Atorvastatin Loaded Solidlipid Nanoparticles: Formulation, Optimization, and in - vitro Characterization

Panakanti Pavan Kumar, Panakanti Gayatri, Reddy Sunil, Somagoni Jagannohan and Yamasai Madhusudan Rao

Abstract—This study describes the formulation of Atorvastatin (ATRS) loaded solid lipid nanoparticles by hot homogenization followed by ultrasonication technique, and optimization of formulation and process parameters to formulate preferred SLN dispersions. The effects of composition of lipid materials, surfactant mixture and sonication time on particle size, PDI, zeta potential, drug entrapment efficiency, and in vitro drug release behavior were investigated. The mean particles size, PDI, zeta potential and entrapment efficiency of optimized formulation (A5) was found to be 50.0± 6.12 nm, 0.08±0.011,10.40± 4.68mV, 88.7± 6.08 % respectively. To characterize the state of drug and lipid modification in ATRS loaded solidlipid nanoparticles, differential scanning calorimetry analysis was performed. Shape and surface morphology was determined by Transmission Electron Microscopy (TEM) which revealed fairly spherical shape of nanoparticles. The in-vitro drug release study demonstrated that ATRS-SLN formulation (A5) possessed controlled drug release over a period of 24 hrs than dispersion of pure drug. Stability studies performed on the selected formulations revealed that there was no physical instability of the developed formulation for a period of 3 months at room and refrigerated temperatures.

Keywords—Solid lipid nanoparticles, Atorvastatin, Ultrasonication and Entrapment Efficiency

INTRODUCTION

Atorvastatin is an antihyperlipidemic drug with poor oral bioavailability (12%) due to the first pass metabolism [1]. Possible methods to avoid first pass metabolism include transdermal, buccal, rectal and parenteral routes of choice for the delivery of drugs, although several factors like pH of GIT, residence time and solubility can affect drug administration by this route. Lymphatic delivery is an alternative choice to avoid first pass metabolism in per oral drug delivery [2]. Enhanced lymphatic transport of drugs reduces the hepatic first-pass metabolism and improves bioavailability, because intestinal lymph vessels drain directly into thoracic duct, further in to the venous blood, thus bypassing the portal circulation [3]. The main function of the lymphatic system is to facilitate absorption of long chain fatty acids via chylomicron formation. Two different lipid based approaches are known to enhance the lymphatic transport, which includes construction of a highly lipopholic prodrug and incorporation of drug in a lipid carrier [4].

Lipid nanoparticles with a solid matrix, such as solid lipid nanoparticles (SLN), are an alternative nanoparticulate carrier system to polymeric nanoparticles, liposomes and o/w emulsions [5-8]. Aqueous SLN dispersions are composed of a lipid which is solid both at body and room temperature, being stabilized by a suitable surfactant. With regard to developing commercial products for the therapy, SLN possess distinct advantages compared to other carriers, e.g., polymeric nanoparticles. Especially for topical and oral administration, all lipids can be used as matrix material, which are currently in use for creams, ointments, tablets, and capsule formulations including the long list of different surfactants/stabilizers employed in these traditional formulations. Thus, there is no problem with the regulatory accepted status of excipients [9-11]. SLN also enjoy more advantages over other colloidal delivery systems with regard to biocompatibility and scale up [12], also the release of drugs from SLN can be modulated in order to optimize their blood levels [13]. These features together make lipid nanoparticles an interesting carrier system for optimized oral delivery of drugs. Reports on the use of SLN for avoiding first pass metabolism of drugs are scanty. Various researchers have studied the gastro-intestinal uptake and transport to lymphatic circulation of labeled SLNs [14], tobramycin [15], clozapine [16] after duodenal administration to rats. The intention of this study was to prepare and evaluate Atorvastatin
(ATRS) loaded solid lipid nanoparticles; and optimize the formulation and process parameters to fabricate SLN dispersions of desired characteristics.

II. MATERIAL AND METHODS

2.1. Materials:
Atorvastatin (ATRS) and Poloxamer 188 was a kind gift from Torrent pharmaceuticals, Ahmadabad, India; Trimyristin (TM) (Dynasan 114) was generously supplied by Sasol (Witten, Germany); Soy phosphatidylcholine 99% (Epikuron 200) was donated by Degussa Texturant Systems (Deutschland, Hamburg); Dialysis membrane-70 were purchased from Hi-Media (Mumbai, India); Centrisart filters (molecular weight cutoff 20,000) were purchased from Sartorius (Goettingen, Germany). All other chemicals were of analytical reagent grade.

2.2. Methods:
2.2.1. Preparation of Atorvastatin loaded SLN:
Atorvastatin, trimyristin, and soy phosphatidylcholine 99% were dissolved in 20 mL mixture of chloroform and methanol (1:1). Organic solvents were completely removed using a rotaevaporator (Laborota 4000, Heidolph, Germany). Drug-embedded lipid layer was melted by heating at 5°C above melting point of the lipid. An aqueous phase was prepared by dissolving Poloxamer 188 in double distilled water (sufficient to produce 20 mL of preparation) and heated to same temperature of oil phase. Hot aqueous phase was added to the oil phase, and homogenization was carried out at 12,000 rpm using a Diax 900 homogenizer (Heidolph, Germany) for 3 min. Coarse hot oil in water emulsion so obtained was ultrasonicated (12T-probe) using a Sonoplus ultrahomogenizer (Bandelien, Germany) for 20 min. ATST-SLN were obtained by allowing hot nanoemulsion to cool to room temperature.

2.3. Selection and optimization of formulation and process parameters:
A set of experiments, carried out in a systematic way, to predict the optimum and desired formulation and process parameters for the development of ATRS loaded solid lipid nanoparticles. Initial studies were undertaken to decide the excipients and their levels. The choice of lipid was done on the basis of solubility and partitioning of Atorvastatin in the lipid. Aqueous phase surfactant and lipid phase surfactant were selected on the basis of stability of dispersion prepared by using different surfactants. The drug: lipid ratio, surfactant (lipid phase) concentration and sonication time were used in the optimization and the responses were the average particle size (PS), PDI, ZP and % Entrapment Efficiency (EE). These three factors that might affect the designed characteristic of nanoparticles formulation.

2.4. Evaluation of solid lipid nanoparticles:
2.4.1. Measurement of particle size and zeta potential of SLN:
The size and zeta potential of SLN were measured by photon correlation spectroscopy using a Zetasizer 3000 HSA (Malvern, UK). Samples were diluted appropriately with the aqueous phase of the formulation to get optimum kilo counts per second (Kcps) of 50–200 for measurements, and the pH of diluted samples ranged from 6.9 to 7.2. Zeta potential measurements were carried out at 25°C, and the electric field strength was around 23.2 V/cm.

2.5. Determination of entrapment efficiency:
Entrapment efficiency (EE %) was determined by measuring the concentration of free drug (unentrapped) in aqueous medium [17] The aqueous medium was separated by ultra-filtration using centerisart tubes (Sartorius, Germany), which consists of filter membrane (molecular weight cutoff 20,000 Da) at the base of the sample recovery chamber. About 1mL of the formulation was placed in the outer chamber and sample recovery chamber placed on top of the sample and centrifuged at 4000 rpm for 15 min. The SLN along with encapsulated drug remained in the outer chamber and aqueous phase moved into the sample recovery chamber through filter membrane. The amount of ATRS in the aqueous phase was estimated by high performance liquid chromatography (HPLC) method and the entrapment efficiency was calculated by the equation:

\[
\text{Entrapment Efficiency} = \frac{\text{analyzed weight of drug in SLN}}{\text{theoretical weight of drug loaded in SLN}} \times 100
\]

Drug entrapment efficiency (%) = theoretical weight of drug loaded in SLN

2.6. Determination of drug content (Assay):
For estimation of assay, 50 µL of formulation was diluted to 1 mL with chloroform: methanol (2:1). The final dilution was made with the mobile phase and ATRS content was determined by HPLC.

2.7. HPLC methodology for Atorvastatin:
HPLC determination of ATRS was performed using Shimadzu LC 20AT solvent delivery pump equipped with a 20 µL loop and rhodyne sample injector and UV-Visible detector. Samples were chromatographed on a stainless steel C-18 reverse phase column (250 x 4.6 mm) packed with 5 µm particle (Inertsil column). Elution was conducted with a mobile phase of acetonitrile: double distilled water containing
1% v/v triethylamine. (pH was adjusted to 5.6 with orthophosphoric acid) at a ratio of 55:45 v/v. The elute was monitored at 230 nm, at a flow rate of 1 mL/min. 

2.8. In vitro release of Atorvastatin from SLNs:
In vitro release studies of ATRS-SLN dispersion and dispersion of pure ATRS were performed using modified Franz diffusion cell. Dialysis membrane having pore size 2.4 nm, molecular weight cut off 12,000–14,000 was used. Membrane was soaked in double-distilled water for 12 h before mounting on a Franz diffusion cell. A volume of 4 mL of Atorvastatin loaded SLN formulation was placed in the donor compartment and the receptor compartment was filled with 25 mL of dialysis medium containing pH 6.8 phosphate buffer. An aliquot of 2 mL of sample was withdrawn from receiver compartment through side tube at time intervals of 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h. Fresh medium was replaced each time to maintain constant volume. Samples were analyzed by using UV-Visible spectrophotometer.

2.9. Differential scanning calorimetry (DSC):
Thermal characteristics of the drug, trimyristin (TM), physical mixture (PM), lyophilized ATRS-SLN were studied using a differential scanning calorimeter. DSC thermograms of the drug, TM, PM, lyophilized ATRS-SLN was recorded using Mettler-Toledo DSC (Mettler-Toledo, Viroflay, France). Ultra high pure nitrogen was used at a flow rate of 50 mL/min. Samples were analyzed in crimped aluminum pans and heated from 30-250°C at a linear heating rate of 10°C min⁻¹. The sample size was 3-5 mg for each measurement.

2.4.7. Transmission Electron Microscopic studies:
Images were recorded using Transmission Electron Microscope (Hitachi, Japan, Model H 7500 ID) with magnification: 179000×. ATRS loaded SLN was diluted appropriately with 0.1 M phosphate buffer and centrifuged at 4000 rpm for 5 min. Few drops of the diluted emulsion was placed on grid and stained with 2% uranyl acetate, and then the image was captured.

2.10. Stability Studies:
ATRS loaded SLN was stored at room temperature and refrigerator temperature for 40 days. At different time intervals day 1, 20 and 40 the average size, zeta potential, PDI and entrapment efficiency were determined. The number of samples estimated was in triplicate. (n = 3)

III. RESULTS AND DISCUSSION

3.1. Selection and optimization of formulation and process parameters
On the basis of the results obtained in the preliminary screening studies, the trimyristin (TM), soy phosphatidylcholine 99% and Poloxamer 188 were chosen as lipid, lipid phase surfactant and aqueous phase surfactant respectively for further study. Total 8 (A1 to A8) formulations of ATRS loaded solid lipid nanoparticles were developed for optimization and selection of formulation and process parameters.

3.2. Preparation of ATRS loaded SLN:
Homogenization followed by ultrasonication is a reliable, simple and reproducible method for preparing SLN [18]. We used trimyristin as solid lipid, and a mixture of Soy phosphatidylcholine 99% and Poloxamer 188 as surfactant. It is known that the employ of two surfactants, respectively of lipophilic and hydrophilic nature, yields a better stabilization of the disperse system. The solvent mixture of chloroform and methanol (1:1) was found to be effective in homogenously dispersing the drug in the lipid phase. Rota evaporation at 5°C above the melting point of lipid ensured the complete removal of the traces of organic solvents. Homogenization of the lipid phase with hot aqueous Poloxamer 188 solution for 3 min was sufficient to produce a coarse emulsion with average particle size between 2.52 and 2.83 μm. Further increase in homogenization time did not show any significant decrease in the particle size. Thus a homogenization time of 3 min was selected for all the formulations and further reduction of size was preceded with sonication. To optimize the sonication time, all the developed formulations were ultrasonicated (12T-probe) using a Sonoplus ultrahomogenizer (Bandelin, Germany) for 10 min, 20 min and 25 min. Sonication of the coarse emulsion for 20 min resulted in particle size between 50 and 125 nm with narrow size distribution. Hence 20 min sonication time was preferred for all the formulations. The effect of sonication time on particle size was presented in Fig 7. In order to optimize the lipid to drug ratio, two different amounts of trimyristin (250 and 300 mg) were tried with fixed amount of ATRS (10 mg) and soy phosphatidylcholine 99% (100 mg). The final composition of the investigated SLN dispersions was shown in Table 1.

3.3. Measurement of size and zeta potential:
All the formulations were analyzed in sequence to determine their particle size distribution and zeta potential values. In all formulations, the particle sizes ranged from 50.0±4.12 to 125.1±6.12 nm, and zeta potentials were 5.82±2.22 to 12.4±4.72 (Table 2). The increasing concentration of surfactant in SLN formulations could reduce the interfacial tension between lipid matrix and dispersion medium (aqueous phase), consequently favor the formation of SLN with smaller particle size. Poloxamer 188 also provides a steric stability for maintaining the stability of SLN [18]. To obtain stable and smaller SLN, Poloxamer 188 concentration was varied from 0.75 to 1.5 % (150 to 300 mg). It is evident from the results obtained that the
Poloxamer 188 concentration (1.5%) was effective in producing smaller particle size. Further increase in Poloxamer 188 concentration to 2% did not reduce the particle size. These results clearly suggest that an optimum concentration of 1.5% Poloxamer 188 was sufficient to cover the surface of nanoparticles effectively and prevent agglomeration during the homogenization process. High concentration of surfactant (2%) was avoided to prevent decrease in the entrapment efficiency and also toxic effects associated with surfactant [19].

Zeta potential is a key factor to evaluate the stability of colloidal dispersion. It was currently admitted that zeta potentials above 30mV were required for full electrostatic stabilization. However, many experiments demonstrated that not only electrostatic repulsion dominated the stability of nanoparticles; the use of steric stabilizer also favored the formation of stable nanoparticle dispersion. In these studies, it seemed that the value of zeta potential of ATRS loaded SLN was not sufficient to keep the particles dispersing stably. However, the particle size did not change significantly within 40 days, which should contribute to the following point. High surfactant mixture can easily compensate for missing electrostatic repulsion to stabilize the dispersion for long time. Poloxamer 188 provides a steric stability for maintaining the stability of SLNs.

3.4. Entrapment efficiency:

Entrapment efficiency of SLN formulations are shown in Table 2. Drug expulsion in SLN can occur when the lipid matrix transforms from high energy modifications, characterized by the presence of many imperfections, to the β-modification forming a perfect crystal with no room for guest molecules. This phenomenon is even more pronounced when high purity lipids are used. Among the SLN formulations highest entrapment efficiency (88.7±6.08 %) was observed with formulation A5, whereas formulation A4 showed lowest entrapment efficiency (81.7±8.22 %). As the lipid concentration is decreased there is a decline in entrapment efficiency of the SLN formulations.

3.5. Effect of surfactant mixture on particle size, zeta potential and PDI:

The effect of surfactant composition on the size, zeta potential and PDI of SLN are presented in Fig 1. The surfactant amount in SLN is an important factor for determining the physicochemical characteristics due to the surface active properties. The zeta potential of SLN formulations (A1 to A8) was decreased with decrease in the total amount of surfactant mixture (soy phosphatidylcholine & Poloxamer 188) from 400 to 250 mg. As the amount of surfactant mixture was increased, the decrease in the particle size was observed. The amount and type of emulsifier affects the particle size and the stability of the formulation. The amount of emulsifier should be optimum to cover the surface of the nanoparticles and the combination of hydrophilic and lipophilic surfactants used in the formulation improves the stability of the formulation.

3.6. In Vitro release Study:

The in vitro drug release profiles of ATRS-loaded SLNs with different formulations and dispersion of pure ATRS were shown in Fig 2a-b. In order to evaluate the controlled release potential of the investigated formulations, the release of ATRS from the lipid particles was investigated over 24h. Cumulative percent of drug release from the formulations A1 to A4 showed drug release of 7.98%, 6.52%, 6.59% and 6.60% in 0.1N hydrochloric acid and 33.4.6%, 14.7%, 14.2% and 13.4% in phosphate buffer pH 6.8 respectively; Whereas the formulations A 5 to A 8 drug release was found to be 5.90%, 7.12%, 7.32% and 8.20% in 0.1N hydrochloric acid and 40.68%, 33.7%, 12.8%, 13.4% in phosphate buffer pH 6.8 respectively; Formulations A4 and A8 showed similar drug release in 24h, whereas A3 and A7 also showed one and the same drug release pattern in 24h, this may be due to the concentration of Poloxamer 188 was unchanged in both the formulations. These findings imply that the release behavior was governed by the concentration of the Poloxamer 188 in the formulation but not the concentration of the lipid. To evaluate the above outcomes, drug release pattern of formulations A2 and A6 in which the concentration of Poloxamer 188 was also equal, were tested, and expected to show the similar drug release in 24hr, but the release was found to be dissimilar, this may be due to the distinction in the concentration of lipid in the formulations. To authenticate the obtained results, assessment was done on the formulations A1 and A5. The effect of lipid material and surfactant concentration on in vitro drug release performance of these two formulations revealed that the, release was not only governed by the concentration of surfactant alone and also depend upon the concentration of lipid material. The amount and type of lipid and emulsifier affects the particle size, drug loading capacity and the stability of the formulation. The amount of lipid ought to optimum to encapsulate maximum amount of the drug and also should turn out minimum size lipid particles with narrow size distribution. The quantity of emulsifier should be optimum to cover the surface of the nanoparticles effectively and prevent agglomeration during the homogenization process, consequently favor the formation of SLN with smaller particle size and also be a factor to prevent decrease in the entrapment efficiency. Formulation A5 containing 1.5% Poloxamer 188 and 250 mg of TM was found to be Optimum and showed highest drug release i.e. 40.68% of ATRS and the release was extended up to 24hrs. As the surfactant concentration is decreasing from 1.5% to 0.75% there was decrease in controlled release properties of the SLN formulations. Whereas decrease in lipid concentration from 300mg to 250 mg results, decrease in particle size and have an influence on the drug release behavior form the SLN formulations but did not show any significant effect on % entrapment efficiency and stability of the formulations. In present
investigation ATRS-SLN dispersion of optimized formulation (A5) showed significantly low release of Atorvastatin (40.68%) than dispersion of pure drug (98.4%). The drug release data of most of the SLN formulations fitted well into the Higuchi square root release kinetics and weibull dissolution kinetics ($r^2$ values ranging from 0.92 to 0.99). This indicates that the test product follows matrix diffusion based release kinetics. The obtained results advise that the lipid material and surfactant concentration were the critical formulation variables and sonication time was the important process aspect to prepare preferred SLN dispersions of ATRS.

3.7. DSC Analysis:
DSC thermograms of ATRS, trimyristin, physical mixture of drug and lipid, SLN are shown in Fig 3. The melting endotherm of the drug was completely absent in the thermograms of ATRS loaded SLN, which indicates that ATRS was completely solubilized inside the lipid matrix of the SLN. Incorporation of ATRS inside the lipid matrix results in an increase in the number of defects in the lipid crystal lattice, and hence causes a decrease in the melting point of the lipid in the final SLN formulations. Bulk trimyristin showed a sharp endothermic event, ascribing to the melting, around 58.68 °C (minimum) with an extrapolated onset of the melting peak 53.28 °C (the difference between onset and minimum can be taken as a measure for the width of the peak). When the raw material was formulated as nanoparticles, the endothermic happened at a slightly lower temperature. These differences are generally ascribed to the nanometric size of the particles, having then a high specific surface area [20]. A certain effect due to the surfactant should be taken into account as well.

3.8. Visualization by Transmission Electron Microscopy (TEM):
The result of TEM imaging of ATRS loaded SLN, which was shown in Fig 4, indicated that the particles had nanometer-size spherical shapes and no drug crystal (irregular crystallization with the vast majority of needle or rod crystal in the length range from 10 micron to a few dozen microns) was visible. The average particle size of optimized ATRS loaded SLN was found to be 50.0 nm to 125.0 nm respectively.

3.9. Stability study:
All the formulations are stored in amber colored bottles at room temperature and refrigerator temperature. As A3, A4, A7 and A8 formulations are not stable, so rest of formulations that is A1, A2, A5, A6 are analysed for particle size, zeta potential and PDI after stored at room temperature and refrigerator temperature for the Day 1, 20 and 40. A1, A2, A5, A6 are analysed for entrapment efficiency on the different time intervals (day 1, 20 and 40) after stored at room temperature. The effect of duration on storage and storage condition on particle size, zeta potential and PDI of SLNs are showed in Fig 5 and Table 3. There is no significant difference in particle size, zeta potential, and PDI of SLN after stored at room temperature and refrigerator temperature for the Day 1, 20 and 40. The good stability might derive from the slow transition of lipid in nano formulations and the steric effect of Poloxamer 188. These results clearly suggest that an optimum Poloxamer 188 concentration of 1.5% was sufficient to cover the surface of nanoparticles effectively and prevent agglomeration during the homogenization process.

During long-term storage, triglycerides undergo degradation to fatty acids and mono- and diglycerides, which could compete with formulation surfactants for positioning on the surface. Fatty acids and monoglycerides can form mixed micelles that might enhance the partitioning of hydrophobic drug out of the nanoparticle. Therefore, the concentration of excipients and possible degradation products need to be determined to understand the stability of nanoparticle [21]. The effect of duration of storage and storage condition at room temperature on entrapment efficiency of ATRS SLN is presented in Fig 6. As the duration of storage increased the entrapment efficiency was found to be decreased.

IV. CONCLUSION
Homogenization followed by ultrasonication method is suitable to produce SLN of 50–125 nm size ranges. Lipophilic drugs like ATRS can be successfully loaded with triglycerides, nontoxic surfactants like Poloxamer 188 and phosphatidylcholine. The entrapment efficiency and the drug release profile depend on the concentration of lipid and surfactant mixture employed. The drug release rate decreases for SLN with a higher lipid concentration, which is explained by the physical morphology of the lipid particles. As the surfactant concentration is decreasing from 1.5% to 0.75% there is decrease in controlled release properties of the SLN formulations. DSC analysis showed amorphous state of ATRS in SLN. The results of the in-vitro drug release studies demonstrated, significantly low release of ATRS (40.68%) from ATRS-SLN as compared to dispersion of pure drug (98.4%). In vitro release of ATRS followed Higuchi and Weibull equations better than first-order equation. This system is most suitable for exploiting lymphatic transport pathway for improving oral bioavailability of ATRS. Stability studies revealed that after 40 days of storage at different temperatures the mean diameters of SLNs remain practically the same, which emphasizes the physical stability of these lipid particles. These data collectively support that SLNs are the promising delivery systems for poorly water soluble drugs, such as ATRS.
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REFERENCES

[19]. Muller Rainer H., karsten Mader, sven gohla. solid lipid nanoparticles (SLN) for controlled drug delivery a review of the state of the art. European journal of Pharmaceutics and Biopharmaceutics, 50(2000) 161-177.

Table 1: Composition of ATRS-SLN formulations

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
<th>A8</th>
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<tr>
<td>ATRS (mg)</td>
<td>80</td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>400</td>
<td>80</td>
<td>100</td>
<td>200</td>
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<tr>
<td>TM (mg)a</td>
<td>80</td>
<td>150</td>
<td>250</td>
<td>350</td>
<td>450</td>
<td>80</td>
<td>100</td>
<td>200</td>
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<tr>
<td>SP 99% (mg)b</td>
<td>100</td>
<td>100</td>
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<td>300</td>
<td>400</td>
<td>100</td>
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<td>200</td>
</tr>
<tr>
<td>SS (mL)c</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Poloxomar180 (mg)d</td>
<td>300</td>
<td>250</td>
<td>200</td>
<td>150</td>
<td>300</td>
<td>250</td>
<td>200</td>
<td>150</td>
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Table 2: Zeta size, PDI, zeta potential and entrapment efficiency (%EE) of SLN Formulations (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zeta size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>%EE</th>
<th>Assay (%)</th>
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<tbody>
<tr>
<td>A 1</td>
<td>103.6 ± 2.33</td>
<td>0.09 ± 0.012</td>
<td>9.76 ± 3.61</td>
<td>84.9 ± 4.49</td>
<td>98.5</td>
</tr>
<tr>
<td>A 2</td>
<td>115.6 ± 6.47</td>
<td>0.11 ± 0.018</td>
<td>8.44 ± 2.48</td>
<td>83.8 ± 5.88</td>
<td>97.6</td>
</tr>
<tr>
<td>A 3</td>
<td>120.1 ± 5.12</td>
<td>0.12 ± 0.015</td>
<td>7.94 ± 2.15</td>
<td>82.7 ± 6.76</td>
<td>97.9</td>
</tr>
<tr>
<td>A 4</td>
<td>125.8 ± 6.66</td>
<td>0.10 ± 0.026</td>
<td>7.82 ± 1.22</td>
<td>81.0 ± 6.75</td>
<td>98.7</td>
</tr>
<tr>
<td>A 5</td>
<td>50.0 ± 6.12</td>
<td>0.08 ± 0.081</td>
<td>10.4 ± 4.72</td>
<td>87.7 ± 6.08</td>
<td>98.3</td>
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<tr>
<td>A 6</td>
<td>61.3 ± 5.55</td>
<td>0.08 ± 0.011</td>
<td>10.6 ± 4.68</td>
<td>85.2 ± 5.43</td>
<td>98.3</td>
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<tr>
<td>A 7</td>
<td>74.8 ± 9.91</td>
<td>0.10 ± 0.08</td>
<td>9.26 ± 3.11</td>
<td>84.3 ± 5.61</td>
<td>97.6</td>
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<tr>
<td>A 8</td>
<td>93.4 ± 4.89</td>
<td>0.11 ± 0.021</td>
<td>8.27 ± 2.58</td>
<td>82.6 ± 6.23</td>
<td>98.2</td>
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Table 3: Influence of storage condition and storage duration on zeta size, PDI and zeta potential of SLN

<table>
<thead>
<tr>
<th>Storage condition</th>
<th>Duration</th>
<th>Formulation</th>
<th>Zeta Size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Room temperature</strong></td>
<td>Day 1</td>
<td>A1</td>
<td>103.6 ± 2.33</td>
<td>0.09 ± 0.012</td>
<td>9.76 ± 2.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>115.6 ± 6.47</td>
<td>0.11 ± 0.018</td>
<td>8.84 ± 2.68</td>
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<tr>
<td></td>
<td>Day 20</td>
<td>A1</td>
<td>113.8 ± 2.23</td>
<td>0.11 ± 0.013</td>
<td>9.76 ± 3.61</td>
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<tr>
<td></td>
<td></td>
<td>A2</td>
<td>125.5 ± 6.57</td>
<td>0.13 ± 0.016</td>
<td>8.64 ± 2.58</td>
</tr>
<tr>
<td></td>
<td>Day 40</td>
<td>A1</td>
<td>121.6 ± 2.33</td>
<td>0.15 ± 0.011</td>
<td>9.46 ± 3.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>129.1 ± 6.38</td>
<td>0.17 ± 0.017</td>
<td>8.64 ± 2.48</td>
</tr>
<tr>
<td><strong>Refrigerator temperature</strong></td>
<td>Day 1</td>
<td>A1</td>
<td>103.6 ± 2.33</td>
<td>0.09 ± 0.012</td>
<td>9.76 ± 3.61</td>
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<tr>
<td></td>
<td></td>
<td>A2</td>
<td>115.6 ± 6.47</td>
<td>0.11 ± 0.018</td>
<td>8.44 ± 2.48</td>
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<tr>
<td></td>
<td>Day 20</td>
<td>A1</td>
<td>110.5 ± 2.13</td>
<td>0.12 ± 0.011</td>
<td>9.66 ± 3.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>119.9 ± 5.47</td>
<td>0.13 ± 0.015</td>
<td>8.48 ± 2.58</td>
</tr>
<tr>
<td></td>
<td>Day 40</td>
<td>A1</td>
<td>123.4 ± 2.34</td>
<td>0.14 ± 0.016</td>
<td>8.76 ± 3.52</td>
</tr>
<tr>
<td></td>
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<td>A2</td>
<td>125.1 ± 6.27</td>
<td>0.21 ± 0.016</td>
<td>8.46 ± 2.38</td>
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<tr>
<td><strong>Room temperature</strong></td>
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<td>A5</td>
<td>50.0 ± 6.12</td>
<td>0.08 ± 0.08</td>
<td>10.4 ± 4.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A6</td>
<td>61.3 ± 5.55</td>
<td>0.08 ± 0.011</td>
<td>10.6 ± 4.68</td>
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<tr>
<td></td>
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<td>55.36 ± 1.9</td>
<td>0.09 ± 0.045</td>
<td>9.14 ± 3.3</td>
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<td>0.10 ± 0.068</td>
<td>9.31 ± 3.1</td>
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<td>0.12 ± 0.072</td>
<td>9.94 ± 2.4</td>
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<td>0.13 ± 0.018</td>
<td>9.91 ± 2.4</td>
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<td><strong>Refrigerator temperature</strong></td>
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<td>10.4 ± 4.72</td>
</tr>
<tr>
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<td>A6</td>
<td>61.3 ± 5.55</td>
<td>0.08 ± 0.011</td>
<td>10.6 ± 4.68</td>
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<tr>
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<td>Day 20</td>
<td>A5</td>
<td>61.08 ± 4.5</td>
<td>0.12 ± 0.048</td>
<td>9.46 ± 2.3</td>
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<td>66.49 ± 5.1</td>
<td>0.13 ± 0.033</td>
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<td>66.16 ± 7.5</td>
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<td>68.26 ± 7.3</td>
<td>0.13 ± 0.044</td>
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Figure 1: Effect of surfactant composition on the zeta size, PDI and zeta potential of SLN formulations

Figure 2a: In vitro drug release profile of SLN formulations in 0.1 N HCl

Figure 2b: In vitro drug release profile of SLN formulations in phosphate buffer pH 6.8
Figure 3: DSC thermograms of ATRS (A), trimyristin (B), physical mixture of ATRS and TM (C), and lyophilized ATRS-SLN (D)

Figure 4: Transmission electron micrograph of ATRS loaded SLN

Figure 5: Influence of storage condition and duration of storage on zeta size, PDI and zeta potential of SLN formulation.
Atorvastatin Loaded Solid lipid Nanoparticles: Formulation, Optimization...

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**Figure 6**: Influence of duration and Storage condition at room and Refrigerator temperature on % entrapment efficiency of SLN

**Figure 7**: Influence of sonication time on particle size of SLN formulations