



Research Article

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Development and *in vivo* Evaluation of Lovastatin Loaded Transdermal Proniosomal Gel by Design of Experiment

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ABSTRACT

The objectives of the present study were to define effects on the hypercholesterolemia activity and pharmacokinetics of a novel transdermal proniosomal gel incorporating Lovastatin prepared by coacervation phase separation method. 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 Tween 80 on dependent variables (particle size, % entrapment efficiency, and % of drug release). Lovastatin optimized proniosomal formulation F1 shown better particle size and % entrapment efficiency and drug release of 99.49% within 24 h in slow and controlled manner when compared with control. The particle size and zeta potential of the optimized lovastatin proniosomal gel was found to be 138.82 nm and -11.4 mV respectively. Optimized batch of Proniosomes was used for the preparation of Lovastatin-based proniosomal hydrogel by incorporating hydrated Proniosomes to Carbopol matrix to enhance the stability and viscosity of the system. From *in vivo* studies the maximal concentrations (C_{max}) of drug was significantly reduced while the areas under the plasma concentration-time curve (AUC) and $t_{1/2}$ were evidently increased and extended. The results suggest that proniosomes can act as promising carrier which offers an alternative approach for transdermal delivery of Lovastatin. The study demonstrated the effectiveness of proniosomal preparation of Lovastatin for effective management of hypercholesterolemia to reduce risk of cardiovascular disease.

Keywords: Lovastatin, Proniosomal gel, Box Behnken Design, Soya lecithin, Tween 80.

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INTRODUCTION

The role of the novel drug delivery system is not only limited to a drug package convenience and ease of

administration but along with this it is also needed to provide better therapeutic efficacy and safety by delivering the drug molecules to the target site in the

most convenient manner. These novel carriers provide sustained drug release for prolonged duration in targeted tissue thus resulting in enhanced therapeutic efficacy and minimized side effects. [1]

Drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs. [2]

Transdermal drug delivery system (TDDS) is among the most widely employed system to overcome the issues associated with oral route. TDDS increases the therapeutic efficacy of many drugs by preventing their conversion to undesirable metabolites and also helps in maintaining uniform plasma levels *in-vivo*. [3-4] Among various strategies, vesicular systems like niosomes exhibits substantial potential to overcome such barrier. These vesicles interact with the horny layer and improve the permeability of drug across stratum corneum. It also acts as drug reservoir and provides the controlled release of drug. Further, the release rate can be adjusted by either changing the composition or by surface modification. However, sedimentation, aggregation or fusion and leakage of vesicles are one of the common drawbacks associated with niosomal preparations. [5-6] 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 niosomes. Proniosome gel when applied to skin under occlusive conditions, they get hydrated with the skin moisture and converted to niosomes. [7]

Lovastatin is an antihyperlipidemic drug used to reduce cholesterol in the treatment of hyper lipidemias particularly in type - 2a and 2b hyper lipoproteinaemias. The absorption of Lovastatin following oral administration is approximately 30%. It undergoes high first pass metabolism. [8]

In the present study the coacervation phase separation method was used for the preparation and optimization of Lovastatin proniosomes, as this method is simple and easy to scale up. The proniosomes are thus of interest from a technical viewpoint and allow a wider scope to be used to study the influence of various formulation variables. To enhance the stability and viscosity of the system, the proniosomes were mixed with carbopol gel. [9]

MATERIALS AND METHODS

Materials

Lovastatin calcium was received as a gift sample from Aurobindo Pharma Ltd, Hyderabad. Tween 80 and Soya lecithin were purchased from SD Fine Chemicals (Mumbai, India). Cholesterol 95% stabilized was purchased from Granules India Limited, Hyderabad. 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 optimum lie. Among all the non-ionic surfactants Tween 80 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. The independent factors and the dependent variables used in the design are listed in Table 1. Through preliminary screening the concentrations of surfactant, cholesterol and Soya lecithin were identified as the most significant variables within the range of 50-200 mg, 10-50 mg, and 50-150 mg, respectively.

Preparation of proniosomal gel

Lovastatin Proniosomes were prepared by Coacervation phase separation method. [10] The non-ionic surfactant (Tween 80), cholesterol and soya lecithin were taken in appropriate amount as shown in Table 2. All the components were taken in a wide mouth glass tube and dissolved in ether. The drug, Lovastatin 10mg was dissolved in ethanol (2.5 ml). After mixing all ingredients, the open end of the glass vial was covered with a lid to prevent loss of solvent, and the glass vial warmed in a water bath at $65 \pm 3^\circ\text{C}$, to dissolve all the ingredients. The aqueous phase (0.1% glycerol solution) was added and warmed in a water bath until a clear solution is formed that on cooling converted into a proniosomal gel. The gel obtained was preserved in the same glass tubes in dark for characterization. The proniosomal structure was a liquid crystalline-compact Proniosomes hybrid that was converted into Proniosomes upon hydration. The system hydrated by 10 ml of phosphate buffer (pH 6.8) under sonication (Ultrasonic Bath Sonicator, Make - Frontline Electronics, Model- FS-2) at 37°C .

Characterization of proniosomal gel

Surface morphology

Surface morphology of Proniosomal gel was done by using optical microscopy with reported method. [11]

Entrapment efficiency

The entrapment efficiency was determined by separating the untrapped drug. Proniosomal gel (100 mg) was hydrated with 10 ml of distilled water by manual shaking for 5 minutes, to form niosomal dispersion. The percentage entrapment efficiency of lovastatin 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 untrapped drug was withdrawn and analysed for free drug content by measuring absorbance at λ_{max} 238 nm using UV-Visible spectrophotometer. [12-13]

Table 1: List of dependent and independent variables in in Box-Behnken design

Independent variables			Levels		
Variable	Name	Units	Low	Mid dle	High
A	Concentration of Tween 80	mg	50	125	200
B	Concentration of cholesterol	mg	10	30	50
C	Concentration of soya lecithin	mg	50	100	150
Dependent variable			Goal		
Y1	Particle size	nm	Minimize		
Y2	Entrapment efficiency	%	Maximize		

Table 2: Box-Behnken experimental design and observed responses

Run	Factor A Concentration of Tween 80	Factor B Concentration of cholesterol	Factor C Concentration of soya lecithin	Response Y1 Particle size	Response Y2 Entrapment efficiency
1	125	30	100	176.72	92.34
2	125	30	100	175.98	93.12
3	50	30	50	134.83	88.76
4	125	50	150	173.65	78.92
5	50	30	150	140.48	89.12
6	200	50	100	222.89	81.96
7	125	10	50	182.76	97.13
8	125	50	50	188.44	72.82
9	50	50	100	148.66	76.46
10	125	30	100	175.52	93.42
11	125	30	100	176.36	92.54
12	50	10	100	137.28	98.12
13	200	30	150	230.92	86.29
14	125	30	100	175.12	93.63
15	125	10	150	168.71	94.85
16	200	30	50	206.16	82.53
17	200	10	100	212.62	95.81

*Each formulation containing Lovastatin 10 mg

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). [14-15]

In vitro drug release studies

In vitro release studies of hydrated lovastatin proniosomal dispersions were done using home-made 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 5.5 containing 1% Tween 20, for 12 h before mounting into a Franz diffusion cell. A

2.5 ml hydrated lovastatin 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 24 h 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 lovastatin concentration using UV spectrophotometry at $\lambda_{\max}$ 238 nm. Measurements were carried out in triplicate. [16]

Stability studies

The stability study of proniosomes 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 ($4 \pm 1^\circ\text{C}$) and at room temperature ($25 \pm 2^\circ\text{C}$) and were analyzed for Particle size and entrapment efficiency after 3 months. [17]

Formulation of Lovastatin - based proniosomal hydrogel

Based on the previously mentioned characterization, and the results of the main effects of the adopted factorial design a candidate formula F1 with adequate Particle size, highest entrapment efficiency and high % of drug released after 5 h was selected. The selected F1 hydrated proniosomal formulation was formulated into hydrogel by adding 1% (w/w) Carbopol P 934 under magnetic stirring at 800 rpm to enhance the stability and viscosity of the system. Stirring was continued until Carbopol was dispersed. The dispersions were neutralized using triethanolamine solution. [18]

Ex vivo permeation study

Ex vivo permeation study was carried out using male wistar rat skin as reported by Ibrahim *et al.* [19]

Data analysis

Data are expressed as the mean $\pm$ 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 $25 \pm 2^\circ\text{C}$, Relative Humidity $45\% \pm 5\%$ 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 Lovastatin drug suspension and optimized preparation of Lovastatin proniosomal gel were evaluated using twelve healthy

Male Wister rats weighing 250 g. Rats were divided in to two groups at random, each group containing six animals. First group was administered Lovastatin suspension; second group was administered optimized preparation of Lovastatin proniosomal gel. About 2 cm² of skin was shaved on the abdominal side of rats. They were fasted for the period of 12 h for observations of any unwanted effects and applied the gel equivalent to 10 mg of Lovastatin. Blood samples were withdrawn at time intervals of 1, 2, 4, 8, 12, 24 hours from retro-orbital venous plexus under ether anesthesia using glass capillaries into sodium citrate containing eppendorf micro-centrifuge tubes. Plasma was separated by centrifugation using Centrifuge and stored in vials at -70°C until further analysis. [20]

Determination of Lovastatin in Rat plasma by HPLC method

Determination of Lovastatin by high performance liquid chromatography using C8 (4.6 × 250 mm, 5µm) column with the mobile phase containing acetonitrile: phosphate buffer (pH 4.0 ± 0.5) in the ratio of 65:35 v/v, the optimized flow rate was 0.7 ml/min and the UV detection was carried out at 240 nm. The retention time of Lovastatin and niacin (Internal standard) were found to be 3.093 min and 6.196 min respectively. [21]

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

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

Where, AUC_{test} and AUC_{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). Dose_{test} and Dose_{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 Lovastatin proniosomes

In this study, the Proniosomes of lovastatin 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, Tween 80 was selected as suitable surfactant for the preparation of lovastatin Proniosomes.

Optimization of formulation variables

Through preliminary experiments the Concentration of Tween (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 Tween 80 (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.

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

Response	Regression equation
Y1	106.75908 + 0.51890A + 0.20169B + 0.003925C 88.45427 + 0.044000 A - 0.42058B + 0.29670 C
Y2	+1.30167 AB + 2.095 BC -3.71333 A ² -7.08437 B ² - 1.69850 C ²

The particle size of proniosomes was found to be in the range of 134.83 nm to 230.92 nm. The mathematical model generated for Particle size (Y1) was found to be significant with Model F-value of 86.98. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise (*P* < 0.0001) and R² value of 0.9525. The model indicated the linear relationship between particle size and independent variables. The independent variables A, B, and C have linear effects on the 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 A and B on the particle size (Y1) at fixed level of C. At low levels of B (concentration of cholesterol), Y1 increases from 137.28 nm to 212.62 nm. Similarly, at high levels of B, Y1 increases from 148.66 nm to 222.89 nm.

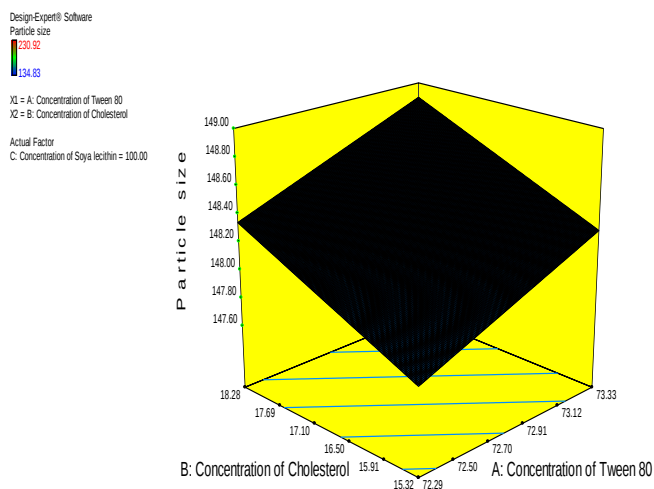


Fig. 1: Response surface plot showing the influence of concentration of tween 80 and concentration of cholesterol on particle size

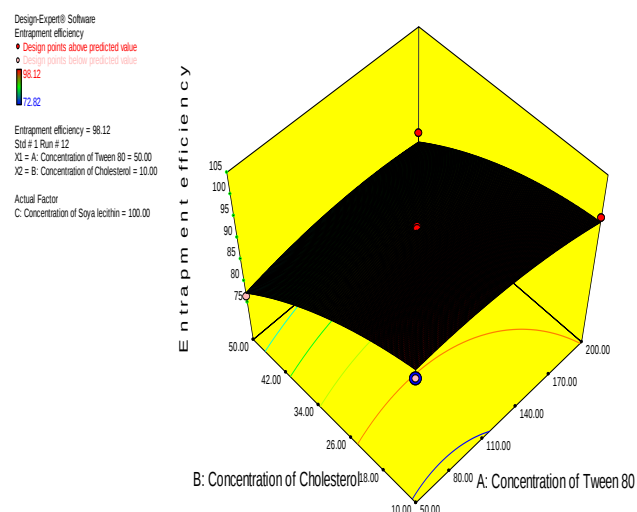


Fig. 2: Response surface plots showing the interactions between concentration of tween 80 and concentration of cholesterol on entrapment efficiency

Entrapment efficiency of Proniosomes was found to be in the range of 72.82 – 98.12% as shown in Table 2. The polynomial equation for entrapment efficiency exhibited a good correlation coefficient (0.9724) and the Model F-value of 35.18 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. 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.

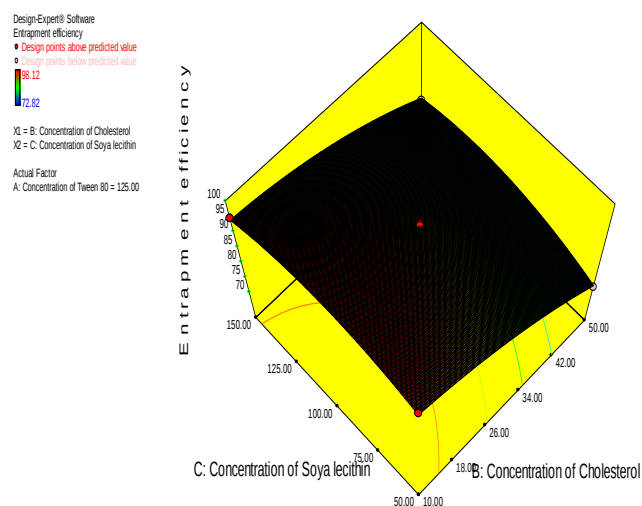


Fig. 3: Response surface plots showing the interactions between concentration of cholesterol and concentration of soya lecithin on entrapment efficiency

Optimization and confirmation experiments

A numerical optimization technique using the desirability approach was employed to prepare lovastatin 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. All the three batches of obtained lovastatin Proniosomes were subjected to further characterization.

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)	50.00			F1	138.82	98.345
				F2	139.12	97.146
Concentration of cholesterol (B)	10.00	134.987	98.1204	F3	138.36	97.435
Concentration of soya lecithin (C)	57.58					

n=3; Conc. of drug = 10 mg

The Particle size of diluted Proniosomes are shown in Table 4, it ranges from 138.36 ± 1.43 nm to 139.12 ± 2.13 nm with unimodal particle size distribution, which favours transdermal delivery of Lovastatin. Since the smaller Particle size is advantageous to decrease the irritation and improve the penetration of Particles into the skin. The polydispersity index in all the formulations was low (< 0.730), which indicated the

homogeneity of the preparation. 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 $\pm$ SD (nm)	PDI	ZP $\pm$ SD (mV)	% EE $\pm$ SD
1	138.82 $\pm$ 0.56	0.730	-11.4 $\pm$ 0.12	98.345 $\pm$ 0.78
2	139.12 $\pm$ 2.13	0.605	-10.5 $\pm$ 0.18	97.146 $\pm$ 0.17
3	138.36 $\pm$ 1.43	0.782	-13.5 $\pm$ 0.82	97.435 $\pm$ 0.12

n = 3 (p < 0.05)

Lovastatin loaded proniosomal gel formulation prepared using optimum ratio of lecithin and cholesterol demonstrated lamellar structures under compound microscope. Hydration of this gel with saline solution leads to swelling of bilayers as well as Particles due to interaction of water with polar groups of surfactant. The bilayer tends to form spherical structure randomly giving rise to multilamellar, multivesicular structures. When shaken with aqueous phase, complete hydration takes place leading to formation of Proniosomes. Observation under an optical microscope revealed that proniosomal gel was progressively, but rapidly converted to Proniosomes almost completely within minutes.

SEM for Lovastatin proniosomes

Scanning electron microscope studies of optimized formulation of Lovastatin revealed oval shaped globules. The size is within nanometers. There are clear liquid droplets without any pores (Figure 4).

Drug release study

From Figure 5, it is clear that cumulative release of drug from control (Lovastatin in 30% PEG) was faster than from the Proniosomes. This was due to the fact that lovastatin was sufficiently lipophilic and it partitions in favour of the Proniosomes, which resulted in the slower release of lovastatin 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 Lovastatin-based proniosomal hydrogel

Lovastatin proniosomal hydrogel was prepared using Carbopol P 934.

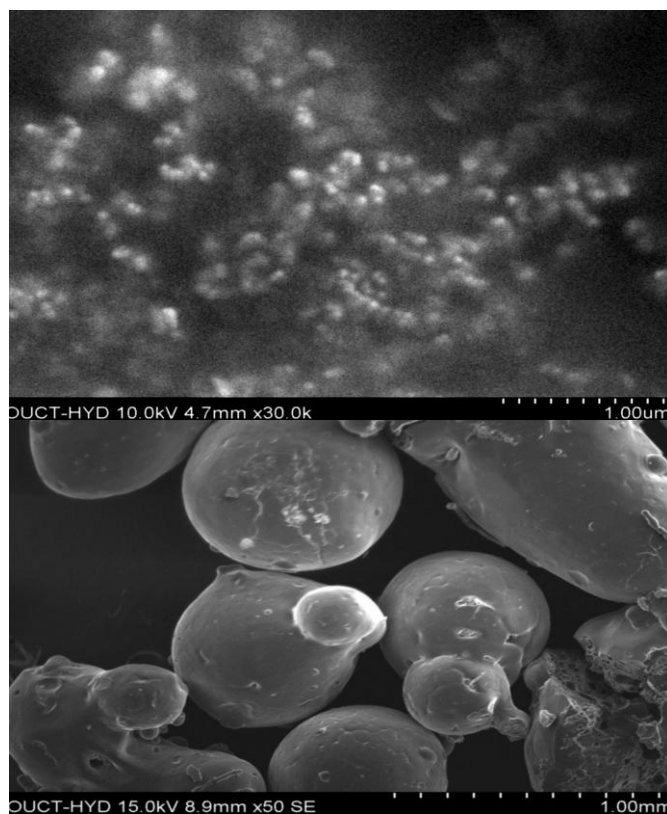


Fig. 4: SEM photographs of Lovastatin proniosomes

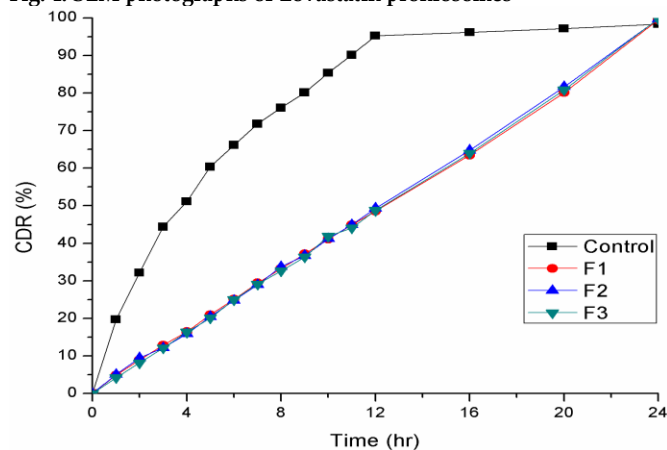


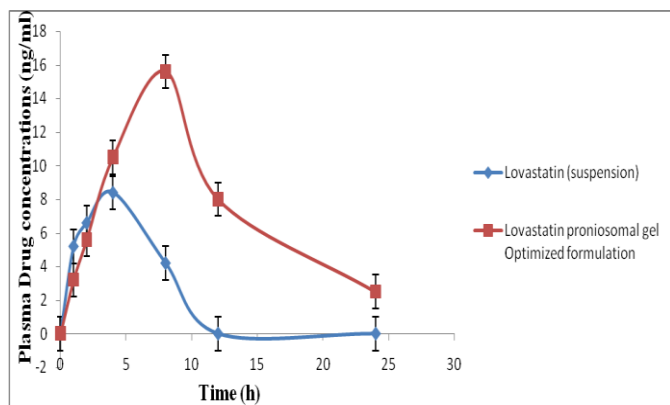
Fig. 5: In vitro release of lovastatin from Proniosomes.

Ex vivo permeation study

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 proniosome 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 lovastatin 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 Lovastatin from optimized Proniosomes

Formulation	Flux ($\mu\text{g cm}^{-2} \text{h}^{-1}$)	
	Egg membrane	Rat skin
F1	51.286 $\pm$ 4.67	216.234 $\pm$ 2.457
Control	426.2125 $\pm$ 3.33	72.112 $\pm$ 2.672

**Fig. 6:** shows the plasma profiles of Lovastatin in rats after the oral administration of the reference (pure drug suspension), optimized preparation of Lovastatin proniosomal gel**Table 7: Pharmacokinetic Parameters of Lovastatin proniosomal gel, Lovastatin (suspension)**

Pharmacokinetic Parameters	Lovastatin (suspension)	Lovastatin proniosomal gel
$C_{\max}$ (ng/ml)	9.4 $\pm$ 0.56	7.5 $\pm$ 0.15
AUC_{0-t} (ng h/ml)	62 $\pm$ 1.55	104 $\pm$ 1.74
$AUC_{0-\infty}$ (ng h/ml)	105 $\pm$ 4.24	145 $\pm$ 3.45
$T_{\max}$ (h)	2.00 $\pm$ 0.4	4.00 $\pm$ 0.12
$t_{1/2}$ (h)	4.32 $\pm$ 0.0	6.30 $\pm$ 0.0

Skin Irritation studies

The irritation studies were 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. The incidences of erythema and edema was significantly lower (0.23 ± 0.421 ; $p<0.05$) 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

The Lovastatin plasma concentrations in rats treated with optimized preparation of proniosomal gel was significantly higher than those treated with pure drug suspension. Pharmacokinetic parameters of Lovastatin after administration of the formulations to Wister rats are shown in Table 7 and Figure 6. $C_{\max}$ of the optimized formulation of proniosomal gel was $7.5 \pm 0.15 \text{ ng/ml}$, was lower as compared to $C_{\max}$ of the pure drug suspension, i.e., $9.4 \pm 0.56 \text{ ng/ml}$. $T_{\max}$ of optimized preparation of proniosomal gel and pure drug suspension was 4.00 ± 0.12 and 2.00 ± 0.4 hours 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. $AUC_{0-\infty}$ for optimized proniosomal gel formulation was higher ($145 \pm 3.45 \text{ ng h/ml}$)

h/ml) than $AUC_{0-\infty}$ of the pure drug suspension $105 \pm 4.24 \text{ ng h/ml}$. Statistically, AUC_{0-t} of the optimized preparation of proniosomal gel was significantly higher ($p<0.05$) as compared to pure drug suspension. Higher amount of drug concentration in blood indicated better systemic and prolong absorption of Lovastatin from optimized proniosomal gel formulation as compared to the pure drug suspension.

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 lovastatin Proniosomes by Coacervation phase separation method. Optimized batch of Proniosomes was used for the preparation of Lovastatin - based proniosomal hydrogel by incorporating hydrated Proniosomes to Carbopol matrix. The particle size and zeta potential of the optimized formulation was found to be 138.82 nm and -11.4 mV respectively. Optimized batch of Proniosomes was used for the preparation of lovastatin - based proniosomal hydrogel by incorporating hydrated Proniosomes to Carbopol matrix to enhance the stability and viscosity of the system. Lovastatin optimized proniosomal formulation F1 shown better drug release of 99.72% within 24 h in slow and controlled manner when compared with control. From *in vivo* studies of the optimized proniosomal gel, the maximal concentration ($C_{\max}$) was significantly reduced while the areas under the plasma concentration-time curve (AUC) and $t_{1/2}$ were evidently increased and extended. The results suggest that proniosomes can act as promising carrier which offers an alternative approach for transdermal delivery of Lovastatin. The enhanced skin permeation for prolonged period of time, may lead to improved efficacy and better patient compliance than the conventional formulations.

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