ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

# *Invivo* Pharmacokientic and Pharamacodynamic Studies of Optimized Antihyperlipidemic Drug Loaded Solid Lipid Nanoparticle

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*Article History:*

Received on: 02 Oct 2020
Revised on: 03 Nov 2020
Accepted on: 09 Nov 2020

*Keywords:*

Invivo pharmacokinetic, Antihyperlipidemic, Solid lipid nanoparticle, Particle size, Zeta potential, Polydispersity index

ABSTRACT

The purpose of this research is to increase bioavailability by solid lipid nanoparticle (SLN) carrier for low bioavailable drugs (< 5%) such as Lovastatin. Eight SLN loaded Lovastatin was designed and optimised by variables such as Particle Size (PS in nm) and Zeta Potential (ZP in mV) using a micro emulsification technique. SLN 7 was chosen as the optimised formulation according to the findings obtained and the same was chosen for invivo pharmacokinetic and triton-induced antihyperlipidemic operation. SLN7 confirms an improvement in bioavailability of 3.15 percent by an improvement in AUC compared to conventional dosage type (Altoprev) from the pharmacokinetic invivo results. SLN was also an appropriate career in drug delivery for Lovastatin by enhancing bioavailability and therapeutic response. The stability studies of SLN7 revealed that the evaluation parameters of SLN did not change significantly. It was verified from the data that the drug-loaded SLN was stable under varying temperature and humidity conditions. While compare to 25°c±2°c/ 60% RH, SLN are more stable in 4°c±2°c and shows good reproducible reports in Particle Size (nm), Zeta potential (mV), PI and EE% data. Therefore, Solid Lipid Nanoparticle is a viable drug carrier mechanism for low bioavailable Lovastatin to improve their bioavailability through efficiently permeating them.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i4.3935

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## INTRODUCTION

Lovastatin is a 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) inhibitor, an enzyme catalyzes the mevalonate alteration of HMG-CoA. Mevalonate is an essential building block for the biosynthesis of cholesterol and Lovastatin interferes with its development by acting as a competitive HMG-CoA reversible inhibitor that binds to the reductase of HMG-CoA (Suresh *et al.*, 2007; Chen *et al.*, 2010). Lovastatin is a prodrug, an inert lactone in its natural form, hydrolyzed in vivo to the open ring form of $\beta$-hydroxy acid, the gamma-lactone closed

ring form in which it is delivered, which is the active form. It is part of a Class-II compound of low aqueous solubility and reasonable biomembrane permeability. These are metabolites of HMG-CoA reductase. By blocking enzyme HMG-CoA reductase, it decreases the cholesterol level in the blood and stops and treats cardiovascular disease (Reiter, 2004; Henwood and Lovastatin, 1988). The rationale for selecting Lovastatin as a drug candidate is that it has a poor bioavailability of around 5 percent, 98 percent protein binding, and hepatic (CYP3A and CYP2C8 substrate) metabolism with a biological half-life of 2 to 5 hours. It has a severe 10 percent renal and nearly 83 percent unchanged faecal form excretion (Radha and Lakshmanan, 2013).

SLN typically is spherical with average diameters ranging from 50 to 500 nanometers. SLN has a solid central matrix of solubilizable lipophilic molecules (Yang *et al.*, 2011; Nair *et al.*, 2012). The lipid core is stabilised by surfactants(emulsifiers). In order to minimise carrier cytotoxicity, the use of biological lipids is hypothesised and the solid lipid state is postulated to allow more controlled release of the medication due to increased resistance to mass transfer (Fang *et al.*, 2008). SLN is typically spherical in structure and consists of a solid lipid core stabilised by a surfactant interface zone. (zur Mühlen *et al.*, 1998; Freitas and Müller, 1999).

The main hypothesis of this research is to encapsulate the Lovastatin in SLN and to enhance its bioavailability in Albino wistar rat's plasma.

## MATERIALS AND METHODS

Lovastatin was procured from Microlabs Pvt. Ltd. India. Spans 80, Stearic acid were obtained from LOBA chemie vt. Ltd 107, Mumbai, India. Different instruments were used in formulation and evaluation of SLN like Magnetic Stirrer (REMI, India), Nanoparticles Size Analyzer (HORIBA, Japan), and Scanning electron microscopy (Zeiss Evo, USA).

### Methodology

### Preparation of SLN by Micro emulsification method

Solid Lipid Nanoparticle (50-500 nm) was developed using Micro emulsification method by applying $2^3$ factorial design as in Table 1 and Table 2, by varying the concentration of solid lipid, surfactant and homogenization time (Heng *et al.*, 2003; Sailaja *et al.*, 2011). As formula weighed quantity of the solid lipid mixture (Stearic acid) are melted. a beaker contain the necessary level of distilled water; to it weighed quantity of surfactant and cosurfactant (Span 80 and Polaxomer 188-5% common for all formulation) was allowed to gradually dissolve with the aid of magnetic stirrers (Meghana *et al.*, 2012; Khan *et al.*, 2012). This results in transparent microemulsion which is thermodynamically stable. It then slowly dispersed the weighed drug into the microemulsion that was prepared. With High Speed Homogenizer, CAT, Germany, the prepared hot microemulsion is then dispersed under extreme homogenization at 10,000 RPM with specified time in 2-4 $^\circ$ C cold water. Then the prepared emulsion is diluted with water in a 1:20 ratio and stored at 4$^\circ$ C for further use (Hecq *et al.*, 2005; Sawant *et al.*, 2011).

### Evaluation of SLN

### Particle characterization

The average particle size of SLN at an angle of $90^o$ was observed by Dynamic Light Scattering (DLS) and the temperature of the sample holders is approximately $25^0$C by use (Nanopartica SZ-100 HORIBA Test, Japan). The sample was diluted by double distilled water (1:10 v/v) to ensure the dispersion power of light in the instrument set. The Zeta potential is defined as the difference in potential between the surface of solid particles scattered or immersed in a conductive liquid. One of the key variables affecting the stability of SLN dispersion is the zeta potential; the Zetasizer (Nanopartica SZ-100 HORIBA Science, Japan) was used to calculate the zeta potential. The zeta potential should be $\pm$30 to $\pm$60 mV for a stable nanoparticle and the poly dispersion index should be < 0.7 to disperse the nanoparticle over an extended period of time in the continuous process (Shinde *et al.*, 2013).

### Scanning Electron Microscopy studies (SEM)

To evaluate SLN surface morphology, size, and shape with direct nanoparticle depiction, the SEM (Zeiss Evo, USA) technique is mainly used. When it comes to sizing and morphological analysis, the SEM has various benefits. The SLN solution is initially turned into a dry powder and then placed on a sample holder later covered with a conductive metal such as gold. The electron beam is first centered in sample surface, resulting in the secondary electron being released from the sample surface, which defines the morphology of the sample. There could be a risk of disruption to the Lipid for the nanoparticles that have to be with stand vacuum. The mean average size of the SEM is compared with the effects of elastic light dispersion (Müller-Goymann, 2004).

### *In-vivo* Pharmacokinetic studies of Lovastatin SLN

Using PK solver software, the Pharmacokinetic (PK) efficiency of optimized SLN7 following oral admin-

**Table 1: Preferred variables for formulation of SLN by $2^3$ optimization design**

| Optimization Model | | Factorial Model | |
|---|---|---|---|
| Model | | Factorial design | |
| technique | | Linear Regression | |
| chosen Critical Material Attribute (CMA) | | Selected Critical process parameter (CPP) | |
| Formulation technique | Microemulsification | Homogenization speed | 10000 RPM |
| Solid Lipid | tearic acid | | |
| Surfactant | Span 80 | | |
| Co-surfactant | Poloxamer 188 | | |
| **Factor** | **Variable** | **Level** | |
| | | **-1 (low)** | **1 (high)** |
| X1 | Solid lipid (mg) | 2.5 | 5 |
| X2 | Surfactant concentration (%) | 0.5 | 1.0 |
| X3 | Homogenization Speed 10000 RPM fixation time (min) | 15 | 30 |
| **Response** | **Variable** | **Constraint** | |
| Y1 | Particle Size ( PS nm) | 10-500 nm (Minimize) | |
| Y2 | Zeta Potential (ZP mV) | ±10 to ±30 Incipient instability ±30 to ±60 Good Stability | |
| Y3 | Entrapment efficiency (EE %) | 85-115 % (Maximize) | |

**Table 2: Optimization Design of SLN by $2^3$ factorial design**

| Run | Lovastatin in mg | CMA and CPP (Level Codes and its concentration) | | |
|---|---|---|---|---|
| | | Factor A: Lipid Conc. Stearic acid mg | Factor B: Conc. of Surfactant Span 80 % | Factor C: Homogenization Time min. |
| SLN1 | 10 | -1 / 2.5 | -1 / 0.5 | -1 / 15 |
| SLN2 | 10 | 1 / 5 | -1 / 0.5 | -1 / 15 |
| SLN 3 | 10 | -1 / 2.5 | 1 / 1.0 | -1 / 15 |
| SLN 4 | 10 | 1 / 5 | 1 / 1.0 | -1 / 15 |
| SLN 5 | 10 | -1 / 2.5 | -1 / 0.5 | 1 / 30 |
| SLN 6 | 10 | 1 / 5 | -1 / 0.5 | 1 / 30 |
| SLN 7 | 10 | -1 / 2.5 | 1 / 1.0 | 1 / 30 |
| SLN 8 | 10 | 1 / 5 | 1 / 1.0 | 1 / 30 |

**Table 3: Optimization of Solid Lipid Nanoparticle by $2^3$ factorial design and evaluation of effect CMA and CPP on CQA by $2^3$ factorial design**

| Run | CMA and CPP (Level Codes and its concentration) | | | CQA | |
|---|---|---|---|---|---|
| | Factor A: Lipid Conc. Stearic acid mg | Factor B: Surfactant Conc. Span 80 % | Factor C: Homogenization Time min. | PS* Y1 nm | ZP * Y2 mV |
| SLN1 | -1 / 2.5 | -1 / 0.5 | -1 / 15 | 754.6 ± 20.2 | -20.6 ± 1.12 |
| SLN2 | 1 / 5 | -1 / 0.5 | -1 / 15 | 1154.4 ± 18.6 | -2.4 ± 1.24 |
| SLN 3 | -1 / 2.5 | 1 / 1.0 | -1 / 15 | 285.8 ± 22.4 | -38.6 ± 1.22 |
| SLN 4 | 1 / 5 | 1 / 1.0 | -1 / 15 | 665.3 ± 20.2 | -25.4 ± 1.26 |
| SLN 5 | -1 / 2.5 | -1 / 0.5 | 1 / 30 | 690.6 ± 24.6 | -24.4 ± 1.24 |
| SLN 6 | 1 / 5 | -1 / 0.5 | 1 / 30 | 960.2 ± 26.2 | -15.2 ± 1.26 |
| SLN 7 | -1 / 2.5 | 1 / 1.0 | 1 / 30 | 180.9 ± 30.2 | -44.5 ± 1.22 |
| SLN 8 | 1 / 5 | 1 / 1.0 | 1 / 30 | 394.6 ± 10.2 | -40.6 ± 1.32 |

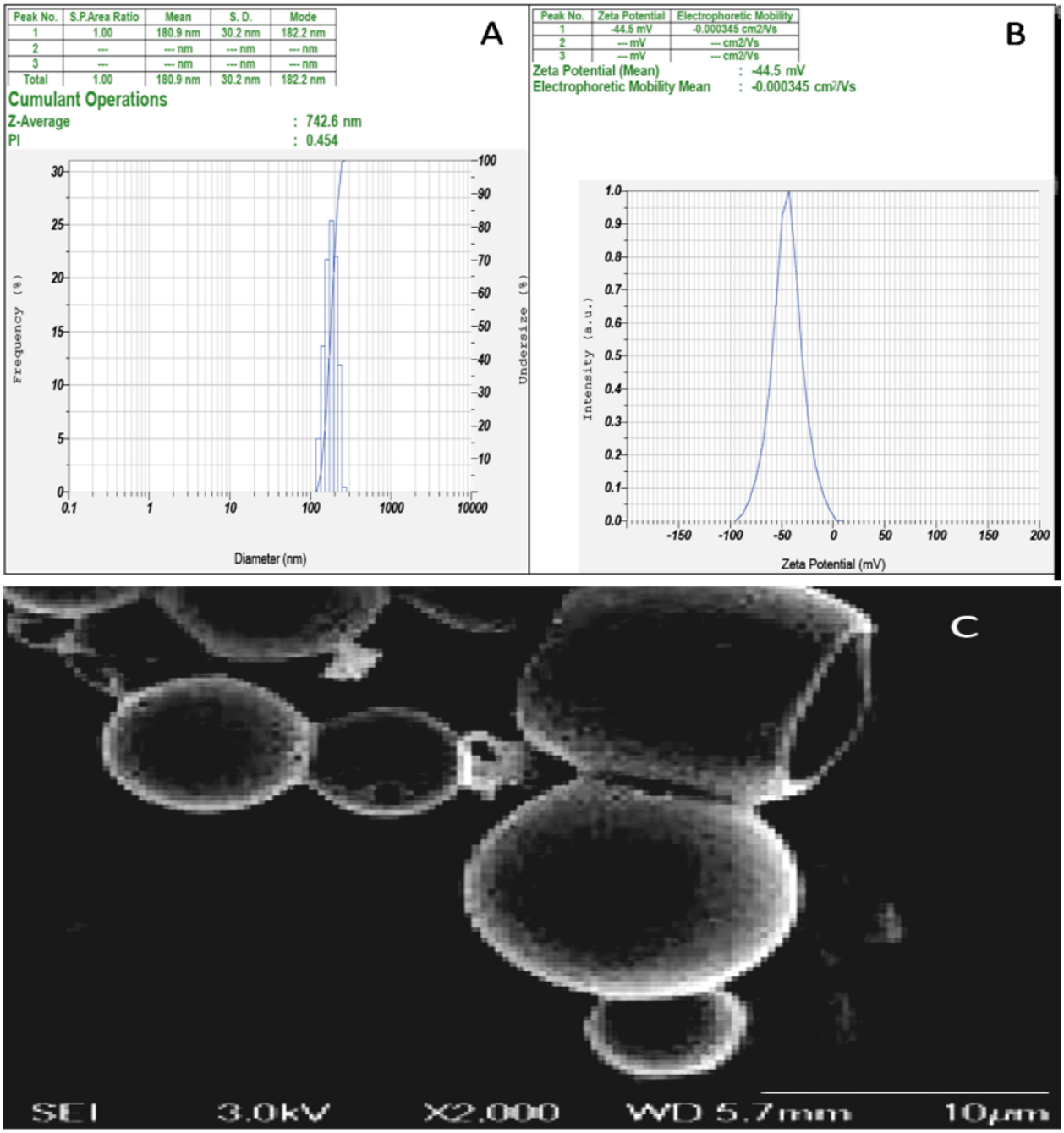


**Figure 1: Particle Characteristics; (A) PS, PI; (B) ZP of SLN 7; (C) SEM images of optimized solid lipid Nnanoparticle (SLN7)**

**Table 4: Relative in-vivo pharmacokinetic studies among Lovastatin formulations (Marketed and SLN 7) treatment groups**

| Parameter | G1:Altoprev ER (4 mg/kg) (Marketed Lovastatin Tablet) – Administered Orally | G2:SLN 7 Suspension (4 mg/kg) – Administered Orally |
|---|---|---|
| Tmax (h) | 1 | 2 |
| Cmax ($\mu$g/ml) | 0.49 | 0.6 |
| AUC 0-$\alpha$ ($\mu$g/ml/h) | 324.40 | 1024.42 |
| AUMC 0-$\alpha$ ($\mu$g/ml/h) | 1336.54 | 3420.52 |
| MRT 0-$\alpha$ (h) | 5.99 | 12.64 |

Enhance in $AUC_{0-\infty}$ ; MRT; Tmax; reducein Cmax in Lovastatin loaded SLN show improved bioavailability than marketed tablet.

**Table 5: Impact on serum lipid profile in triton-induced hyperlipidemic rats with different Lovastatin formulations**

| Group | Treatment | TC (mg/dl) * | TG (mg/dl)* | HDL (mg/dl)* | LDL (mg/dl)* |
|---|---|---|---|---|---|
| Control | Only access to water | 112.40 ± 3.42 | 122.50 ± 5.42 | 50.42 ± 2.48 | 98.34 ± 4.68 |
| Negative Control | Only With Triton (400 mg/kg) Induction (14 days) | 276.42 ± 5.04 *** (a) | 264.50 ± 5.66 *** (a) | 9.58 ± 2.80 ***(a) | 205.98± 6.42 *** (a) |
| Positive Control | Lovastatin (Altoprev) (4mg/kg) in CMC + Triton (400mg/kg) induction | 188.50 ± 2.42 ** (b) | 176.68 ± 4.98 * (b) | 38.94± 2.66 ***(b) | 142.42 ± 6.40 *** (b) |
| Test 1 | Lovastatin SLN (SLN7) (4mg/kg) +Triton (400mg/kg) induction | 126.60 ± 2.46 *** (a,b) | 133.42 ± 6.78 *** (a,b) | 51.68 ± 2.64 *** (a,b) | 120.68± 3.54 *** (a,b) |
| Normal value | | < 200 mg/dL | < 200 mg/dL | >50 mg/dL | < 130 mg/dL |

* The values are described as mean ± SEM, n=6. ANOVA performed a statistically valid test for comparison. A-Comparison of group I Vs ll, * * * p<0.05 with 95% differential CI; b- Comparison of group II Vs All treatment groups, * * *p<0.05 with 95% differential CI; b- Comparison of group II Vs All treatment groups, * * * p<0.05 with 95% differential CI;

**Table 6: Stability studies for Lovastatin SLN (SLN 7) by Long term studies**

| Parameters | Stability studies data of Lovastatin SLN* (mean ± SD, n=3.) | | | |
|---|---|---|---|---|
| SLN 7 | Temp | Initial | After 3 month | After 6 month |
| Particle Size (nm) | 4°c ± 2°c | 180.9 ± 30.2 | 181.0± 8.2 | 182.2± 3.4 |
| | 25°c ± 2°c/ 60% RH | 180.9 ± 30.2 | 182.0± 6.4 | 183.0± 4.4 |
| Zeta potential (mV) | 4°c ± 2°c | -44.5 ± 1.22 | -43.5± 8.12 | -43.0± 4.2 |
| | 25°c ± 2°c/ 60% RH | -44.5 ± 1.22 | -43.4± 8.34 | -40.5± 4.2 |
| PI | 4°c ± 2°c | 0.454 ± 0.12 | 0.455 ± 0.024 | 0.500 ± 0.022 |
| | 25°c ± 2°c / 60% RH | 0.454 ± 0.12 | 0.504 ± 0.026 | 0.544 ± 0.024 |
| EE % | 4°c ± 2°c | 98.42 ± 2.84 | 96.60± 4.42 | 94.80± 3.44 |
| | 25°c ± 2°c/ 60% RH | 98.42 ± 2.84 | 97.60± 4.34 | 88.60± 4.54 |

*All values are expressed as mean ± SD, n=3.

istration was examined. Healthy male Wistar adult albino rats, 180-250 gm in weight, were used. A single dose analysis in 2 groups of 6 animals was divided as follows:-

**B1: Positive Control** - Lovastatin – Altoprev Tablets(4mg/kg) in CMC - Oral administration

**B2: Test 2** - Lovastatin SLN(4mg/kg) - Oral administration

Animals were fasted 24 hours before drug formulations were delivered but had free access to water. A drug solution with the aid of an oral feeding needle was delivered orally. Blood samples of approximately 0.5ml volume were obtained at intervals of 0, 1, 2, 4, 6, 8, 10, 12 h after oral administration by retro-orbital puncture. Samples were collected from a retro orbital puncture using capillary tubes on heparinized glass tubes containing anticoagulant ammonium oxalate (1% solution). With the aid of micro centrifugation, the plasma was immediately isolated at 5000 RPM and deposited at -20$^{o}$C until the HPLC study was performed (Shah *et al.*, 2014; Beloqui *et al.*, 2013).

**Lovastatin plasma drug concentration sample preparation**

At 5000 RPM for 5min, 1.5ml of processed animal blood was subjected to centrifugation and 0.75ml plasma was collected into the Eppendorf tube. To this sample, 0.5ml of 10% trichloroacetic acid was added. The material was separated from plasma by centrifugation at 4000 RPM for 15min at 4°C. The supernatant solution has been syphoned out and injected into HPLC to measure the Lovastatin

found in the plasma obtained specimen as follows (Chakraborty *et al.*, 2009; Andrade *et al.*, 2014).

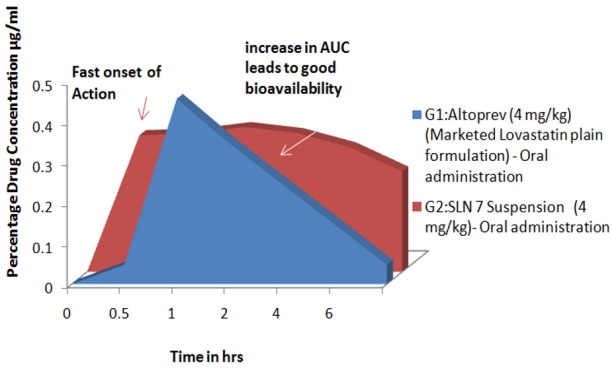


**Figure 2: Graph of Comparative in-vivo pharmacokinetic study for Lovastatin formulations (Marketed Altoprev ER and SLN 7)**

### Quantification of Lovastatin in Plasma

The Lovastatin complete wavelength calculation was carried out using the following parameters with a run time of 10-20 min and a sample of 238 nm was observed. The calibration curve (concentration in $\mu$g / ml on the X-axis Vs peak area in percent on the Y-axis) was performed with concentrations ranging from 0.02-0.16 $\mu$g / ml at 248 nm for 8 solutions of Lovastatin in phosphate buffer solution pH 7.4 and the regression value was found to be r2=0.999. To obtain the sample concentration, the unknown concentration was calculated by picking the peak area of the unknown sample and interpolating it to the X-axis (Manjunath and Venkateswarlu, 2005).

### *In-vivo* Pharmacodynamic studies for Lovastatin SLN

Antihyperlipidemic activity was performed for a total of 28 days. In Albino Wistar rats, the first 14 days were used to cause hyperlipidemia through the application of triton and the remaining fourteen days were used to treat diseased rats.

### Diet-induced hyperlipidemic model

The animals were picked, measured and then tagged for individual identification. In this model, rats were made hyperlipidemic by oral administration of an atherogenic diet by mixing ghee with a standard pellet diet for 14 days and free access to water was provided to rats. Both participants have obtained an atherogenic diet at the same dosage as previously prescribed during the therapy phase. The control animals were fed with their normal diet and free access to water. Different biochemical parameters such as serum total cholesterol (TC), triglycerides ( TG), high density lipoprotein ( HDL) and very low density lipoprotein (VLDL) in mg/dl were analysed by the animals at the treatment end period (Shin *et al.*, 2010).

### Triton-induced hyperlipidemic model

In order to induce hyperlipidemic disease, animals were kept fasting for 24 hours and injected with Triton saline at a dosage of 400 mg/kg given intraperitoneally as a single dose per day for 14 days. By estimating the lipid profile, pharmacodynamic (PD) output in animals following oral administration of Lovastatin SLN was assessed. Healthy Male Albino Wistar rats were picked based on their body weight ranging from 180-250gms and animals were divided into classes. Triton induction of cholesterol and a high fat diet were carried out over a span of 14 days.

The animals were fasted for 24hrs prior to the administration of medication formulations but had free access to water. Animals were left for one hour and rats were administered the drug. After 14 days of oral administration of Lovastatin formulations, blood samples with a volume of approximately 1 ml were obtained at intervals of 0.5, 2, 4, 8, 12, 24 h from the vein of the tail. To remove the serum from the blood, the blood samples collected were centrifuged for 10 min at 4000 rpm.

The 0.5 ml supernatant serum was isolated and by using industrial assay kits to test the following lipid profiles, such as Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL) and Very Low Density Lipoprotein (VLDL) in mg/dl. To test the serum lipid profile, the samples were extracted and diluted with cholesterol, HDL and TG reagents (Wang *et al.*, 2015; Ramachandran *et al.*, 2011).

### Statistical analysis

Data from Antihyperlipidemic Activity were expressed as mean $\pm$ SEM in Albino Wistar Rats. In order to determine the variations between treatment profiles and treatment groups, statistical research was done using one-way ANOVA studies (i.e., comparison of each treatment group with the control group). Using Graph Pad Prism version 5 for Windows (Graph Pad App, San Diego, San Diego), statistical analysis was done. The level of significance was set to be $P < 0.05$. (Asija and Singh, 2016).

### Stability Studies

For this analysis, an optimised sln dispersion was chosen. The formula was split into two batches. Each batch comprises two batches of samples taken from test tubes. The 3rd month and 6th month of each test tube were named. These test tubes are securely capped and closed with an aluminium foil mask to protect them from light degradation. At 2–

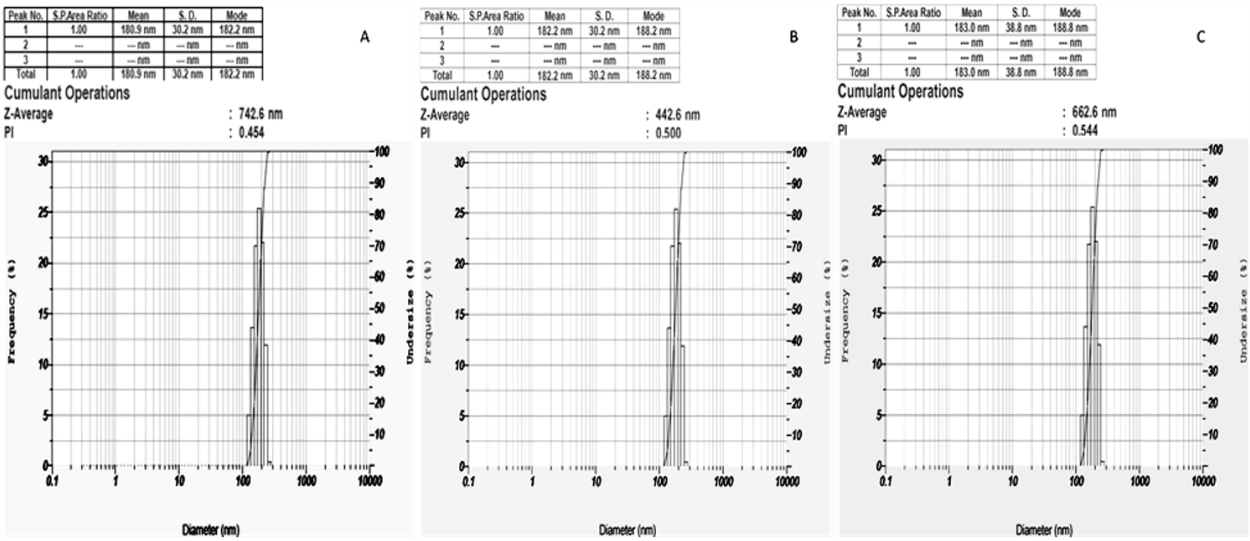


**Figure 3: Stability studies showing PS of SLN7 in different temperature condition: (A) Initial PS at $4^0C \pm 2^0C$ / $25^0C \pm 2^0C$/ 60 ± 5% shows at 180.9 ± 30.2 Nm; (b) Ps at $4^0C \pm 2^0C$ in 3 months is 182.2 ± 3.4nm; (c) Ps at $25^0C \pm 2^0C$ / 60 ± 5% in 6 months is 183.0 ± 4.4 nm**

6°C, one batch was stored in a refrigerator (Souto *et al.*, 2004). At 25°C ± 2°C with 60 percent RH, another batch was stored at room temperature. Each sample from both storage conditions was analysed and measured for the determination of particle size (nm), zeta potential, polydispersity index (PI), Entrapment efficiency (percent) at a given time span. Each formulation was checked for the reproducible results (Üner *et al.*, 2014; Freitas and Müller, 1999).

## RESULTS AND DISCUSSION

### Particle size

The mean particle sizes are defined in Table 3 and Figure 1 for all formulations. Usually, particle sizes for all Lovastatin SLN formulations have been found to be in the 180.9 ± 30.2 to 1154.4 ± 18.6 nm range, based on the effect of CMA and CPP on the formulation process. However, the acceptance specifications of the Solid Lipid Nanoparticle PS should be 50-500 nm. According to the acceptance criteria, the formula SLN3 (2.5 mg A factor; 1.0 per cent of factor B and 15 min of factor C) indicates 285.8 ± 22.4 nm. The SLN7 (2.5 mg factor A; 1.0 percent factor B and 30 min factor C) formulation shows 180.9 ± 30.2 nm. The SLN8 formulation shows 394.6 ± 10.2 nm (5 mg A factor; 1 percent B factor, and 30 min C factor). The remaining formulation shows more than the desired particle size. The chosen CMA's and CPP's have had a major influence on the CQA. Three CMA and CPPs, i.e. X1= lipid concentration, X2= surfactant concentration, X3= homogenization time, were selected and a micro emulsification technique was used to prepare 8 batches. The statistical study of the data indicated whether the fitness of the model to the data was meaningful at p-value < 0.05 and the results were interpreted on the basis of the F-test, p-value at 95 percent CI, coefficient of determination. The results showed a high value of the determination coefficient with R2=0.999 for particle size, and with R2=0.999 for zeta potential. Hence, strong regression (R2) value of the desired model. Lack of response fit values, PS is 0.0267, and ZP is 0.0053 (P > F). Therefore, it was not significant (P-value > 0.05) which indicates that by using the regression model, data can be well explained. P-value was estimated by F-test in ANOVA for response PS and ZP.

### Zeta potential

In general, the zeta potential was found to be in the range of -2.4 ± 1.24mV to -44.5 ± 1.22mV for all Lovastatin SLN, mainly based on the surfactant effect during the formulation process. Nevertheless, the approval criteria for the Solid Lipid Nanoparticle ZP are ±30 and ±60 mV. The SLN3 formulation (2.5 mg A factor; 1.0% B factor and 15 min C factor) shows -38.6 ± 1.22mV according to the acceptance criteria. The SLN7 formulation (2.5 mg A factor; 1.0% B factor, and 30 min C factor) indicates -44.5 ± 1.22mV. The SLN8 formulation (5mg A factor; 1% B factor, and 30 min C factor) shows -40.6 ± 1.32mV. The remaining formulations were found to be less than the range required. CMA has a significant influence on the zeta potential, such as surfactant concentration, lipid concentration, and CPP, such as homogenization time. To predict interactions among the CMA's, CPP's on CQA, the model is sufficiently relevant. The R2 square value is 0.999,

 

i.e. 99 percent of the total variance is represented by this model.

The invivo pharmacokinetic parameters of SLN are shown in Table 4. *Invivo* pharmacokinetic plasma drug concentration profiles were shown in Figure 2. Using a validated HPLC procedure, the concentration of the drug in the blood was calculated at 8 hours. Lovastatin has maximum plasma concentration (Cmax) i.e.,0.49$\mu$g/ml, by administering marketed dosage form (Altoprev tablet). The maximum drug concentration declines rapidly in conventional dosage form due to faster clearance. The higher clearance concentration may be due to unchanged drug will be cleared out of body, due to inability and fluctuation of drug concentration in plasma to reach bioavailable dose and target site receptors. Following oral administration of Lovastatin SLN, the peak plasma concentration of the compound was observed to be 0.4$\mu$g / ml for a period of 2h. The AUC concentration of drug after the administration of Lovastatin tablet Altoprev, and Lovastatin SLN through oral administration was found to be 324.40$\mu$g/ml/hr; 1024.42$\mu$g/ml/hr for 8h respectively. The AUMC concentration of drug after the administration of Lovastatin tablet Altoprev and Lovastatin SLN through oral administration was found to be 1336.54$\mu$g/ml/hr; 3420.52$\mu$g/ml/hr for 8 h respectively. SLN formulations attain a notable maximum plasma concentration and enhance $t_{1/2}$ when compared to other marketed formulation. This leads to longer mean residence time (MRT = 12.64 h) of drug through SLN administration and provides an opportunity for enhanced systemic bioavailability of Lovastatin i.e., 3.15%. This enhanced bioavailability and absorption of drug from Lovastatin SLN was due to small particle size, chain and block like lipid nature of SLN and protection of drug from degradation pathways like first pass metabolism and enzymatic degradation. These discussed data proves that SLN confirms the enhancement of bioavailability by 3.15% when compared to the conventional dosage form. Hence, SLN was a suitable drug delivery career for Lovastatin which enhance the bioavailability.

### Antihyperlipidemic activity of various Lovastatin formulations in triton induced albino wistar rats

Table 5 demonstrates the impact of different Lovastatin formulations on serum lipid levels in Albino Wistar rats. In order to compare statistically significant variations between treatment groups, an ANOVA test was carried out. Initially, at the time of cholesterol induction, the lipid profile of each category was insignificant, i.e., $p > 0.05$, and the maximal TC content was achieved at the end of induction. During the study period, the negative control group reached TC values of around 276.42 $\pm$ 5.04 mg/dl. Test group 1 reported a considerable increase in TC levels of approximately 188.50 $\pm$ 2.42 mg/ml and 126.60 $\pm$ 2.46 mg/ml ($p<0.05$ with 95 percent CI of diff.); Positive control and Test 1 groups showed significant inhibition of TC relative to negative control groups. The highest concentration (264.50 $\pm$ 5.66mg / dL) of serum TG during the induction phase was found in the negative control group. The Lovastatin SLN group demonstrated better influence over serum TG levels of approximately 133.42 $\pm$ 6.78 mg/ml relative to Altoprev (176.68 $\pm$ 4.98 mg/ml) after treatment. At concentrations of 205.98$\pm$ 6.42mg / dL, the negative control group showed higher serum LDL levels. However, Altoprev and Lovastatin loaded SLN treated groups showed a significant reduction in LDL levels of about 142.42$\pm$6.40 mg/dL and 120.68$\pm$3.54mg/dl. A small shift in HDL concentration was observed at the end of induction, particularly in the negative group with low HDL concentration of 9.58 $\pm$ 2.80 mg / dL ($p < 0.05$). Increased HDL concentrations of approximately 38.94$\pm$2.66mg / mL and 51.68$\pm$2.64 mg/ml were observed after treatment with Altoprev and Lovastatin loaded SLN (SLN7) treated classes, showing that Lovastatin SLN can increase blood HDL levels. It was verified on the basis of the results obtained that Lovastatin loaded solid lipid nanoparticle (SLN 7) would prevent atherosclerosis-related risks for an extended period of time, i.e. for 24 hours.

### Stability Studies for Optimized Lovastatin SLN formulation

From the findings of the stability tests, as seen in Table 6 and Figure 3, it was found that the evaluation parameters of SLN did not change significantly. It was verified from the data that the drug-loaded SLN was stable under varying temperature and humidity conditions. While compare to 25°c $\pm$ 2°c/ 60% RH, SLN are more stable in 4°c $\pm$ 2°c and shows good reproducible reports in Particle Size (nm), Zeta potential (mV), PI and EE % data.

## CONCLUSION

The goal of this research is to get better the bioavailability of low bioavailable loavastain (< 5 percent) in the form of an SLN carrier and also to choose the best optimized Lovastatin SLN formulation technique. The SLN was formulated by micro emulsification technique and optimized by evaluating the outcome of CMA and CPPs on CQA such as Particle Size (PS in nm), and Zeta Potential (ZP in mV). From the results obtained, it was concluded that

the micro emulsification technique was an optimal lovastatin-containing SLN preparation technique, with selected formulation variables such as stearic acid as solid lipid, Span 80 as surfactant, and method variables such as homogenization speed for 30 minutes at 10000 RPM. SLN 7 confirms enhancement of bioavailability by 3.15% by increase in AUC when compared to conventional dosage form. Hence, SLN was a suitable drug delivery career for Lovastatin which enhance the bioavailability and SLN are more stable in 4°c $\pm$ 2°c. From the research findings and in-vivo pharmacokinetic evidence, it was hypothesized that the SLN loaded with Lovastatin showed improved bioavailability than the conventional marketed dosage form, by improving the plasma drug concentration profile of AUC and MRT. The chosen and reproducible method for the formulation of SLN was concluded as micro emulsification technique. Therefore, for poorly bioavailable BCS class II drug such as Lovastatin, Solid Lipid Nanoparticle would be a capable drug delivery system.

**ACKNOWLEDGEMENT**

The Authors acknowledge to the Department of Pharmaceutics of GITAM Institute of Pharmacy, Rushikonda, Visakhapatnam, Andhra Pradesh for providing the facilities to do this research in a successful manner.

**Conflict of interest**

The authors declare that they have no conflict of interest for this study.

**Funding support**

The authors declare that they have no funding support for this study.

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