Formulation and In Vivo Evaluation of Proniosomal Gel Based Transdermal Delivery of Atorvastatin Calcium

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ABSTRACT

Atorvastatin calcium is a HMG-CoA reductase inhibitor used in the treatment of hyperlipidaemia. It has oral bioavailability of less than 12%. It also undergoes high first pass metabolism. The objective of the present work was to formulate, optimize and in vivo evaluation of the potential novel proniosomal gel containing atorvastatin for transdermal delivery. On the basis of the preliminary trials a 3-factor, 3-level Box–Behnken design was employed to study the effect of Cholesterol, soya lecithin and Span 60 independent variable on dependent variables (particle size and % entrapment efficiency). Atorvastatin optimized proniosomal formulation F2 shown better particle size and % entrapment efficiency and also the drug release was 99.72% within 24h in slow and controlled manner when compared with control. The particle size and Zeta potential of the optimized atorvastatin proniosomal gel was found to be 65.72 and -10.5 respectively. Optimized batch of Proniosomes was used for the preparation of Atorvastatin - based proniosomal hydrogel by incorporating hydrated Proniosomes to Carbopol matrix to enhance the stability and viscosity of the system. The enhanced skin permeation for prolonged period of time, may lead to improved efficacy and better patient compliance. From in vivo studies the maximal concentrations (Cₘₐₓ) of drug was significantly reduced while the areas under the plasma concentration–time curve (AUC) and t₁/₂ were evidently increased and extended. This study suggests that proniosomal gel of atorvastatin would be a promising alternative to improve the bioavailability problems of atorvastatin.

Keywords: Atorvastatin, Proniosomes, Box Behnken Design, Span 60, zeta potential

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INTRODUCTION

Modification of the particle composition or surface can adjust the affinity for the target site and the drug release rate, and the slowing drug release rate may reduce the toxicity of drug. So, these carriers play an increasingly important role in drug delivery 1.

The use of nonionic surfactant vesicles (niosomes) as drug carrier systems has distinct advantages over conventional dosage 2. They can increase the drug efficacy, reduce drug side effects, increase the drug solubility, and develop an effective topical delivery 3.

Transdermal drug delivery system (TDDS) is among the most widely employed system to overcome the issues associated with oral route. TDDS offers two advantages firstly, self-administration and secondly of termination of drug exposure at any point of time, if required4. Due to which it exhibits high level of patient compliance with low levels of intra and inter-patient variability5. Among various strategies, vesicular systems like niosomes exhibits substantial potential to overcome such barrier 6, 7. Proniosomes was introduced to overcome such problems as it provides ease of transportation, distribution, storage and dosing. Proniosomes are usually dry powder or gel, which can be hydrated just before use resulting in the formation of proniosomes. Proniosome gel when applied to skin under occlusive conditions, they get hydrated with the skin moisture and converted to niosomes 8.

Atorvastatin calcium is a HMG-CoA reductase inhibitor used in the treatment of hyperlipidaemia 9. It has oral bioavailability of less than 12%. It also undergoes high first pass metabolism. It is highly soluble in acidic pH and absorbed more in the upper part of the GIT10.

In the present study preparation and in vivo evaluation of Atorvastatin proniosomes to increase bioavailability and controlled release up to 24h. To enhance the stability and viscosity of the system, the proniosomes were mixed with carbopol gel11.

MATERIALS AND METHOD

Materials

Atorvastatin calcium was received as a gift sample from Aurobindo Pharma Ltd, Hyderabad. Span 60 and Soya lecithin were purchased from SD Fine Chemicals (Mumbai, India). Cholesterol 95% stabilized was purchased from Acros Organics. Carbopol P 934 was obtained from MSN Laboratories, Hyderabad. Dialysis tubing was purchased from Hi-Media Laboratories (Mumbai, India). All other chemicals and solvents were of analytical grade and were used without further purification.
Methodology

Design of experiments

Initially, preliminary experiments (one factor at a time approach) were performed to determine the main factors and the appropriate ranges in which the optima lie. Among all the non-ionic surfactants Span 60 was selected based on results of the preliminary experiments. Further, the effect of three factors (Concentrations of surfactant, cholesterol and Soya lecithin) on the particle size and % entrapment efficiency was tested. Through preliminary screening the concentrations of surfactant, cholesterol and Soya lecithin were identified as the most significant variables within the range of 100-300 mg, 10-50mg, and 50-200 mg, respectively.

On the basis of the preliminary trials a 3-factor, 3-level Box–Behnken design was employed to study the effect of each independent variable on dependent variables (particle size and % entrapment efficiency). The independent factors and the dependent variables used in the design are listed in Table 1. The experiments were conducted as for the design and the obtained responses for the dependent variables were given in Table 2. The response surfaces of the variables inside the experimental domain were analyzed using Stat-Ease Design Expert ® software V8.0.1. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

Preparation of proniosomal gel

Atorvastatin Proniosomes were prepared by Coacervation phase separation method 12. The non-ionic surfactant, cholesterol and soya lecithin were taken in appropriate amount as shown in Table 2.

| Independent variables | Levels |
|-----------------------|--------|
| Variable Name         | Units  |
| A Concentration of surfactant (Span 60) | mg     |
| B Concentration of cholesterol | mg |
| C Concentration of soya lecithin | mg |

| Dependent variable | Goal |
|--------------------|------|
| Y1 Particle size   | nm   |
| Y2 Entrapment efficiency | % |

Table 2: Box–Behnken experimental design and observed responses

| Run | Factor A Concentration of Span 60 | Factor B Concentration of cholesterol | Factor C Concentration of Soya lecithin | Response Y1 Particle size | Response Y2 Entrapment efficiency |
|-----|----------------------------------|--------------------------------------|----------------------------------------|---------------------------|----------------------------------|
| 1   | 200                              | 30                                   | 125                                    | 73.21                     | 93.23                            |
Characterisation of proniosomal gel

Surface morphology
Surface morphology of Proniosomal gel was done by using optical microscopy with reported method\textsuperscript{13, 14}.

Entrapment efficiency
The entrapment efficiency was determined by separating the unentrapped drug. Proniosomal gel (100mg) was hydrated with 10 ml of distilled water by manual shaking for 5 minutes, to form niosomal dispersion. The percentage entrapment efficiency of atorvastatin in hydrated Proniosomes was determined by centrifugation technique. The eppendorff containing hydrated Proniosomes was centrifuged at 14,000 rpm, at 4 °C for 30 min (Pico 21 centrifuge, Thermo Scientific HERAE US). The supernatant containing unentrapped drug was withdrawn and analysed for free drug content by measuring absorbance at $\lambda_{\text{max}}$ 246 nm using UV spectrophotometer\textsuperscript{15,16}.

Particle size and size distribution analysis
For all the batches of Proniosomes Particle size analysis was carried out using Malvern Zeta sizer Nano ZS (Malvern Instruments, UK). The freshly prepared hydrated Proniosomes were dispersed in double distilled water (DDW) and was used to characterize the Particle size. Size measurements were done in triplicate for each sample. Polydispersity Index (PDI) was also determined as a measure of homogeneity. Zeta potential of the Proniosomes formulations was determined to estimate stability of the formulations.
SEM analysis of Proniosomes

Scanning electron microscopy (SEM) was applied to assess the shape and surface morphology of microspheres (HITACHI, S-3700N) 17, 18.

In-vitro drug release studies

In-vitro release studies of hydrated atorvastatin proniosomal dispersions were done using homemade static Franz glass diffusion cells. These cells consist of donor and receptor chambers separated by a cellulose membrane with molecular weight cut-off of 12 000–14 000 (Spectrum Medical Inc., Los Angeles, CA); the area of diffusion was 1.7 cm². The dialysis membrane was hydrated in the receptor medium, which consisted of a phosphate buffer pH 6.8, for 24 h before mounting into a Franz diffusion cell. A 2.5 ml hydrated atorvastatin proniosomal dispersion was placed in the donor chamber and the receptor chamber was filled with 7.5 ml receptor medium and stirred continuously at 100 rpm at 37 °C and samples were withdrawn at different time intervals for 24h from the receptor chamber through a side-arm tube. After each withdrawal of sample, an equal volume of receptor medium was added to the receptor chamber to maintain a constant volume throughout the study. Samples were analyzed for atorvastatin concentration using UV spectrophotometer at λ_{max} 246 nm. measurements were carried out in triplicate 19,20.

Stability studies

The stability study was performed according to ICH guidelines. The proniosomal gel formulations were filled in tightly closed glass vials and subjected to stability testing. The formulations were kept at refrigerated conditions (4 ± 1 °C) and at room temperature (25 ± 2 °C) and were analyzed for Particle size and entrapment efficiency after 3 months 22.

Formulation of Atorvastatin - based proniosomal hydrogel

Based on the previously mentioned characterization, and the results of the main effects of the adopted factorial design a candidate formula F2 with adequate Particle size, highest entrapment efficiency and high % of drug released after 5 h was selected. The selected F2 hydrated proniosomal formulation was formulated into hydrogel by adding 1% (w/w) Carbopol P 934 under magnetic stirring at 800 rpm. Stirring was continued until Carbopol was dispersed. The dispersions were neutralized using triethanolamine solution 23.

Ex-vivo permeation study

Ex-vivo permeation study was carried out using male wistar rat skin as reported by Ibrahim et al. 24.
Skin irritation study of Proniosomal hydrogel:
0.5 gm Proniosomes gel was applied to 3 rats on an area of skin approximately 1” x 1” (2.54 x 2.54 cm) square. Animals were returned to their cages. After a 24 hour exposure, the Proniosomes gel was removed. The test sites were wiped with tap water to remove any remaining test article residue\(^{25}\).

Data analysis
Data are expressed as the mean±standard deviation (SD) of the mean and statistical analysis was carried out employing the one-way analysis of variance (ANOVA). A value of \(p<0.05\) was considered statistically significant.

Pharmacokinetic studies

Animal preparation
Healthy male Wistar rats were (weighing approximately 250±25 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25\(^{0}\)C±2\(^{0}\)C, Relative Humidity 45\%±5\%RH and 12 h alternate light and dark cycle) with 100 \% fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water ad libitum.

Pharmacokinetic study
The pharmacokinetic characteristics for Atorvastatin drug suspension and optimized preparation of Atorvastatin proniosomal gel were evaluated using twelve healthy male Wister rats weighing 250g. Rats were divided in to two groups at random, each group containing six animals. First group was administered Atorvastatin suspension by oral route, second group was administered optimized preparation of Atorvastatin proniosomal gel. About 2cm\(^2\) of skin was shaved on the abdominal side of rats. They were fasted for the period of 12h for observations of any unwanted effects and applied the gel equivalent to 10mg of Atorvastatin. Blood samples were withdrawn at time intervals of 0, 1, 2, 4, 8, 12 and 24hrs from retro-orbital venous plexus under ether anaesthesia using glass capillaries into sodium citrate containing eppendorf micro-centrifuge tubes. Plasma was separated by centrifugation using Centrifuge and stored in vials at -70\(^{0}\)C until further analysis\(^{26}\).

Determination of Atorvastatin in Rat plasma by HPLC method
Determination of Atorvastatin by high performance liquid chromatography using \(C_{18} (100\times4.6\) mm, 5 \(\mu m\) and mobile phase was composed of dibasic phosphate buffer (pH 3.0): acetonitrile in the ratio of 55:45 at a flow rate of 1ml/min with UV detection monitored at 240 nm. The
selected Chromatographic conditions were found to effectively separate amlodipine (Internal standard) (5.1 min) and Atorvastatin (12.1 min) 27.

**Pharmacokinetic data analysis for optimized preparation of proniosomal gel and pure drug suspension**

The area under the drug concentration-time curve from zero to 24h (AUC) was calculated using the trapezoidal rule. The maximum plasma concentration of the drug ($C_{\text{max}}$) and the time to reach $C_{\text{max}}$ ($T_{\text{max}}$) was obtained directly from the plasma profiles.

Where, $\text{AUC}_{\text{test}}$ and $\text{AUC}_{\text{reference}}$ are AUCs obtained after the topical application of the optimized preparation of proniosomal gel formulation and oral administration of the reference standard (pure drug suspension). $\text{Dose}_{\text{test}}$ and $\text{Dose}_{\text{reference}}$ are the doses of the two preparations.

The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

**RESULTS AND DISCUSSION**

**Preparation of Atorvastatin proniosomes**

In this study, the Proniosomes of atorvastatin were formulated, optimized and evaluated for its efficacy in transdermal drug delivery to overcome the major issues associated with its oral delivery. Preliminary experiments conducted using different non-toxic and biocompatible non-ionic surfactants like spans and tweens together with cholesterol and soya lecithin. The phase transition temperature plays a crucial role in the Proniosomes gel formation. Based on the results of preliminary experiments, Span 60 was selected as suitable surfactant for the preparation of atorvastatin Proniosomes.

**Optimization of formulation variables**

Through preliminary experiments the Concentration of surfactant (A), concentration of cholesterol (B) and concentration of soya lecithin (C) were identified as the most significant variables influence the Particle size and entrapment efficiency. The formulations were further optimized by considering the parameters like smaller Particle size, and maximum entrapment efficiency.

Seventeen experiments were required for the response surface methodology based on the Box–Behnken design. Based on the experimental design, the factor combinations yielded different
responses as presented in Table 2. These results clearly specify that the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among all the 17 batches. Data were analyzed using Stat-Ease Design Expert ® software V8.0.1 to obtain analysis of variance (ANOVA), regression coefficients and regression equation. Mathematical relationships were generated using multiple linear regression analysis for the mentioned variables as shown in Table 3. These equations represent the quantitative effect of concentration of surfactant (A), concentration of cholesterol (B) and concentration of soya lecithin (C) and their interaction on Particle size (Y1) and entrapment efficiency (Y2). The values of the coefficients of A, B and C are related to the effect of these variables on the responses Y1 and Y2. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationship respectively. A positive sign represent synergistic effect, while a negative sign indicate antagonistic effect. A backward elimination procedure was adopted to fit the data to the quadratic model. Both the polynomial equations were found to be statistically significant (P<0.01), as determined using ANOVA, as per the provisions of Design Expert software.

Table 3: Regression equations for the responses – Particle size and entrapment efficiency

| Response | Regression equation |
|----------|---------------------|
| Y1       | $72.99 + 45.60 A + 1.43 B + 27.10 C - 13.81 BC + 19.67 A^2 + 29.69 B^2 + 33.84 C^2$ |
| Y2       | $93.30 + 3.13 A - 16.21 B - 0.51 C - 8.54 A^2 - 8.08 B^2 - 8.32 C^2$ |

Figure 1: Response surface plot showing the influence of concentration of cholesterol and concentration of soya lecithin on particle size

The influence of the main and interactive effects of independent variables on the particle size was further elucidated using the perturbation and 3D response surface plots. The relationship between
the dependent and independent variables was further elucidated using 3D response surface plots. **Figure 1** shows the interactive effect of B and C on the particle size (Y1) at fixed level of C. At low levels of A (concentration of surfactant), Y1 increases from 64.68 nm to 97.46 nm. Similarly, at high levels of A, Y1 increases from 158.12 nm to 182.18 nm.

Entrapment efficiency of Proniosomes was found to be in the range of 56.1-96.12 % as shown in **Table 2**. The polynomial equation for entrapment efficiency exhibited a good correlation coefficient (0.9832) and the Model F-value of 97.77 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of ‘Prob>F’ less than 0.0005 indicate model terms are significant. In this case A, B, C and the quadratic term of A^2, B^2 and C^2 are significant model terms as shown in **Table 5**. Results of the equation indicate that the effect of B (concentration of cholesterol) is more significant than A and C. Two variables B & C having the negative effect on the entrapment efficiency, which means these factors, are inversely proportional to the response, whereas, A has positive effect on entrapment efficiency.

The influence of the main and interactive effects of independent variables on the entrapment efficiency was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on entrapment efficiency are as shown in **Figure 2**. It is found that all the variables are having interactive effects for the response Y2. The 3D response surfaces plots of the response Y2 are shown in **Figure 2** to depict the interactive effects of independent variables on response Y2, one variable was kept constant while the other two variables varied in a certain range. The shapes of response surfaces plots reveal the nature and extent of the interaction between different factors. The interaction between A and B on entrapment efficiency at a fixed level of C is shown in **Figure 4**. At low levels of A, Y2 reduced from 92.34 % to 56.1 %. Similarly at high levels of A, Y2 reduced from 96.12 % to 62.18%. At low levels of B, Y2 reduced from 96.12 % to 91.74 %. Similarly at high levels of B, Y2 reduced from 64.32 % to 56.1%. At low levels of C, Y2 reduced from 91.74 % to 59.72 %. Similarly at high levels of C, Y2 reduced from 91.82 % to 64.32 %.

**Optimization and confirmation experiments**

A numerical optimization technique using the desirability approach was employed to prepare atorvastatin Proniosomes with the desired responses. Constraints like minimizing the particle size in addition to maximizing the entrapment efficiency were set as goals to locate the optimum settings of independent variables. The optimized levels and predicted values of Y1 and Y2 are shown in **Table 4**. Obtained Y1 and Y2 values were in a close agreement with the predicted values. This demonstrated the reliability of the optimization procedure in predicting the operating
parameters for the preparation of atorvastatin Proniosomes. All the three batches of obtained atorvastatin Proniosomes were subjected to further characterisation.

![Response surface plots showing the interactions between concentration of surfactant and concentration of cholesterol on entrapment efficiency](image)

**Figure 2:** Response surface plots showing the interactions between concentration of surfactant and concentration of cholesterol on entrapment efficiency

**Table 4:** Optimized values obtained by the constraints applies on Y1 and Y2

| Independent variable | Nominal values | Predicted values | Observed values |
|----------------------|----------------|------------------|-----------------|
|                      |                | Particle size (Y1) | Entrapment efficiency (Y2) | Batch | Particle size (Y1) | Entrapment efficiency (Y2) |
| Concentration of surfactant (A) | 166.20 | 63.2398 | 97.74 | F1 | 66.12 | 96.34 |
| Concentration of cholesterol (B) | 17.46 | 65.72 | 97.42 |
| Concentration of soya lecithin (C) | 103.94 | 65.12 | 97.13 |

The Particle size of diluted Proniosomes are shown in **Table 4**, it ranges from 65.12 ± 3.43 nm to 66.12 ± 4.12 nm with unimodal particle size distribution, which favours transdermal delivery of Atorvastatin. Since the smaller Particle size is advantageous to decrease the irritation and improve the penetration of Particles into the skin. All the Proniosomes formulations were negatively charged, which was due to the negative charge present on the soya lecithin. The zeta potential values were high in all the formulations (**Table 5**). The high zeta potential increases the repulsion between the Particles and thus prevents their aggregation and flocculation. So it electrically stabilizes the system.
Table 5: The mean Particle size, PDI, Entrapment efficiency and zeta potential of optimized formulations

| Batch | MVS ± SD (nm) | PDI   | ZP ± SD (mV) | % EE ± SD |
|-------|---------------|-------|--------------|-----------|
| 1     | 66.12 ± 1.62  | 0.712 | -11.8 ± 0.73 | 96.34 ± 0.18 |
| 2     | 65.72 ± 2.12  | 0.605 | -10.5 ± 0.18 | 97.42 ± 0.37 |
| 3     | 65.12 ± 1.13  | 0.782 | -13.5 ± 0.82 | 97.13 ± 0.82 |

n = 3 (p < 0.05)

Surface morphology

Atorvastatin loaded proniosomal gel formulation prepared using optimum ratio of lecithin and cholesterol (9:1) demonstrated lamellar structures under compound microscope. Observation under an optical microscope revealed that proniosomal gel was progressively, but rapidly converted to Proniosomes almost completely within minutes.

Scanning electron microscopy (SEM) for Atorvastatin proniosomes

Scanning electron microscope studies of optimized formulation of atorvastain proniosomes revealed oval shaped globules. The size is within nanometers. There are clear liquid droplets without any pores (Figure 3).

Drug release study

From Figure 4, it is clear that cumulative release of drug from control was higher than from the Proniosomes. This was due to the fact that atorvastatin was sufficiently lipophilic and it partitions in favour of the Proniosomes, which resulted in the slower release of atorvastatin from Proniosomes.
Figure 4: In-vitro release of atorvastatin from Proniosomes.

Stability study

The purpose of stability testing is to provide the evidence on how the quality of drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity and light, stability studies indicates that no significant difference (p < 0.05) was found in entrapment efficiency and particle size of optimized formulation stored at refrigerated conditions and at room temperature for 3 months.

Formulation of Atorvastatin - based proniosomal hydrogel

Atorvastatin proniosomal hydrogel was prepared using Carbopol P 934. Optimized batch of Proniosomes was used for the preparation of Atorvastatin - based proniosomal hydrogel by incorporating hydrated Proniosomes to Carbopol matrix. It is well known fact that the Proniosomes get hydrated by skin moisture to form Proniosomes, which have the ability to alter the transportation of drug across the skin. Adsorption and fusion of pronisome onto the skin surface would facilitate the permeation of drug, barrier properties of stratum corneum are modified because non-ionic surfactant act as penetration enhancers as it causes the disruption of tightly packed lipids that are filled in the extracellular spaces of stratum corneum. The release rate (flux) of atorvastatin across the membrane and excised skin differs significantly (Table 6), which indicates about the barrier properties of skin. Interaction between skin and Proniosomes component may justify these differences. Association and fusion of Proniosomes to the skin surface resulted in higher flux due to the direct transfer of drug from the Particles.
### Table 6: Flux of Atorvastatin from Proniosomes

| Formulation | Flux (μg cm⁻² h⁻¹) | Egg membrane | Rat skin |
|-------------|---------------------|--------------|----------|
| F2          | 43.231 ± 2.68       | 193.754 ± 3.126 |
| Control     | 312.115 ± 2.37      | 55.635 ± 5.12  |

### Skin Irritation studies

The irritation studies was conducted with animal ethical committee approval bearing No:02/IAEC/VIPER/Ph.D/2017-18, on male wistar rats (n= 3). Formalin was applied as standard irritant. The rats were scored for erythema and edema scale [21, 33]. The incidences of erythema and edema was significantly lower (0.18 ± 0.112; p < 0.05) in Proniosomes formulations treated rats than those treated with standard irritant, formalin. So, it was concluded that the formulations were non-irritant and safe.

### Pharmacokinetic study

**Figure 5:** shows the plasma profiles of Atorvastatin in rats after the oral administration of the reference (pure drug suspension), optimized preparation of Atorvastatin proniosomal gel

#### Table 7: Pharmacokinetic Parameters of Atorvastatin proniosomal gel, Atorvastatin (suspension)

| Pharmacokinetic Parameters | Atorvastatin (Suspension) | Atorvastatin proniosomal gel |
|----------------------------|---------------------------|-----------------------------|
| C max (ng/ml)              | 10.5±0.15                 | 8.4±0.12                    |
| AUC 0-t (ng h/ml)          | 62±1.55                   | 85±1.74                     |
| AUC 0-inf (ng h/ml)        | 85±3.24                   | 117±2.45                    |
| T max (h)                  | 2.00±0.03                 | 4.00±0.02                   |
| t 1/2 (h)                  | 3.50±0.01                 | 7.00±0.04                   |
The Atorvastatin plasma concentrations in rats treated with optimized preparation of proniosomal gel was significantly higher than those treated with pure drug suspension. Plasma pharmacokinetic parameters of Atorvastatin after administration of the formulations to Wister rats are shown in Table 7 & Figure 5. \( C_{\text{max}} \) of the optimized preparation of proniosomal gel was 8.4±0.12ng /ml, was lower as compared to \( C_{\text{max}} \) of the pure drug suspension, i.e., 10.5±0.15ng/ml. \( T_{\text{max}} \) of optimized preparation of proniosomal gel, and pure drug suspension was 4.00±0.02hr, 2.00±0.03hr respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. AUC_{0-inf} for optimized proniosomal gel formulation was higher (117±2.45ng h/ml) than the pure drug suspension 85±3.24ng h/ml. Statistically, AUC_{0-t} of the optimized preparation of proniosomal gel was significantly higher (p<0.05) as compared to pure drug suspension.

**CONCLUSION**

This work has demonstrated the use of a 3-factor, 3-level Box–Behnken design, regression analysis, and contour plots in optimizing the formulation variables in the preparation of atorvastatin Proniosomes by Coacervation phase separation method. Atorvastatin optimized proniosomal formulation F2 shown better drug release of 99.28% within 24h in slow and controlled manner when compared with control. The particle size and Zeta potential of the optimized atorvastatin proniosomal gel was found to be 65.72 nm and -10.5 mV respectively. Optimized batch of Proniosomes was used for the preparation of Atorvastatin - based proniosomal hydrogel by incorporating hydrated Proniosomes to Carbopol matrix to enhance the stability and viscosity of the system. The enhanced skin permeation for prolonged period of time, may lead to improved efficacy and better patient compliance than the conventional formulations. From in vivo studies of the optimized proniosomal gel, maximal concentrations (\( C_{\text{max}} \)) of optimized gel was significantly reduced while the areas under the plasma concentration–time curve (AUC) and \( t_{1/2} \) were evidently increased and extended. This study suggests that proniosomal gel of atorvastatin would be a promising alternative to improve the bioavailability problems of atorvastatin.

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