TEM and it was performed at SAIF, Cochin. Samples for TEM were prepared by initially diluting with millipore water, then a drop of nanosuspension was kept on copper grid (carbon coated) and stained with phosphotungstic acid. The grid was air dried and observed at different magnification under TEM.\textsuperscript{21}

**In-vitro Drug Release Study:**
The in-vitro drug release of Lovastatin was performed by using the dialysis bag diffusion technique. An accurately weighed quantity of nanosuspension (equivalent to 10 mg of drug) was placed in a dialysis bag and the bag was sealed. Then, the bag is suspended initially for 1 h in a basket containing 900 ml of pH 6.8 phosphate buffer. Aliquots of 5 ml of the sample were withdrawn at pre-determined intervals from the compartment and the same amount was replaced by fresh buffer. The sample was analyzed spectrophotometrically after suitable dilution by determining the absorbance at 238 nm.\textsuperscript{22}

**In-vivo Bioavailability Study:**
Healthy Wistar rats weighing 180-200 g were housed in polypropylene cages and maintained at room temperature for 12 hr dark/light cycles. They were fed with standard pelleted diet and water. The animals were acclimatized for one week under laboratory conditions before experiments on the animals. Ethical clearance was obtained from the Institutional Animal Ethics Committee (Resolution No: KLE/ECOP/ CPCSEA/ Re.no.221/ PO/ RE/S/2000/CPCSEA Res.27-24/12/2018) prior to the beginning of research work. In-vivo study was aimed mainly to estimate the amount of drug in the blood withdrawn from rats at various time intervals.\textsuperscript{23}

Twelve healthy male wistar rats weighing 180-200 were selected and divided into 2 groups each containing 6 rats. Group 1 received marketed product of Lovastatin and group 2 received Lovastatin nanosuspension equivalent to 0.108mg in normal saline through oral route. After 0.5, 2, 4, 6, 8, 10 and 24 h, 0.5-1 ml blood was collected from tail-vein into eppendorf tube containing 10 μl of EDTA and centrifuged at 5000 rpm for 20 min. Supernatant plasma was collected, filtered through 0.45 μm membrane into clean vials and analyzed spectrophotometrically to determine the concentration.

**Short Term Stability Studies:**
The optimized formulation was subjected to stability studies as per ICH guidelines. The formulation was exposed to varying conditions of temperature and relative humidity i.e. 25°C/ 65% RH and 4°C / 65 % RH for a period of three months in Humidity Control Oven. The samples were collected and evaluated for particle size and percent cumulative drug release at the interval of zero, one month, two month and third month respectively.
RESULTS AND DISCUSSION:

Optimization of Process Parameters:
For the drug Lovastatin, maximum solubility was observed in methanol and least solubility was observed in water. Hence, methanol was selected as solvent and water as anti-solvent. Based on effect of solvent/antisolvent volume ratio on particle size, 1:4 ratio was considered optimum as the particle size was obtained in nano range. Based on best and reproducible results, the optimized sonication time was found to be 20 mins which produced particles in nano range. Polymer screening was performed using various polymers like HPMC K15M, HPMC K100M and PVPK 30. Particle size was measured immediately after precipitation. HPMC K15M was found to be more effective in inhibiting the crystal growth as compared to particles prepared without the use of polymer. Hence, HPMC K15M was selected as the stabilizer for the experiment. Pluronic F68 surfactant was found to be more potential as the obtained particle size of nanosuspension was observed in nano range.

It was observed that increase in flow rate (2 to 8ml/min) increases the particle size from 487nm to 1023nm because of large crystal formation. Hence, flow rate of 2ml/min was found to be optimum to get the particle size in nano range. The process optimization parameters and their evaluation results are depicted in Table 1.

Formulation of Nano-suspension by Solvent/ Anti-Solvent Technique:
Lovastatin nanosuspension was successfully prepared by Solvent/ Anti-solvent method using probe sonication technique. A total of six formulations were prepared using HPMC K15M and Pluronic F68 as stabilizers. In the technique, addition of drug solution to the anti-solvent leads to higher supersaturation. This produces large number of nuclei as a result of high nucleation rate, which in turn reduces the mass of solute for subsequent growth. Sub-micron particles are thus produced provided that the nucleating crystals growth can be arrested by the stabilizer via stearic or electrostatic mechanism. Lovastatin being a hydrophobic drug, most generally used anti-solvent is water. With respect to solvent, it is more potential if it solubilizes higher amount of drug and possesses a greater diffusion rate to the anti-solvent, while the stabilizer should possess good affinity towards drug particles and result in fast diffusion rate as well as sufficient adsorption onto the surface of drug particles in the solvent-water mixture. Hence, pair of solvent-stabilizer is crucial to achieve sub-micron particles.

Evaluation of Lovastatin Loaded Nanosuspension:
Particle Size Analysis:
Particle size of the prepared formulation was determined to confirm the production of particles in nano range. All the formulations were found to be in the range of 127-401 nm (Table 3). It was observed that particle size was decreased from 284 nm to 127 nm as the HPMC concentration increased from 1.0 mg/ml to 1.5 mg/ml. This is attributed to the good affinity of hydrophobic portion of HPMC for drug particles, which leads to effective stearic barrier against growth. Similarly, when the concentration of Pluronic F68 increased from 1.0 mg/ml to 1.5 mg/ml, particle size decreased from 401nm to 239 nm. This result may be due to high affinity of Lovastatin particles towards this stabilizer which provided an effective stearic barrier against crystal growth. In-addition, particle size of formulations containing combination of both HPMC K15M and Pluronic F68 (F5 and F6) were in nano range.

Polydispersity Index:
PDI determines particle size distribution which ranges from 0 to 1. Sample is said to be monodisperse when the PDI value is close to zero. When PDI value <0.2, it is regarded as narrow size distribution. PDI of all the formulations are shown in Table 3. However, when PDI
value >0.2, it is considered as polydisperse distribution. Amongst the prepared nanosuspension formulation, F1 and F3 showed monodisperse size distribution with PDI values 0.28 and 0.048 respectively. Whereas, F2, F4, F5 and F6 was found to exhibit polydisperse size distribution with PDI values 0.492, 0.81, 0.61 and 0.30 respectively.

**Zeta Potential:**
The zeta potential was analyzed to determine the stability of the optimized formulation. Zeta potential of the optimized formulation was found to be -37.9 mV which indicates good stability.

**Transmission Electron Spectroscopy:**
TEM images of the optimized formulation F2 revealed that the particles were spherical in shape, evenly distributed and were found to be in the range of 64.62 -157.82 nm which is close to the value obtained from particle size analyzer (Nanotrac). TEM images are shown in Figure 2.

**Drug Content:**
Drug content was calculated for the formulations F1-F6 and it was found to be in the range of 73% - 86.29%. Drug content was found to be maximum for formulation F2 and least for formulation F3.

**In-vitro Drug Release Study:**
*In-vitro* drug release studies performed in pH 6.8 phosphate buffer for 1 hr to check the release of drug and is depicted in Figure 3. Results indicated that the formulation F2 with least particle size showed maximum release rate. On the basis of drug content, particle size and drug release profile, formulation F2 was selected as an optimized formulation. The release profile of optimized formulation was then compared with the release profile of pure drug and marketed formulation as shown in Figure 4. The percent cumulative drug release obtained at the end of 1hr for optimized, marketed and pure drug formulations were 92.83 %, 60.47 % and 39.73 % respectively. This is because smaller the particle size, larger will be the surface area. Hence, the drug which is at or near the surface can be easily released.

**In-vivo Bioavailability Study:**
The study was performed in Wistar rats to compare plasma concentration of optimized formulation F2 with that of marketed product given orally in normal saline. The average concentration was obtained at regular intervals for both the formulations. Comparative graph of plasma concentration v/s time of optimized formulation F2 and marketed product was plotted as shown in Figure 5. Graph revealed that, the formulation F2 showed greater bioavailability than that of marketed product. AUC of 29.34 μg h/ml, C_max of 4.9 μg/ml and T_max of 4 h was observed for marketed product when given orally. Whereas, optimized formulation showed AUC of 63.05μg h/ml, C_max of 6.5 μg/ml and T_max of 1 h, which was calculated by Trapezoidal method and are shown in Table 4. Thus, Lovastatin nanosuspension was able to improve bioavailability by approximately 2.5 folds when compared to marketed product.

Though we discussed the previous studies conducted on Lovastatin in introduction section, we will discuss few more studies conducted with respect to bioavailability enhancement of Lovastatin. For instance, Roshan KP et al., performed comparative *in-vivo* pharmacokinetic studies among Solid lipid nanoparticle (SLN) carrier and Nanostructured lipid carrier (NLC) and concludes that the Lovastatin encapsulated Nanostructured lipid carrier presented an increased bioavailability (10.56%) than SLN (7.5%).24 Keerthi P et al., developed self nanoemulsifying drug delivery system (SNEDDS) of Lovastatin to increase the solubility and bioavailability. This study concluded that, optimized formulation of SNEDDS significantly improved the oral bioavailability of Lovastatin as compared to pure drug (no comparison with marketed product).24 Recently, Gaber DA prepared and optimized nanoparticles by
ultrasonication-assisted precipitation method. In vivo studies confirmed that there was 1.45-fold enhancement in Cmax of Lovastatin nanoparticles as compared to marketed tablets. These studies have also led to enhanced bioavailability of drug but not to the extent of 2.5-fold increment compared marketed product.\textsuperscript{25} Thus, developed nanosuspension by solvent-antisolvent method could be a potential technique to produce submicron particles of poorly-water soluble drugs thereby enhancing the oral bioavailability.

**Short Term Stability Studies:**
Stability studies were conducted for optimized formulation F2 as per ICH guidelines for a period of 3 months. Physical appearance of F2 changed slightly when samples were stored at room temperature 25±2°C/ RH 65±5% for 3 months. A sediment of thin layer was observed. However, it disappeared immediately with slight shaking. No change in the physical appearance was observed when nanoparticles were stored in refrigerator at 4±2°C/RH 65±5% for 3 months. By comparing the stability study data with initial data, it was observed that there was not much change in particle size and in vitro drug release for the formulation stored at 4±2°C/RH 65±5% to that stored at room temperature. Thus, formulation stored at 4±2°C/ RH 65±5% showed better stability as compared to the formulation stored at 25±2°C/ RH 65±5%.

**CONCLUSION:**
In present study, solvent-antisolvent method was employed to formulate nanosuspension of Lovastatin, for enhancing the solubility, dissolution rate and thereby its oral bioavailability. Lovastatin nanosuspension was formulated using methanol as solvent, water as an anti-solvent and HPMC K15M and Pluronic F68 as stabilizers. FT-IR and DSC study showed no interaction between the drug and the excipients which were used in the formulation (results not shown). On the basis of drug content, particle size and drug release profile, formulation F2 was selected as optimized formulation. TEM images suggest that the particle size of all the formulations were in nano range. Zeta potential of optimized formulation F2 was found to be -37.9 mV which showed good stability. The dissolution rate depends upon the particle size. The smaller the particle size, faster will be the dissolution. Formulation F2 showed maximum dissolution rate of 92.83% in 1 hr as compared to pure drug and marketed formulation which showed 39.73% and 60.47% dissolution respectively, in the same time period. Pharmacokinetic analysis of in vivo bioavailability data indicated 2.5 fold increase in comparison to marketed formulation. Stability studies conducted according to ICH guidelines for optimized formulation F2 and it was more stable at 4±2°C. Thus, it can be concluded that, solvent-antisolvent method is a simple and potential technique to produce submicron particles of poorly-water soluble drugs thereby enhancing the oral bioavailability for commercial production.

**ACKNOWLEDGEMENT:**
The authors would sincerely like to thank Lupin Pharmaceuticals, Goa for providing a gift sample of lovastatin. The authors are also thankful to the principal KLE College of Pharmacy, Belagavi and Dr. Prabhakar Kore Basic Science Research Centre, Belagavi for providing laboratory facilities. The authors are thankful to Kesar Control System, AICTE-MODROB 2017-2018 for providing stability chamber to carry out the stability studies in research work. Authors are also thankful AICTE-MODROBE for providing Ultrasonic probe sonicator VC750.

**Conflict of interests:** The authors declare that they have no any competing interests.

**References:**

**Optimization of Solvent/Anti-Solvent Ratio and Sonication Time**

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<tr>
<th>Solvent (Methanol): Antisolvent (Water) Ratio</th>
<th>1:1</th>
<th>1:2</th>
<th>1:3</th>
<th>1:4</th>
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<tr>
<td>Sonication time (mins)</td>
<td>05</td>
<td>10</td>
<td>15</td>
<td>20</td>
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<tr>
<td>Particle size (nm)</td>
<td>1595</td>
<td>623</td>
<td>407</td>
<td>379</td>
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<tr>
<td>PDI</td>
<td>1.6</td>
<td>1.5</td>
<td>0.04</td>
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**Effect of Polymer on Particle Size and PDI**

<table>
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<tr>
<th>Polymer</th>
<th>Particle size (nm)</th>
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<tbody>
<tr>
<td>HPMC K15M</td>
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<td>HPMC K100M</td>
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<td>PVP k30</td>
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**Effect of Surfactant on Particle Size and PDI**

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<th>Surfactant</th>
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<tr>
<td>PEG 6000</td>
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<tr>
<td>Pluronic F127</td>
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<td>Pluronic F68</td>
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**Effect of Flow Rate on Particle Size and PDI**

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<tr>
<td>2ml/min</td>
<td>487</td>
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<td>4ml/min</td>
<td>604</td>
<td>0.5</td>
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<tr>
<td>6ml/min</td>
<td>798</td>
<td>0.5</td>
</tr>
<tr>
<td>8ml/min</td>
<td>1023</td>
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Table 1. Optimization of Process Parameters and Their Evaluation
Table 2: Formulation Design of Lovastatin Nanosuspension Using Solvent/Anti-Solvent Method

<table>
<thead>
<tr>
<th>Formulation number</th>
<th>Lovastatin (mg)</th>
<th>HPMC K15M (mg/ml)</th>
<th>Pluronic F68 (mg/ml)</th>
<th>Methanol (ml)</th>
<th>Water (ml)</th>
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<tbody>
<tr>
<td>F1</td>
<td>10</td>
<td>1.0</td>
<td>--</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>F2</td>
<td>10</td>
<td>1.5</td>
<td>--</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>F3</td>
<td>10</td>
<td>--</td>
<td>1.0</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>F4</td>
<td>10</td>
<td>--</td>
<td>1.5</td>
<td>10</td>
<td>50</td>
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<tr>
<td>F5</td>
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<td>1.5</td>
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<td>F6</td>
<td>10</td>
<td>1.5</td>
<td>1.0</td>
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Table 3: Particle Size, Polydispersity index, Percent Drug Content and Percent CDR of The Nanosuspension Formulations F1-F6
Table 4: Pharmacokinetic Parameters of Marketed Formulation and Optimized Formulation F2

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle size (nm)</th>
<th>PDI (Mv)</th>
<th>% Drug Content</th>
<th>% CDR</th>
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<tbody>
<tr>
<td>F1</td>
<td>284 ± 0.04</td>
<td>0.28±0.022</td>
<td>76.90±08</td>
<td>87.44±06</td>
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<tr>
<td>F2</td>
<td>127 ± 0.01</td>
<td>0.492±0.001</td>
<td>86.33±07</td>
<td>92.83±08</td>
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<td>F3</td>
<td>401 ± 0.06</td>
<td>0.048 ±0.004</td>
<td>73.25±06</td>
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<td>F4</td>
<td>239 ±0.002</td>
<td>0.81 ±0.003</td>
<td>78.02±06</td>
<td>89.53±05</td>
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<tr>
<td>F5</td>
<td>224 ±0.02</td>
<td>0.61 ± 0.005</td>
<td>82.51±08</td>
<td>90.23±06</td>
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<tr>
<td>F6</td>
<td>319 ±0.03</td>
<td>0.30 ±0.02</td>
<td>74.00±05</td>
<td>80.25±05</td>
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Data are expressed as Mean ±S.D. (n=3)

Table 4: Pharmacokinetic Parameters of Marketed Formulation and Optimized Formulation F2

<table>
<thead>
<tr>
<th>Formulation</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-t&lt;/sub&gt; (µg/ml.h)</th>
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<tr>
<td>F2</td>
<td>6.5</td>
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<td>Marketed Product</td>
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Figure 1. Formulation of Nano-suspension by Solvent/ Anti Solvent Technique
Figure 2. TEM Images of Optimized Formulation F2
Figure 3. *In-vitro* Drug Release Profile for All the Formulations
Figure 4. Comparison of In-vitro Drug Release Profile of Optimized Formulation (F2) with That of Pure Drug and Marketed Formulation
Figure 5. *In-vivo* Plasma Drug Concentration Time Curve of Optimized Formulation (F2) and Marketed Formulation