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Formulation Development and Characterization of Proniosomal Gel Loaded With Adapalene for Acne Vulgaris

Dr.A.Abdul Hasan Sathali *, R.Ajay Kumar
College of Pharmacy, Madurai Medical College, Madurai, Tamilnadu

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ABSTRACT

The study aimed to develop a proniosomal gel loaded with Adapalene for sustained drug release and reduced side effects. The formulation was prepared using the coacervation phase separation method with varying concentrations of surfactants (Tween-80), cholesterol, and soya lecithin. The optimal concentrations of these factors were determined using a 3-factor, 3-level Box Behnken design, with entrapment efficiency and in vitro drug release as response parameters. The optimized proniosomes exhibited a particle size of 156.2 nm, a zeta potential of -22.6 mV, and an entrapment efficiency of 82.69%. SEM analysis confirmed the spherical and discrete morphology of the proniosomes. The optimized proniosomal formulation was incorporated into a 2% Carbopol gel base to create a topical gel delivery system. The formulated Adapalene proniosomal gel was characterized for physical appearance, pH, viscosity, drug content, in vitro drug diffusion, and in vitro anti-inflammatory activity. The in vitro diffusion study demonstrated a sustained release of 43.26% after 12 hours, indicating prolonged drug release. The release kinetics of the optimized formulation followed the Korsmeyer-Peppas model, suggesting non-Fickian or anomalous transport. Furthermore, the optimized formulation exhibited anti-inflammatory activity with an IC50 value of 236.2 µg/mL. In conclusion, this study provides evidence that proniosomes are a promising approach for the topical delivery of Adapalene. They enhance drug penetration through the stratum corneum, sustain drug action, and reduce side effects by targeting the pilosebaceous unit.

KEYWORDS: Proniosomal gel, Adapalene, anti-inflammatory activity, site targeting.

I. INTRODUCTION

One of the most common dermatological conditions is acne vulgaris. Although it is thought to be a minor cosmetic problem, it can seriously impair the quality of life [1]. The normal causes of acne are excessive sebum production, and unusual keratinocyte proliferation and differentiation, which block sebaceous follicle discharge and serve as a growth medium for Propionibacterium acnes and an immune-mediated reaction to the bacterial antigens, cytokines, and free fatty acids they produce [2]. The most effective comedolytic agents are retinoids, which are the primary choice in mild to moderate instances. They work by restoring normal follicular keratinization, preventing the growth of fresh micro-comedones, and reducing the formation of inflammatory lesions [3].

Adapalene (ADA), also known as 6-3-1-adamantyl-4-methoxyphenyl-2-napthoic acid, is a second-generation retinoid that binds well to the retinoic acid receptors. The most frequent side effects of topical ADA therapy were "retinoid reactions," which are characterized by erythema, burning, and scaling feelings and are dose-dependent. The incidence of free carboxylic acid near the end of the polar region of the agent has been a manifestation of these reactions [4]. Irritation may cause patients to stop taking their acne medication, which must be continued for a few weeks.

The stratum corneum, the highest layer of skin for topical distribution of medication actives, is present in the skin, the biggest organ of the body, which has a surface area of 1.8 to 2 m2. This acts as a primary barrier. The stratum corneum is dense, which renders it less permeable to topically administered medications in the form of customary creams and ointments. By using cutting-edge drug delivery devices that allow for regulated medication release through the skin, these restrictions can be removed. With few adverse effects, this will essentially maintain the effective drug level at the target spot. Niosomes, ethosomes, lipoproteins, and microcapsules are a few examples of new carriers that can slowly degrade to target particular areas [5]. Additionally, using novel drug delivery vehicles, such as proniosomes for loading ADA, is necessary to prevent the medication from being released in a

high dose quickly, enable a sustained and regulated drug release pattern, and lessen side effects. The control of follicular diseases like acne is made easier by the fact that ADA has a very low percutaneous absorption and that nanoparticles will assist it to pass through the stratum corneum and become stuck in the epidermis and hair follicles [6].

Proniosomes are either water-soluble carriers coated with suitable noise-forming surfactants to create liquid crystals or anhydrous free-flowing formulations having a jelly-like consistency. They can be hydrated in body compartments to form niosomal vesicles or reconstituted with an aqueous phase before delivery. Niosomes produced from proniosomes are superior to traditional niosomes [7]. Proniosomes are now employed in addition to traditional niosomes to improve drug delivery. A rate-limiting barrier for medication absorption is the proniosomal system. These systems can get through the skin's barrier to permeation and help the medications penetrate deeper.

The hydrophilic ends of the surfactant bilayer that makes up the niosome are exposed on both the outside and inside of the vesicle, while the hydrophobic chains are oriented in opposition to one another. [8]. As a result, the hydrophobic medications are entrenched into the bilayer itself, while the hydrophilic pharmaceuticals are held within the vesicle's contained interior [9]. Cholesterol addition causes the creation of an ordered liquid phase, which gives the bilayer stiffness and leads to fewer leaky niosomes. Compared to liposomes, niosomes offer special benefits. Even in their emulsified state, niosomes are remarkably stable structures. Niosomes' hydrodynamic diameter and trapping effectiveness are increased when cholesterol is included in them [10-12].

In the current work, proniosomal gel was created utilizing the coacervation phase separation method and various concentrations of the non-ionic surfactant tween 80, cholesterol, and lecithin. It was then examined using SEM and tested for in vitro drug release. The chosen proniosomal gel formulation was combined with carbopol gel in order to further boost the stability and viscosity of the system. Their release study and stability study were then carried out, and in vitro, anti-inflammatory activity was compared with a standard preparation.

II. MATERIALS AND METHODS

Adapalene was obtained as a gift sample from Madras Pharmaceuticals Pvt. Ltd., Chennai. Tween-80 was received as a gift sample from Mohini Organics Pvt Ltd, Mumbai. Carbopol 934 was received as a gift sample from Sai Mirra Innopharm Pvt. Ltd. Chennai. Soya lecithin, cholesterol, and Ethanol were purchased from G. S. Scientific Services, Madurai.

Drug-Excipient Compatibility Studies by FT-IR Spectroscopy

FTIR spectroscopy was carried out to check the compatibility between API and excipients used. IR spectra of API (Adapalene), and the physical mixture was studied using an FTIR instrument in the range between 400- 4000 cm^{-1}.

Formulation development

Proniosomes were formulated using the Coacervation phase separation method. In a glass beaker, Adapalene was added along with the surfactant, Soya lecithin, and cholesterol. Add the required quantity of Ethanol and heat it over a water bath at about 60-70^0 C for 5 min by closing the beaker to prevent loss of solvent while shaking until the complete dissolution of cholesterol. After complete dissolution of the solution, add phosphate buffer saline and warm for another 5 min until the formation of a clear or translucent solution and cooled down at room temperature to get a clear milky white solution of proniosomes.[13]

Optimization of Adapalene Proniosomes using Box Behnken Design [14]

To optimize formulation factors and assess their main effects, interaction effects, and quadratic effects, a Box-Behnken statistical design using Design Expert (Version 13) software was used. Box-Behnken statistical design with 3 factors (Cholesterol conc; X_1, Soya lecithin conc; X_2, and Surfactant conc; X_3), and with 3 levels (−1, 0, and 1) was built up to estimate the significant effect of these different variables on the responses namely, Vesicle size (Y_1), PDI(Y_2) and Entrapment efficiency(Y_3), and then predicts the optimized proniosomal formulae. BBD is appropriate to explore quadratic response surfaces and assemble second-order polynomial equations. The dependent and independent variables were tabulated in Table 1. The polynomial equation generated by this experimental are described in eq (1)

$$Y = b_0+ b_1X_1+ b_2X_2+ b_3X_3+ b_{12}X_1X_2 + b_{13}X_1X_3+ b_{23}X_2X_3 + b_{11}X_{12}+ b_{22}X_{22} + b_{33}X_{32} \quad \text{- Eq (1)}$$

Where Y is the dependent variable, b_0 is the intercept, b_1 to b_{33} are the regression coefficient, and X_1, X_2, and X_3 are the independent variables selected from preliminary experiments. The experimental design is summarized in Table 1.

Table 1: Summary of Experimental Design

INDEPENDENT VARIABLES	UNITS	LEVELS		
		LOW (-1)	MEDIUM (0)	HIGH (+1)
X_1 = CHOLESTEROL CONC	mg	50	75	100
X_2 = SOYA LECITHIN CONC	mg	100	150	200
X_3 = SURFACTANT CONC (TWEEN80)	mg	400	600	800
DEPENDENT VARIABLES	**UNITS**	**CONSTRAINTS**		
Y_1 = VESICLE SIZE	nm	Minimize		
Y_2 = PDI	-	Minimize		
Y_3 = ENTRAPMENT EFFICIENCY	%	Maximize		

CHARACTERIZATION OF FORMULATED PRONIOSOMES

a) Determination of vesicle size, size distribution

Particle size (z-average diameter) and polydispersity index (as a measure of particle size distribution) of Adapalene loaded Proniosomes dispersion is performed by Dynamic Light Scattering, also known as Photon Correlation Spectroscopy (PCS) using a Horiba Scientific (Nanoparticle analyzer) SZ-100 at 25ºC. Prior to measurements, all samples were diluted using ultra-purified water to yield a suitable scattering intensity. The diluted Proniosomes dispersion was poured into a disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed.

b) Determination of zeta potential

The Zeta Potential (ZP) is a determinant of the electric charge on the surface of the particles. The ZP values were assessed by determining the surface charge on the Adapalene-loaded Proniosomes using Horiba Scientific using an additional electrode. 1ml of a sample of Adapalene suspension was filled in a clear disposable zeta cell, without air bubble within the sample, the system was set at 25ºC temperature, and results were recorded.

c) Determination of vesicular shape by Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to examine the surface morphology of proniosomes. A one drop of proniosomal suspension was placed on the specimen stub which was coated with carbon and then with gold vapor appeared using a vacuum evaporator. The samples were examined under a scanning electron microscope for vesicular shape and then photographed.

d) Determination of entrapment efficiency by centrifugation

In a test tube, 0.1g of proniosomal suspension was taken, and 10 ml of pH 7.4 isotonic phosphate buffer was added to reconstitute the suspension. The drug-containing niosomes were separated from the dispersion by centrifugation at 3000 rpm for 30 min at 20 °C. The supernatant (1 ml) was taken and diluted with phosphate buffer (in a 10 ml volumetric flask) with appropriate dilution. The drug concentration in the resulting solution was assayed by the UV-visible spectroscopy method at 235nm. The % of drug entrapment efficiency was calculated by using the following formula, [15]

$$EE\,\% = [(Ct - Cr)/Ct] \times 100$$

Where, EE = Entrapment Efficiency,
Ct = Concentration of total drug,
Cr = Concentration of unentrapped drug.

e) Determination of Drug content

Weigh a specific quantity of proniosome suspension containing 1mg of drug and add sufficient qty of IPA and diluted with phosphate buffer pH 7.4 and sonicated until dissolved and filter it. After appropriate dilution, the samples were analysed spectrophotometrically at 235 nm against blank using UV- Visible spectrophotometer.[15]

FORMULATION AND INCORPORATION OF ADAPALENE-LOADED PRONIOSOMES IN THE PREPARED GEL BASE

In distilled water, a solution of Carbopol 934 (2% w/v) was allowed to swell for 3-4 hr. The prepared proniosomal suspension was added to Carbopol 934 and mechanically stirred to combine. To change the pH, triethanolamine was added. It was then sonicated for 15 minutes and left overnight to eliminate air bubbles.[16]

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CHARACTERIZATION OF PRONIOSOMAL LOADED GEL

1. Physical appearance and homogeneity

Visual observations were used to check the produced gels' physical appearance and homogeneity after the gel had been placed in the container. They were analyzed for their appearance and the existence of any aggregates.

2. Measurement of pH

The pH measurements are performed using a digital pH meter.

3. Measurement of Viscosity

Using a Brookfield Viscometer, the viscosity of the produced proniosomal gel was measured. The optimized formulation was measured by rotating the spindle 62 at 10 rpm at 25 °C.

4. Determination of Spreadability [17]

During the measurement using the parallel-plate method, 1 g of the sample was prepared in 48 h before the test is placed between two glass plates 20 x 20 cm. A weight of 500 g is placed on top for 1 minute. Then the diameter of the sample between the plates is measured.

Spreadability is determined by the formula:

$Si = d^2 \times \pi/4$,

Where,

Si– spreading area (mm2) depending on mass,

d– spreading area diameter (mm)

5. Determination of Drug content in gel

Weigh a specific quantity of gel containing 10 mg of the drug and add sufficient qty of IPA and diluted with phosphate buffer pH 7.4 and stir the solution for 2 hours on a magnetic stirrer. The resulting solution was filtered using Whatman filter paper, and after appropriate dilution, the samples were analyzed spectrophotometrically at 235 nm against blank using UV- Visible spectrophotometer.

6. *In vitro* drug diffusion studies

An *in vitro* diffusion study was performed by using Franz diffusion cell assembly. It has two compartments: a donor compartment that contains a proniosomal gel with 10 mg of the medication and one of the receptor chambers that contains a phosphate buffer pH 7.4. A cellophane membrane was previously soaked for 24h. The cellophane membrane was placed in contact with Phosphate buffer pH 7.4 and filled in the receptor compartment to avoid disruption in the ongoing process; it was ensured that no air bubbles were seen between the cellophane membrane and the liquid surface of Phosphate buffer pH 7.4. The temperature was maintained at 37 °C±0.5 using a magnetic stirrer. 0.5 ml of the sample was withdrawn from the receptor chamber side tube at the time interval of 15 min, 30 min, 45 min, 1h, 2h, 3h, 4h, 5h, 6h, 7h, 8h, 10h, and 12h and equilibrated with a new or fresh dissolution medium to maintain a sink state. Suitable dilution was carried out and was spectroscopically analyzed at a λ max of 235 nm using UV-visible spectroscopy.

7. *In vitro* drug release kinetics [18,19]

To study the *in vitro* release kinetics of the optimized formulation, data obtained from the dissolution study was plotted in various kinetics models. To interpret the drug release kinetics from the formulation, a variety of kinetic models were used, including zero order (cumulative % of drug released vs. time), first order (log cumulative % of drug remaining vs. time), Korsmeyer-Peppas model (log cumulative %drug release vs. log time), Higuchi model (cumulative % of drug released vs. square root of time), and Hixson Crowell model (cube root of log cumulative % of drug remaining vs. log time) Based on the highest regression values for correlation coefficients for formulations, the best- fit model was decided.

8. *In vitro* Anti-inflammatory activity (Protein denaturation method) [20,21]

The protein denaturation measure was utilized for the assessment of the anti-inflammatory capability of Optimized Adapalene Proniosomal gel (OPT-ADP gel). To make a reaction mixture of 3 mL, bovine serum albumin (200 µL) was combined with 200 µL of OPT ADP gel at varying concentrations (1000 to 62.5 µg/mL) and 2.6 mL of phosphate buffer at pH 6.4. The mixtures were then incubated for 15 minutes at room temperature before being heated for 5 minutes at 70 to 90 ℃. After cooling the solutions, the absorbance was measured using a vehicle as a blank on a UV spectrophotometer (Labman, V320 Model) at 660 nm. Diclofenac was used as the standard, and distilled water served as the control. The percentage inhibition of protein denaturation was then determined by using the formula,

Protein denaturation % =

$$\frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}}$$

The IC50 values were calculated using AATBIO IC_{50} calculator online tool.

III. RESULTS AND DISCUSSION

Drug-Excipient Compatibility Studies By FT-IR Spectroscopy

The FT-IR spectra of the Adapalene drug molecule are shown in Fig. 1 and the absorption bands are positioned at 2962 cm^{-1} representing the polymer –CH vibration, the Adapalene carboxylic –OH stretching vibration is observed at 2896 cm^{-1}, and band centered at 1771 cm^{-1} reveals that the C=O stretching present in the drug molecule. The band is located at 1684 cm^{-1} which denotes the conjugation of carboxylic acid to the aromatic ring and the C=O stretch presence of Adapalene molecule. The absorption band at 534 - 900 cm^{-1} confirmed the C-H out-of-plane bending of an aromatic ring.

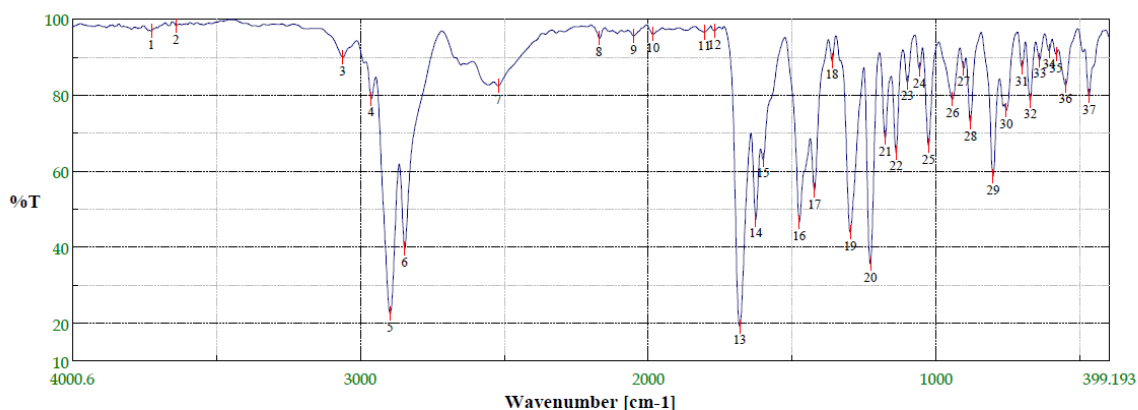

Fig.1: FT-IR Spectra of Adapalene

The FT-IR spectra of the physical mixture are shown in Fig. 2 and it showed all the characteristic peaks of Adapalene (2962, 2896, 1771, and 534 – 900) thus confirming that no interaction of the drug occurred with the excipients of the formulation. The results revealed that there was no interaction between the drug and excipients.

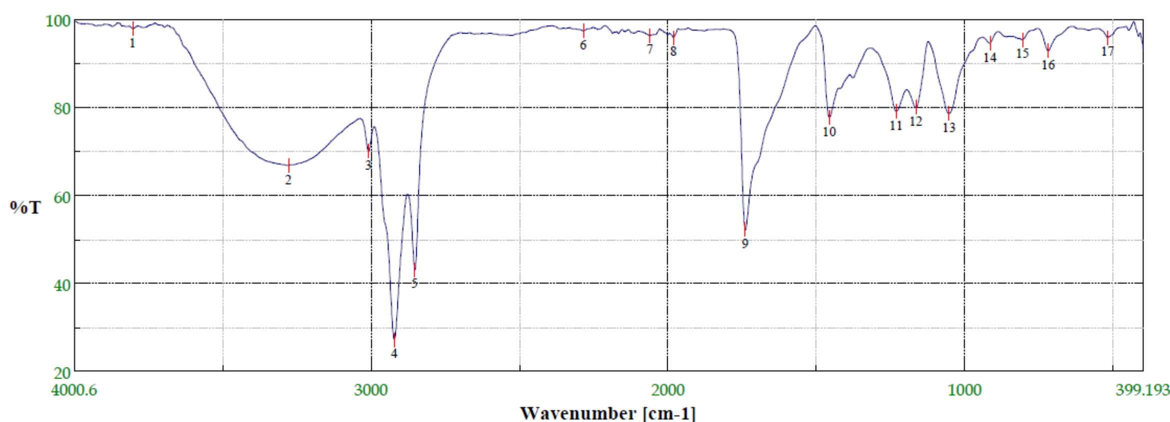

Fig.2: FT-IR Spectra of Physical mixture

Optimization Of Adapalene Proniosomes using Box Behnken Design

The effect of 3 independent variables at 3 levels on various responses was evaluated using the Box-Behnken design. These independent variables included Cholesterol, Soya lecithin, and Surfactant with vesicle size, PDI, and % EE as responses. 15 batches (F1-F15) generated by Design Expert Software (Design Expert 11, Stat-Ease, Minneapolis, MN) were prepared. The measured responses were used to construct 3D response surface plots to establish the relationship between variables and their interaction. Table 2 shows the 15 formulation compositions and their responses.

Table 2: Box-Behnken experimental design with measured responses.

S.NO	FORMULATION CODE	X_1: CHOLESTEROL (mg)	X_2: SOYA LECITHIN (mg)	X_3: SURFACTANT (TWEEN 80) (mg)	Y_1: VESICLE SIZE (nm)	Y_2: PDI	Y_3: EE (%)
1.	F1	75	400	400	246.8	0.356	78.6
2.	F2	75	200	800	170.5	0.285	77.9
3.	F3	100	300	800	177.7	0.315	83.6
4.	F4	75	300	600	341.7	0.586	77.5
5.	F5	100	400	600	205.5	0.324	76.5
6.	F6	75	200	400	329.8	0.467	80.8
7.	F7	75	300	600	321.3	0.543	79.3
8.	F8	50	300	800	322.8	0.486	64.11
9.	F9	50	200	600	278.6	0.267	69.53
10.	F10	75	300	600	364.3	0.574	79.8
11.	F11	100	200	600	190.4	0.305	84.2
12.	F12	50	400	600	298.7	0.365	63.69
13.	F13	75	400	800	220.2	0.378	71.9
14.	F14	50	300	400	375.5	0.387	73.9
15.	F15	100	300	400	358.9	0.532	81.5

Figure.3: Formulation of Adapalene loaded proniosomes

Effect of independent factors on vesicle size

The observed vesicle sizes varied from 170.5 nm to 375.5nm in different formulation batches. In the polynomial Eq, the model F-value was found to be 21.49, hence it can be inferred that the model is significant ($p < 0.0001$). Simultaneously, a quadratic sequential p-value of 0.0018 indicated the significance of the model and the lack of fit F-value (0.6471) implied an insignificant lack of fit. The predicted and adjusted R2 values for the vesicle size were in reasonable agreement. Finally, the precision of 13.7608 indicated a good signal, thereby demonstrating that the model could be used to navigate the design space.

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PARTICLE SIZE = +342.43-42.89A+0.2375B-52.48C-1.25AB-32.13AC+33.17BC-16.12A²-83.02B²-17.59C²

$$\text{PARTICLE SIZE} = +342.43 - 42.89A + 0.2375B - 52.48C - 1.25AB - 32.13AC + 33.17BC - 16.12A^2 - 83.02B^2 - 17.59C^2$$

The positive coefficient of 'B' indicates that there would be an increase in Particle size with the increase in the Soya lecithin concentration, whereas, the negative coefficient of 'A' and 'C' indicates the simultaneous decrease in Particle size with an increase in the concentration of Cholesterol conc and Surfactant conc, respectively.

To find out the factors which affect the response perturbation graphs were plotted in **Fig. 4**. For response *Y*1, factor B show high curvature which indicates that the concentration of soya lecithin has a significant effect on the particle size of Proniosomes. Factors A and C show less curvature which indicates that the conc of cholesterol and surfactant has a less significant effect on the particle size of proniosomes.

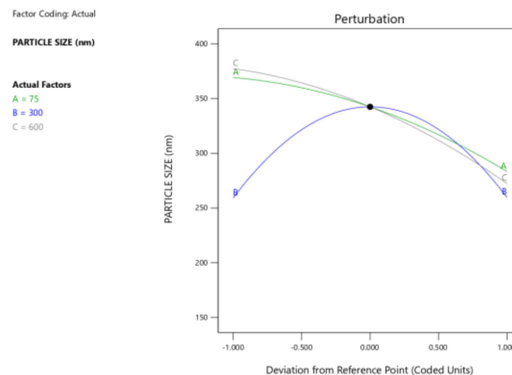

Fig.4: Perturbation plot for Response Y_1.

The effect of the changes of the independent variables on vesicle size is depicted in the 3-dimensional surface plot (Fig.5)

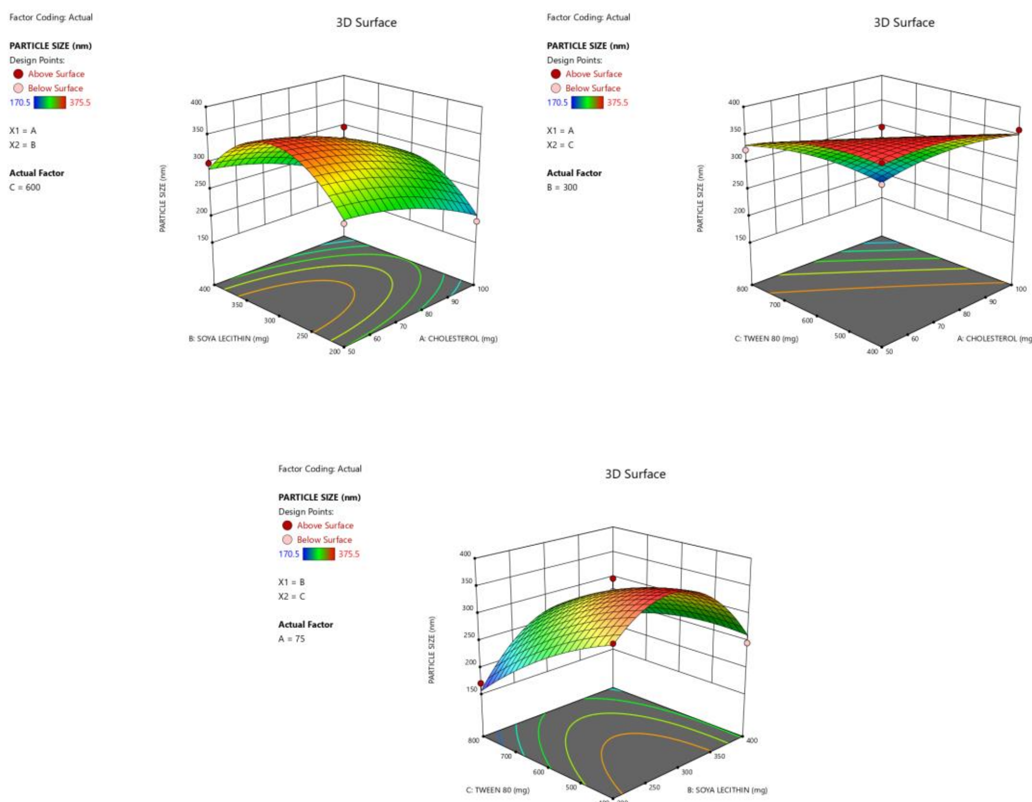

Fig.5: 3D Response surface plot for Response Y_1.

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Effect of independent factors on PDI

The observed PDI varied from 0.267 to 0.586 in different formulation batches, In the polynomial Eq, the model F-value was found to be 25.97, hence it can be inferred that the model is significant ($p < 0.0001$). Simultaneously, a quadratic sequential p-value of 0.0011 indicated the significance of the model and the lack of fit F-value (0.3858) implied an insignificant lack of fit. The predicted and adjusted R2 values for the vesicle size were in reasonable agreement. Finally, the precision of 13.5279 indicated a good signal, thereby demonstrating that the model could be used to navigate the design space.

PDI = +0.5677-0.0036A+0.0124B-0.0348C-0.0197AB-0.0790AC+0.0510BC-0.0970A²-0.1555B²-0.0407C²

$$PDI = +0.5677-0.0036A+0.0124B-0.0348C-0.0197AB-0.0790AC+0.0510BC-0.0970A^2-0.1555B^2-0.0407C^2$$

The positive coefficient of 'B' indicates that there would be an increase in PDI with the increase in the Soya lecithin concentration, whereas, the negative coefficient of 'A' and 'C' indicates a simultaneous decrease in PDI with an increase in the concentration of Cholesterol conc and Surfactant conc, respectively.

To find out the factors which affect the response perturbation graphs were plotted in **Fig. 6**. For response *Y*1, factor B show high curvature which indicates that the concentration of soya lecithin has a significant effect on the PDI of Proniosomes. Factors A and C show less curvature which indicates that the conc of cholesterol and surfactant has a less significant effect on the PDI of proniosomes.

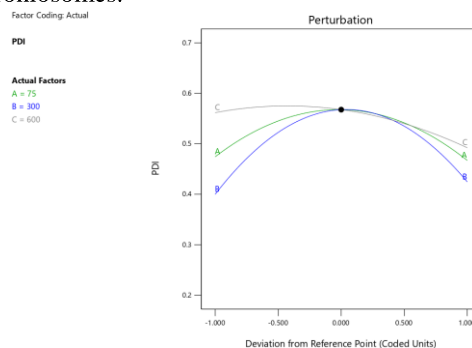

Fig.6: Perturbation plot for Response Y_2.

The effect of the changes of the independent variables on PDI is depicted in the 3-dimensional surface plot (Fig.7)

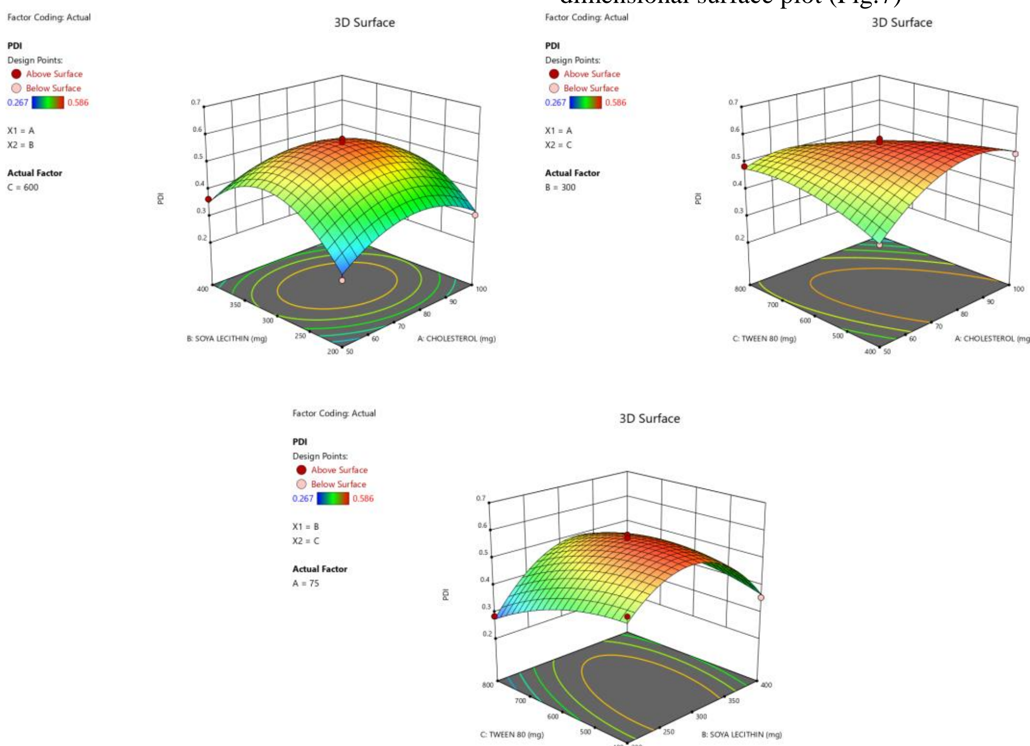

Fig.7: 3D Response surface plot for Response Y_2.

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Effect of independent factors on Entrapment Efficiency

The observed Entrapment efficiency varied from 63.69% to 84.2% in different formulation batches, In the polynomial, the model F-value was found to be 45.06, hence it can be inferred that the model is significant ($p < 0.0001$). Simultaneously, a quadratic sequential p-value of 0.0003 indicated the significance of the model and the lack of fit F-value (0.5584) implied an insignificant lack of fit. The predicted and adjusted R2 values for the vesicle size were in reasonable agreement. Finally, the precision of 20.4007 indicated a good signal, thereby demonstrating that the model could be used to navigate the design space.

ENTRAPMENT EFFICIENCY = $+78.87+6.82A-2.72B-2.16C-0.4650AB+2.97AC-0.9500BC-3.45A^2-1.93B^2+0.3654C^2$ The positive coefficient of 'A' indicates that there would be an increase in entrapment efficiency with the increase in the cholesterol concentration, whereas, the negative coefficient of 'B' and 'C' indicates a simultaneous decrease in entrapment efficiency with an increase in the concentration of soya lecithin conc and Surfactant conc, respectively.

To find out the factors which affect the response perturbation graphs were plotted in **Fig. 8**. For response *Y*1, factor A shows high curvature which indicates that the concentration of cholesterol has a significant effect on the Entrapment efficiency of Proniosomes. Factors B and C show less curvature which indicates that the conc of soya lecithin and surfactant has a less significant effect on the entrapment efficiency of proniosomes.

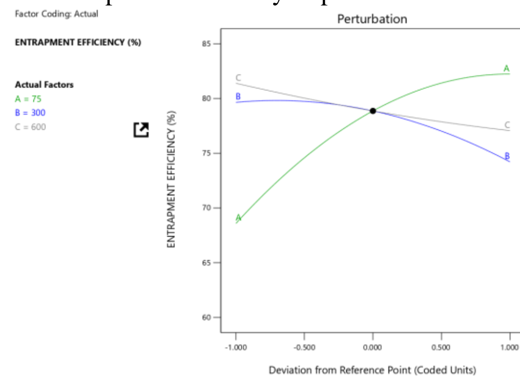

Fig.8: Perturbation plot for Response Y_3.

The effect of the changes of the independent variables on entrapment efficiency is depicted in the 3-dimensional surface plot (Fig.9)

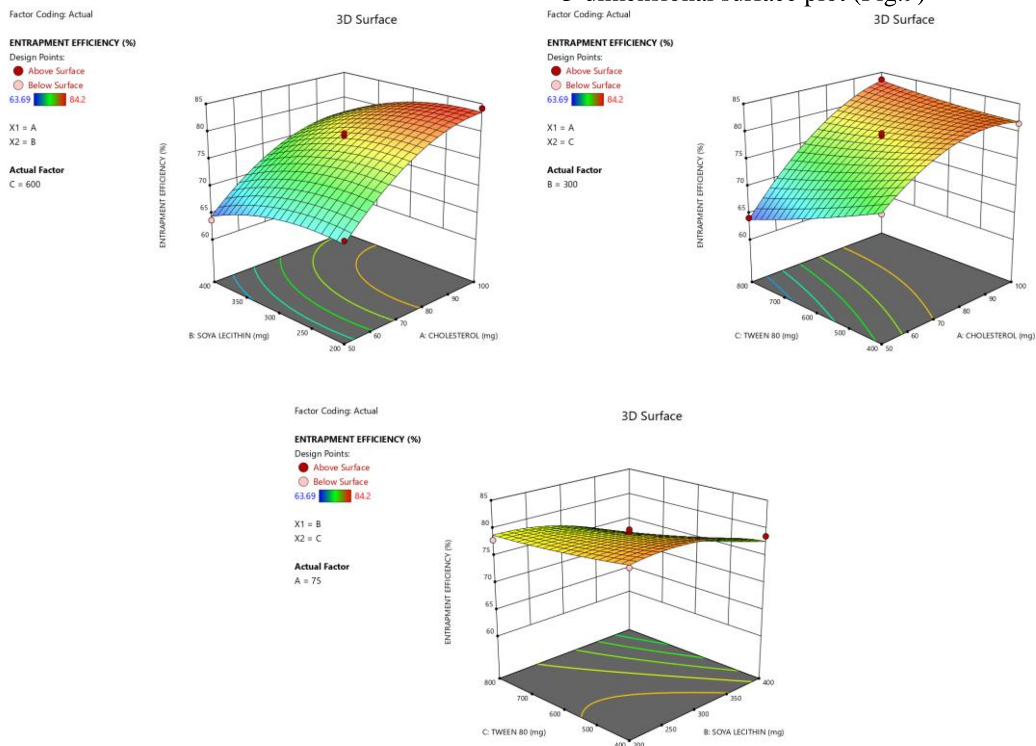

Fig.9: 3D Response surface plot for Response Y_3.

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Selection of optimized batch

The selection of optimized batch for ADP-Proniosomes was done by making various trials with the goal of least particle size (170.5 – 375.5 nm), PDI (0.267 – 0.586), and highest EE (63.69 – 84.2 %). The selection of the batch was done by numeric optimization with a desirability function. The constraints for vesicle size (Y1), and PDI (Y2) was minimized, and for EE (Y3) it was maximized. The optimized batch given by the software was prepared and analyzed. The optimized batch consisted of cholesterol conc (A=99.80 mg), soya lecithin conc (B=200.24 mg), and Surfactant conc (C=679.85 mg) with vesicle size, PDI, and EE predicted to be 152.708 nm, 0.249156, and 84.2334% respectively. The vesicle size observed was 156.2 nm, PDI observed was 0.283, and EE of 82.69 % with a standard error of 1.786 for vesicle size, 0.01692 for PDI, and 0.7717 for EE. Thus, the batch giving minimum vesicle size, minimum PDI, and maximum EE was selected as the optimized batch.

Fig.10: Optimized formulation.

Evaluation of optimized proniosomes

Besides the three responses (Y1, Y2& Y3) the optimized proniosomes were also evaluated for their ZP, drug content, and morphological characteristics.

a) Determination of zeta potential of optimized formulation

The Zeta potential value of the optimized formulation was found to be -22.6 mV.

b) Drug content of the optimized formulation

The drug content of the optimized formulation was found to be 95.68%.

c) Morphology of Optimized Proniosomes by Scanning Electron Microscopy (SEM) analysis

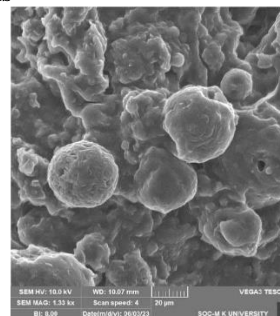

Fig.11: SEM images of optimized formulation

The shape and surface morphology of the optimized formulation was observed in a scanning electron microscope. It shows that the optimized formulation was showing spherical morphology.

INCORPORATION OF OPTIMIZED ADAPALENE-LOADED PRONIOSOMES IN THE PREPARED GEL BASE

The Optimized Adapalene loaded proniosomes formulation (5 ml) was incorporated into 5g of 2% Carbopol 934 as gelling agent containing glycerin, then this solution was mixed by mechanical stirring, and the pH of the gel was adjusted by the addition of triethanolamine. Pronio-somal gel was sonicated for 10-15 minutes and kept overnight to eliminate air bubbles.

CHARACTERIZATION OF OPTIMIZED ADAPALENE PRONIOSOMAL GEL

a) Physical appearance and homogeneity

The optimized Adapalene-loaded proniosomal gel formulations had a pale yellow, transparent appearance, free from the presence of particles, and showed good homogeneity with the absence of lumps.

b) Measurement of pH

The pH of the gel was found to be 6.3 pH, which lies in the normal pH range of the skin, indicating skin compatibility.

c) Measurement of Viscosity

The Viscosity of the Optimized Formulation was found to be 4128 cps.

d) Determination of Spreadability

The spreadability of the optimized formulation was found to be 10.76 cm^2, which shows that the formulation has good spreadability.

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e) Determination of Drug content in gel

The drug content of the optimized formulation was found to be 92.46%.

f) *In vitro* drug diffusion studies

In vitro drug diffusion studies for optimized Adapalene loaded proniosomal gel formulation was carried out through cellophane membrane in phosphate buffer pH 7.4 by using Franz diffusion cell apparatus. The graph was plotted against Time (hrs) on the X axis vs Cumulative % drug release on the Y axis.

Table.3: % Drug release of optimized formulation

TIME (h)	CUMULATIVE (%) DRUG RELEASE	
	OPT- ADP proniosomal gel	Marketed formulation
0	0	0
1	6.87±0.78	16.78±1.59
2	11.54±1.76	28.36±0.41
3	17.34±0.48	44.34±1.17
4	22.5±0.95	60.56±1.65
5	25.16±1.21	73.14±0.73
6	26.75±0.39	84.23±0.32
7	32.5±0.69	89.45±1.48
8	35.58±0.43	96.54±0.79
10	40.64±1.87	-
12	43.26±0.63	-

Values are expressed as mean ± SD (n=3)

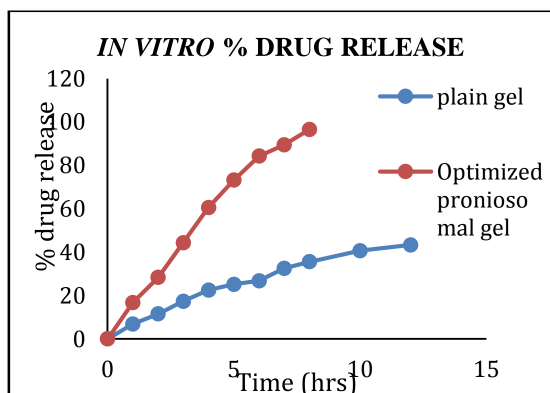

Fig.12: *In vitro* % drug release

In vitro Release profile of the optimized formulation showed 43.26% release up to 12 hrs and plain gel showed 96.54% release up to 8 hours. This confirms that the drug is released from the optimized proniosomal gel in a sustained manner.

g) *In vitro* release kinetics of optimized adp-proniosomal gel formulation

The values obtained from *in vitro* diffusion release study of Adapalene loaded Proniosomal gel were fitted in various kinetic models. Based on the findings, it was determined that the Korsmeyer-Peppas model had the highest correlation coefficient, R^2 (0.9909), and was, therefore, the model that best fit the optimized ADP-proniosomal gel. The fact that the value of "n" was calculated to be 0.7584 (0.45 n 0.89), shows that the drug release from the polymeric matrix follows non-Fickian or anomalous transport. Diffusions and other processes, such as matrix swelling, erosion, or relaxation, are all part of the release mechanism.

h) *In vitro* Anti-inflammatory activity

The anti-inflammatory activity of optimized adapalene proniosomal gel was studied by an *in vitro* protein denaturation assay.Fig.13 depicts the inhibition of protein denaturation induced by the optimized gel. The inhibition of protein denaturation was summarized in Fig.14. The estimated potency was 236.2 µg/mL, and it elicited significant concentration-dependent inhibition with a maximum effect of 84 % at 1000 µg/mL

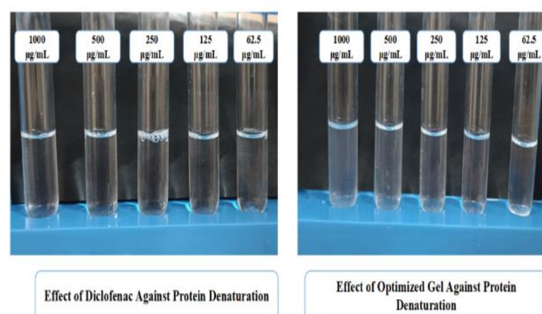

Fig.13: Anti-inflammatory activity of test sample (opt gel) and control drug (Diclofenac)

The results suggest that the optimized proniosomal gel exhibits comparable anti-inflammatory activity through inhibition of protein denaturation to control (Diclofenac), a commonly used anti-inflammatory drug. Further, the results indicate that upon topical application, it can produce a promising anti-inflammatory effect in the management of acne vulgaris.

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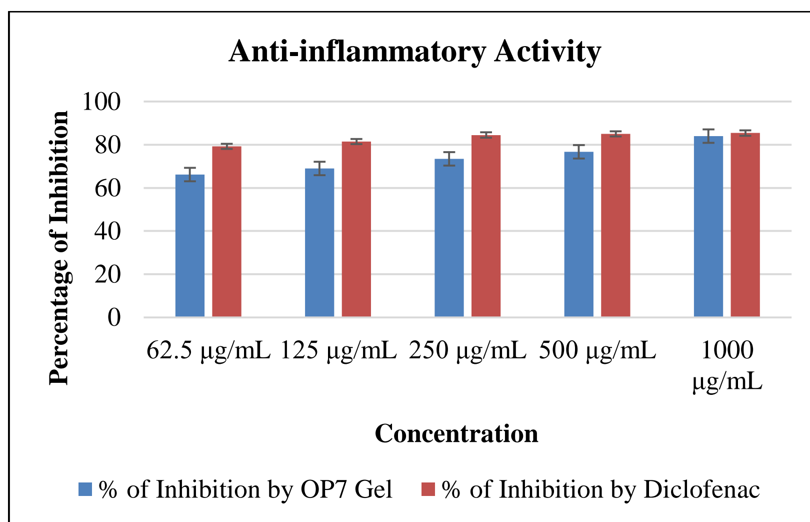

Fig.14: The % effect of Optimized Gel and control against protein denaturation assay

IV. CONCLUSION

Adapalene, a third generation topical retinoid was successfully prepared as proniosomes by Coacervation phase separation method using cholesterol as membrane stabilizer, soya lecithin as phospholipid and tween 80 as non-ionic surfactant. The optimized formulation was selected using Box behnken design, which showed mean particle size of 156.2 nm and a narrow size distribution, suggesting improved permeation through skin stratum corneum. Further to enhance the contact at target area, the optimized adapalene proniosomes were incorporated into 2% carbopol gel base which provided sustained release of drug up to 12 hours. From *in vitro* anti-inflammatory activity studies by Protein denaturation assay, it is also confirmed that the optimized adapalene loaded proniosomal gel having anti-inflammatory activity with IC 50 value of 236.2 µg/mL.

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