Journal of Advances in Science and Technology
Vol.22, Issue No. 4 September-2025, ISSN 2230-9659

Hybrid Vesicular Formulation of Adapalene and Tea Tree Oil: A Targeted Strategy for Acne Management

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Abstract: Acne vulgaris is a multifactorial skin condition that often requires long-term management due to its chronic and relapsing nature. While conventional therapies provide temporary relief, their limitations—including side effects, resistance, and poor skin compatibility—necessitate the development of safer and more effective alternatives. This research focuses on designing a topical delivery system that merges the benefits of synthetic retinoids and herbal actives using niosomal technology. Niosomes, composed of non-ionic surfactants, offer enhanced drug stability, targeted delivery, and controlled release, making them an ideal carrier for cutaneous applications. In this formulation, Adapalene is combined with natural oils such as Tea Tree and Lemongrass—known for their antimicrobial and anti-inflammatory actions—to develop a stable, skin-friendly gel. The proposed system aims to overcome the common drawbacks of existing acne treatments while enhancing therapeutic efficacy and patient compliance. The findings suggest that the developed niosomal gel holds promise as a holistic and advanced approach to acne therapy.

Keywords: Niosomes, Acne vulgaris, Adapalene, Herbal bioactives, Tea tree oil, Lemongrass oil, Topical delivery, Controlled release, Nanocarrier system, Anti-inflammatory formulation

INTRODUCTION

Acne vulgaris remains one of the most prevalent dermatological conditions globally, particularly affecting adolescents and continuing into adulthood in many cases. It is influenced by hormonal changes, genetic predisposition, lifestyle, and microbial activity, especially by *Cutibacterium acnes*. While not life-threatening, the condition significantly impacts quality of life, often leading to scarring and psychological distress. Existing treatments range from topical retinoids to systemic antibiotics and hormonal therapies, yet limitations like resistance, irritation, and recurrence often persist.

In this context, topical drug delivery systems have gained prominence due to their ability to localize therapeutic action, bypass first-pass metabolism, and minimize systemic exposure. Among these, niosomes—non-ionic surfactant-based vesicles—offer advantages such as controlled drug release, enhanced skin penetration, and improved stability over traditional

carriers. Their amphiphilic structure enables the encapsulation of both hydrophilic and lipophilic drugs, making them highly versatile for dermal applications.

The research presented aims to formulate and evaluate herbal bioactives (such as essential oils) loaded in niosomal carriers for the treatment of acne. These systems not only harness the antibacterial and anti-inflammatory properties of natural agents but also benefit from the targeted delivery afforded by the vesicular system. The incorporation of such agents into gel-based systems further enhances patient compliance and skin adherence.

The selected drug—Adapalene, a third-generation retinoid—and the herbal oils (Tea Tree Oil, Lemongrass Oil) have well-documented efficacy in acne management. Their combination in a niosomal gel formulation is expected to produce synergistic effects, reduce side effects, and ensure sustained release. The rationale rests on the need for a stable, non-irritant, and effective topical system that addresses both microbial activity and inflammation with minimal resistance or relapse**.**

MATERIALS AND METHODS:

Materials Used

Table 1: List of Materials Used

‘	Material	Use	Supplier
1.	Adapalene, Tea Tree Oil	API	---
2.	Span 20, Span 40, Span 60, Span 80, Span 65, Span 85.	Surfactants	Vishal Chem, Mumbai
3.	Tween 20, Tween 40, Tween 60, Tween 80, Tween 65, Tween 85 Carbopol 934		Vishal Chem, Mumbai
4.	Carbopol 974P, Carbopol 934 NF Carbopol 934	Gelling Agent (Gel Base)	Vishal Chem

5.	Polysorbate	Co-surfactant	Vishal Chem
6.	Cholesterol	Lipid, Stabilizes noisome Membrane	Vishal Chem
7.	Solulan C24	Stearic Stabilizer	Vishal Chem
8.	DMSO, N- Methyl 2-Pyrrolidone (NMP)	Penetration Enhancer	Vishal Chem
9.	Benzoic Acid, Sorbic Acid, Parabens	Preservatives	Vishal Chem
10.	Ethanol Methanol HPMC	Solvent	Vishal Chem
11.	Deionized water	Vehicle	-------

List of instruments and equipments.

Table 2: List of Instruments and Equipment's

Sr.No.	Equipment/Instrument Name	Model	Company
1.	Digital Weighing Balance	CA 123	Contech, Mumbai
2.	Digital pH meter	ME 962-P	AE MAX
3.	Brookfield viscometer	---	---
4.	Franz diffusion cell system	---	---
5.	UV Visible Spectrophotometer	3000+	Schimadzu
6.	Fourier Transform Infrared Spectrophotometer (FTIR)	8400S	Schimadzu, Japan
7.	Sonicator		Kromtach
8.	Gel strength apparatus	---	---
9.	Brookfield QTS Texture Analyzer	---	---

Pre-formulation Studies

Selection of Drug [1]

Adapalene was selected as API for Formulation Development

Characterization of Drug [2]

Adapalene was obtained as gift sample

Physical Appearance

Adapalene visually checked for its physical appearance

Melting Point Determination [3]

For melting point determination capillary tube method and observed value was compared with standard

UV Spectroscopy [4]

- In a 5 ml-volumetric flask, 5 milligrams of the standard medication Adapalene were precisely measured and mixed with 2.5 milliliters of tetrahydrofuran. To create a 1000 µg/ml stock solution, the same solvent was used to fill the volume to the mark.
- To make a sub-stock solution with a concentration of 100 µg/ml, 0.5 ml was removed from the stock solution and mixed with 5 ml of methanol.
- To make a working solution with a concentration of 10 µg/ml, 0.5 ml was taken from the 100 µg/ml sub-stock solution and mixed with 5 ml of distilled water.
- The solution that was being tested was scanned between 200 and 400 nm with distilled water used as a control.

FTIR Spectroscopy [5]

Sample Preparation: Mix adapalene with potassium bromide (KBr) and press into a pellet.

Scanning: Perform FTIR spectroscopy in the wavenumber range of 4000 cm^{-1} to 400 cm^{-1}.

Analysis: Identify characteristic peaks, such as C-H stretching (~2950 cm^{-1}), C=O stretching (~1720 cm^{-1}), and aromatic C=C stretching (~1600 cm^{-1}).

Result: Use the obtained spectrum to confirm the chemical structure and purity of adapalene.

Analytical Method Development

Calibration Curve of Adapalene [6]

- We used a variety of solvents to conduct a solubility test on Adapalene.
- All of the following are examples of solvents: water, methanol, ethanol, Dimethyl-sulfoxide, and tetrahydrofuran.
- DMSO and tetrahydrofuran were discovered to have solubility for adapalene. Hence, tetrahydrofuran was chosen as the analytical solvent. Further, required dilutions were done with methanol and distilled water
- In a 5 ml-volumetric flask, 5 milligrams of the standard medication Adapalene were precisely measured and mixed with 2.5 milliliters of tetrahydrofuran. To create a 1000 µg/ml stock solution, the same solvent was used to fill the volume to the mark.
- To make a sub-stock solution with a concentration of 100 µg/ml, 0.5 ml was removed from the stock solution and mixed with 5 ml of methanol.
- To make a working solution with a concentration of 10 µg/ml, 0.5 ml was taken from the 100 µg/ml sub-stock solution and mixed with 5 ml of distilled water.
- The solution that was being tested was scanned between 200 and 400 nm with distilled water used as a control.
- The maximum absorbance (λ max) of Adapalene was found at 235 nm, 282 nm, and 335 nm, which are the wavelengths that were recorded. For further research, 235 nm was chosen as the maximum wavelength.

Preparation of standard solutions [7]:

- As mentioned earlier, distilled water was used to create a 10 µg/ml solution of adapalene.
- In distilled water, standard solutions with concentrations of 3 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, and 12 µg/ml were made from this working solution.

- The absorbances of the reference solutions were measured at a constant 235 nm wavelength and a calibration curve that was linear in shape was made.
- Table no.5.3, which is provided below, displays the results of the linearity test.

Table 3 - Concentration and absorbance for Adapalene

Concentration (ppm)	Absorbance at 235 nm
3	0.193 ± 0.0026
5	0.326± 0.0020
7.5	0.505 ± 0.0030
10	0.64 ± 0.0020
12	0.741± 0.0020

Drug and Excipient Compatibility Study [8]

For compatibility study Weigh equal amounts (e.g., 1 mg each) of adapalene, Span 60, and cholesterol. Mix them together, add KBr, grind into a fine powder, and press into a pellet.

Selection of Excipients

Preliminary Trial batches [9]

Measurements of Span 60, cholesterol, tween 80, adapalene, and ethanol were mixed in a beaker and stirred with a magnetic stirrer set to 250 rpm at 60-65°C until the mixture was completely dissolved, yielding a clear solution. The distilled water was added dropwise using a syringe that was equipped with a 10-gauge needle, with the stirring being maintained at 250 rpm. For the purpose of extracting adapalene niosomes, the stirring was maintained at 1000 rpm for an additional fifteen minutes.

Screening of effect of concentration of Span 60 [10]

In order to achieve the best possible entrapment efficiency and polydispersity index (PDI), niosomes were synthesized utilizing the aforementioned approach with changing concertation Span 60.

Table 4 - Optimization of Span 60 concentrations in niosome preparation

Batch no	Ethanol	Water	Drug	Tween 80	Cholesterol	Span 60
1	6 ml	4ml	10 mg	130 mg	20 mg	35 mg
2	6 ml	4ml	10 mg	130 mg	20 mg	40 mg
3	6 ml	4ml	10 mg	130 mg	20 mg	45 mg
4	6 ml	4ml	10 mg	130 mg	20 mg	50 mg
5	6 ml	4ml	10 mg	130 mg	20 mg	55 mg
6	6 ml	4ml	10 mg	130 mg	20 mg	60 mg

One milliliter of the mixture was spun at 10,000 revolutions per minute in a microcentrifuge tube for ten minutes to determine the entrapment efficiency. The pellet was separated from the sample supernatants. A solution was prepared by dissolving the pellets in 200 ul of THF and then adding distilled water to get the amount to 1 ml.The diluted samples were analysed for drug content by UV spectrophotometry at 235 nm as the λ_{max}.

Optimization of Carbopol concentration [11]

The gelling agent and stabilizer carbopol-940 is a man-made high-molecular-weight polymer that has great thickening, suspending, and stabilizing capabilities. It finds widespread application in a wide range of compositions. The finished product's viscosity, spreadability, and performance are all affected by the carbopol content.
To improve the product's use and effectiveness, we adjusted the carbopol content in this research so that we could create a niosomal gel with the rheological qualities we wanted. During optimization, Carbopol concentrations of0.3%,0.5%,0.75%,0.9%, and 1% were used. To provide a thorough study over a broad range of concentrations, these values were chosen based on the literature studies. We tested the formulation's physical properties, such as texture, stability, application ease, and extrudability, at different Carbopol concentrations to determine

the optimal concentration. After achieving the optimal noisome formulation, carbopol was stirred into it and left to hydrate for 6 hours at room temperature. Raising the dispersion's pH to 7 with triethanolamine influenced the gelling. Gentle stirring with a glass rod was used to introduce tea tree oil into the gel once the pH was adjusted.

Table 5 - Optimization of Carbopol Concentration

Ingredient	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Adapalene	10 mg	10 mg	10 mg	10 mg	10 mg
Span 60	40 mg	40 mg	40 mg	40 mg	40 mg
Tween 80	131mg	131mg	131mg	131mg	131mg
Cholesterol	60 mg	60 mg	60 mg	60 mg	60 mg
Ethanol	6 ml	6 ml	6 ml	6 ml	6 ml
Carbopol	0.30%	0.5 %	0.75 %	0.9 %	1 %
Water	4 ml	4 ml	4 ml	4 ml	4 ml

Formulation Development [16]

Full Factorial Design: A 3^2 randomized full factorial design was adopted to optimize the variables. In the design, 2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. The concentrations of,

- Span 60 (X1)
- Cholesterol (X2)

Were chosen as independent variables, as their marked effects were seen on Particle Size and %EE. The response (Y) is measured for each trial.

$Y= \beta_0+\beta_1X_1+\beta_2X_2+\beta_{12}X_1X_2+\beta_{11}X_1^2+\beta_{22}X_2^{\ 2}$

Where, Y is the dependent variable, β0 is arithmetic mean response of the nine runs, β1 is estimated coefficient for factor X1, and β2 is estimated coefficient for factor X2.

In order to examine non-linearity, we use polynomial terms (X1 2 and X2 2), while the main effects (X1 and X2) indicate the average outcome of changing one factor at a time from its

low to high value. The interaction terms (X1X2) demonstrate how the response varies when two variables are modified concurrently.

Once the size and mathematical sign of the coefficient are known, the polynomial equation may be utilized to derive conclusions.

For this research, the researchers used a complete factorial design with 32 random variables. Here we can see the design arrangement together with the coded value of the independent factor. Initial research informed the selection of the criteria.

Full Factorial Design Batches of Niosomal Gel

Table 6: Coded Values of Factor and Level

Sr. No.	Formulation Code	Coded factor Levels	
		X1	X2
1	F1	-1	-1
2	F2	0	-1
3	F3	+1	-1
4	F4	-1	0
5	F5	0	0
6	F6	+1	0
7	F7	-1	+1
8	F8	0	+1
9	F9	+1	+1

Variables for Factorial Design

Table 7: List of Independent and Dependent Variables

Independent variable		Dependent Variables	
X1	X2	Y1	Y2
Span 60 Concentration	Cholesterol Concentration	Particle Size	% Entrapment Efficiency

Table 8: Design Layout

	REAL VALUES		TRAMSFORMED VALUES		DEPENDENT VARIABLE	
BATCH CODE	**Span 60 Concentration (mg)**	**Cholesterol Concentration (mg)**	**X1**	**X2**	**Y1 Particle Size (nm)**	**Y2 Entrapment Efficiency (%)**
F1	40	10	-1	-1	172.3	71
F2	60	10	0	-1	400.9	57
F3	80	10	1	-1	581.3	43
F4	40	20	-1	0	166.2	80
F5	60	20	0	0	477.2	62
F6	80	20	1	0	512.7	54
F7	40	30	-1	1	254.2	75
F8	60	30	0	1	361.6	68
F9	80	30	1	1	641.7	58

Data Analysis and Model Validation [17]

- Statistical validation of the polynomial equations generated by Design Expert 12 was established on the basis of ANOVA in the software. A total 9 runs were generated. The models were evaluated in terms of statistically significant co-efficient and R^2 values. Various feasibility and grid searches were conducted to find the composition of optimized formulations.
- Various 3D response surface graphs were provided by the Design Expert 12 software. By intensive grid search performed over the whole experimental region, one optimum checkpoint formulations were selected to validate the chosen experimental domain and polynomial equations.
- The checkpoint formulation was prepared andevaluated for various response properties.

The resultant experimental values of the responseswere quantitatively compared with the predicted values to validate the equation.

Statistical Analysis

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the response.

$$Y = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 12X1X2 + \beta 11X^2 + \beta 22 X 2^2$$

Contour Plot and Surface Plot of Design

Surface plots (3-D) and contour plots (2-D) were used to optimize the formulation for all dependent variables that were observed. In this case, the Design Expert 12 program was used to create surface plots and contour plots. You may analyze the influence of two variables on the answer at once using these charts.

Preparation of 50 g final formulation for characterization studies

Table 9 - Amount Excipients for 50 ml of formulation

Excipient	Quantity for 50g
Span 60	200 mg
Cholesterol	100 mg
Ethanol	30 ml
Water	20ml
Drug	50 mg
Tween 80	650 mg
Carbopol	337.5Mg
Tea tree oil	2.5ml

Characterization of the Niosomal gel

1. Viscosity testing [18]

For determining the viscosity of the optimized formulation was performed using Brookfield viscometer. The RPM was initially increased and later decreased in a consistent and controlled manner. The readings were noted for change in viscosity (in Cp). A viscosity vs RPM graph was plotted with the upward and downward curves which were later overlayed to investigate the rheological nature of the formulation.

2.The Spread ability test [19]

When assessing the use and comfort of topical formulations like gels, spread ability is an important attribute to look for. The time it takes for a gel to spread between two glass slides under a certain weight is measured in this test. This gives an indicator of the gel's consistency and use. In between two sterile glass slides, the gel was spread out to a consistent thickness. On top of the upper glass slide, a 1 gram weight was inserted to guarantee equal spreading. After setting up, we gave it 5 minutes to relax. The weighing pan that was fastened to the top glass slide had more weight added to it. The top glass slide was allowed to glide over the lower slide for a certain amount of time while the increased weight was applied. A measure of spread ability is the time it takes for the slide to move.

Formula

The spread ability (S) can be calculated using the formula:

$S=m\times l/t$

where:

m = weight tied to the upper slide (in grams)

l = length moved on the glass slide (in cm)

t = time taken (in seconds)

3.Extrusion test [20]

A semisolid formulation's extrusion ease from a tube or container may be assessed using the extrusion test. This includes creams, gels, and ointments. If you want to know how reliable

and easy the product is to use, this is the test for you. The substance was carefully transferred to a clean, empty tube that had a tiny orifice. There shouldn't be any damage or leaks in the tube. After making sure there are no air bubbles within, the tube was filled with the formulation. No air could escape from the tube because of the tight seal. The temperature of the substance was kept constant for the whole test. You may use a holder to ensure the tube remains upright or set it on a level surface. It was determined that 0.75% carbopol had the best uniformity via visual inspection.

4.Entrapment efficiency [21]

The niosomes were first centrifuged to separate them in order to determine the drug loading level. The separated pellet was dissolved in ethanol and the quantity of encapsulation was measured using UV-Visible spectroscopy at 235 nm. Centrifugation at 5,000 rpm for 30 minutes was used to separate the drug-loaded niosomes. To ensure that the niosomes were free of any medication in the supernatant, they were rinsed twice with distilled water. Using ultraviolet-visible spectroscopy, we were able to approximate the medication concentration in the supernatant. By deducting the quantity in the supernatant (i.e., loss) from the initial drug solution, the amount of medication encapsulated in niosomes was directly determined. Triplicate runs of each experiment were carried out.

The % EE was calculated by the following equation:

% EE = Amount of drug-loaded in the noisome / Initial amount of the drug × 100

5.Determination of Vesicle Size [22]:

The Malvern particle size analyzer, a computerized inspection device, was used to estimate the vesicle size using dynamic light scattering. Before analysis, freshly made vesicle batches were diluted tenfold with filtered distilled water. The niosomal dispersion was assessed by measuring the vesicle size and polydispersity index.
One parameter that may be used to determine the breadth of the vesicle distribution is the polydispersity index. The distribution becomes broader as the polydispersity index rises. The zeta potential of the improved niosomal dispersion batches was measured using a Malvern zeta sizer. After that, the batches were diluted with filtered distilled water.

6.Fourier Transform Infrared Spectroscopy (FTIR) [23]

To study the possible chemical interactions between ADP, excipients and the formulation,

Fourier transform infrared (FTIR) spectra were recorded on a Jasco V5300 FTIR (Tokyo, Japan). Samples were mixed with KBr to construct pellets by using a pressure of 150 kg cm^{-2}. FTIR spectra were scanned in the range of 4000–400 cm^{-1} at a resolution of 2 cm^{-1}

7.In-vitro Diffusion Studies

In vitro release studies were carried out utilizing an artificial cellophane membrane (Membra-Cel^{s} MD 34-14; cut-off: 12 kDa; Viskase Co, MS, USA). For this study, a vertical Franz diffusion cell with a reservoir volume of 32 mL and a surface area of 2.54 cm^2 was utilized. The artificial cellophane membrane was firmly mounted between the two halves of the diffusion chamber. The receptor chamber contained ethanol: water (80: 20). The whole system was kept at 372 ^{o}C with constant magnetic stirring at 100 rpm. 1 g of accurately weighed ADP-NM-G gel and the commercial gel were placed on the donor chamber, respectively. The donor compartments were sealed carefully with para films to avoid the evaporation of the solution. At predetermined time points aliquots of the acceptor medium (1 mL) were withdrawn, subsequently filtered through a 0.45-mm membrane filter and replaced with fresh acceptor medium. The collected aliquots were then properly diluted and quantitatively analyzed using an UV spectrophotometer at 235 nm. All measurements were performed in triplicate and their means were reported.

8.Kinetic Modelling and Mechanism of Drug Release

1.Zero Order Release Kinetics

- Data obtained from *in-vitro* drug release study gives following kinetic models:

- In many of the modified release dosage forms, particularly controlled release dosage forms, is zero-order kinetic.

Q=K0t

- Where Q is the fraction of drug release at time t & K0 is the zero order release rate constant. When the plot is done as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K0.

2.First Order Release Kinetics

• The equation describing first order kinetics is,

In (1-Q) = -K1t

• Where Q is the fraction of drug released at time t. And K1 is the release rate constant for first order. Thus, a plot of the logarithm of the fraction of drug remained against time will be linear if the release obeys first order release kinetics.

3.Higuchi Model

• It defines a linear dependence of the active fraction released per unit of surface (Q) on the surface root of time. The following relationship applies,

Q = K2 t 1/2

• Where, K2 is release rate constant. For Obeying Higuchi equation a plot of the fraction of drug released against root of time should be the linear. From these Equation Based on the Fick's Law Drug Release is Described.

4.Korsmeyer- Peppas Model

• In order to define a model, which would represent a better fit for the formulation data was further analyzed by Korsmeyer- Peppas equation,

Mt/M∞ = ktn

• Where, Mt is the amount of drug released at time t and M α is the amount released at Time α, thus the Mt / M∞ is the fraction of drug released at time t, K is the kinetic constant and n is the diffusional exponent. A plot between log of Mt / M∞ against log of time will be linear if the release obeys Peppas & Korsmeyer equation and the slope of this plot represents n value. The n indicates the drug release mechanism. For a slab the value n = 0.5 indicates Fickian diffusion, values of n > 0.5 and ≤ 1.0 indicate non- Fickian mechanism.

5.Hixson-Crowell Model

• The Simplified equation is represented as,

Q0 1/3 - Qt 1/3 = K*t

• Where, Qt = amount of drug released in time (t) Q0 = initial amount of drug in solution K = cube root constant. If geometric shape of the formulation diminishes proportionally over time then graphic representation of cubic root of unreleased fraction of drug versus time will be linear.

6.Differential scanning calorimetry

A constant heating rate of 10 1C $min^{—1}$ was utilized over a temperature range of 20–340 1C for the lyophilized ADP-NM and their individual excipient components with nitrogen purging (50 mL $min^{—1}$). Indium standards were utilized to standardize the enthalpy scale and temperature. Approximately 3–4 mg of samples was used for the differential scanning calorimetry (DSC) study.

7.Surface Morphology

We used transmission electron microscopy (TEM, JEM 1400) to examine the niosomes' morphology. An enhanced sensitivity and resolution compared to more classic thermionic sources like LaB6 or Tungsten filaments are produced by the high brightness field-emission gun (FEG) source, which is part of the 200 KV TEM. Using vitreous ice and imaging at temperatures below -180°C, this method rapidly freezes biological material. As indicated in the figure in the discussion section, the last optimized batch of drug-loaded niosomes was subjected to transmission electron microscopy (TEM) investigation.

8.Stability Study [24]

Accelerated stability studies, conducted in accordance with ICH Q1A(R2) guidelines, are essential for predicting the long-term stability and shelf life of an optimized pharmaceutical batch. By exposing the optimized batch to stress conditions (e.g., 40°C/75% RH), critical quality parameters such as potency, degradation, and microbial stability are closely monitored over time. These results help to quickly assess the product’s stability under normal storage conditions, ensuring regulatory compliance and guaranteeing the safety and efficacy of the optimized formulation throughout its intended shelf life.

Marketed formulation Study [26]

Comparison of Adapalene with tea tree oil niosomal gel with marketed formulation of adapalene i.e. adapalene gel

Drug Diffusion Study for marketed adapalene gel was done and was compared with inhouse prepared formulations.

Result and Discussion:

The formulation utilized key excipients like Adapalene, Tea Tree Oil, and penetration enhancers (DMSO, NMP), which contributed to drug permeation and gel stabilization. Instruments such as UV spectrophotometer, viscometer, and Franz diffusion cell were vital for analyzing drug content, viscosity, and release behavior. Characterization confirmed vesicle stability and uniformity. All trends, comparisons with marketed products, and evaluations using Tables 5.10–5.20 and Figures 6.1–6.23 are discussed in detail.

Pre-formulation Studies [1]

Drug Selection

Adapalene was selected as API for Formulation Development

Drug Characterization [1-5]

The characterization of adapalene confirmed its identity as an off-white powder with a melting point of 319-322°C, soluble in ethanol and DMSO. UV-Visible spectroscopy and FTIR studies validated its purity and structural integrity.

Tea tree oil appeared as a pale-yellow essential oil, miscible in ethanol with a pH of 5.5, making it skin-compatible. FTIR analysis confirmed the presence of key bioactive compounds like terpinen-4-ol, essential for antimicrobial properties.

Lemongrass oil was also pale yellow, miscible in ethanol, with a pH of 6.5. Its FTIR study confirmed the presence of major components like citral, contributing to its antimicrobial and anti-inflammatory effects.

Determination of λmax of Adapalene

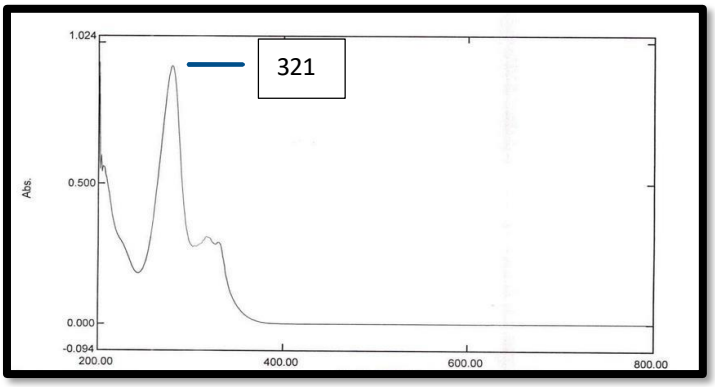

Figure 1: UV Spectroscopy of Adapalene

Discussion:

The UV-Visible spectroscopy of adapalene shows a λmax at 321 nm, indicating its characteristic absorption peak. This confirms its structural integrity and suitability for further formulation studies. The sharp peak suggests good solubility and stability in the selected solvent system.

FTIR Spectroscopy of Adapalene

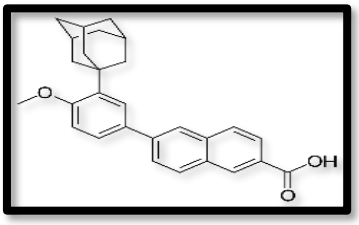

Figure 2: Structure of Adapalene

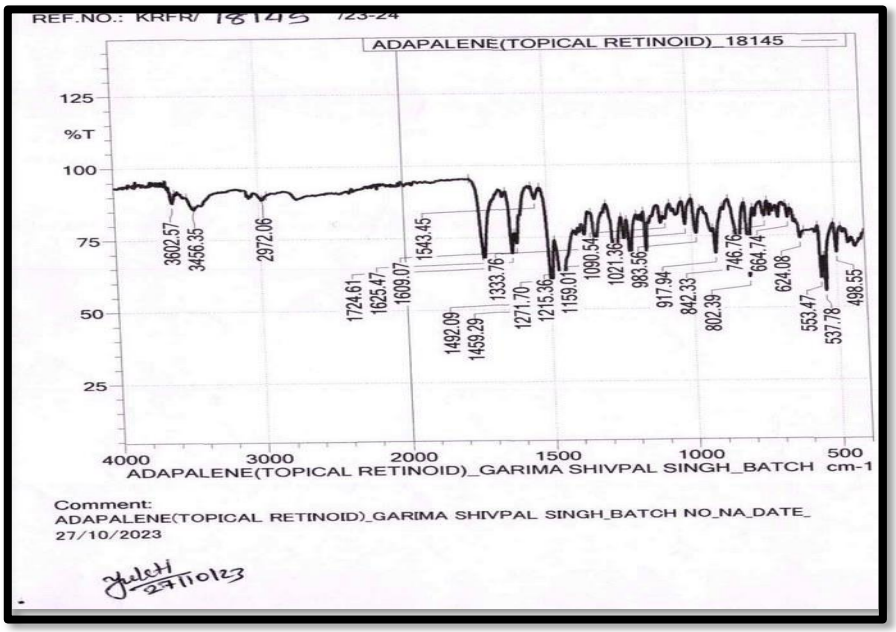

Figure 3: FTIR of Adapalene

Table 10: Characteristic FTIR Peaks of Adapalene

Bond Type	Observed Value (cm^{-1})	Std. Value (cm^{-1})
C_6H_6	2972	3000-2850
COOH	3456	3000-2400
C-O	1021	1300-1000

FTIR Spectroscopy of Tea Tree Oil

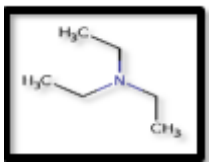

Figure 4: Structure of Tea Tree Oil

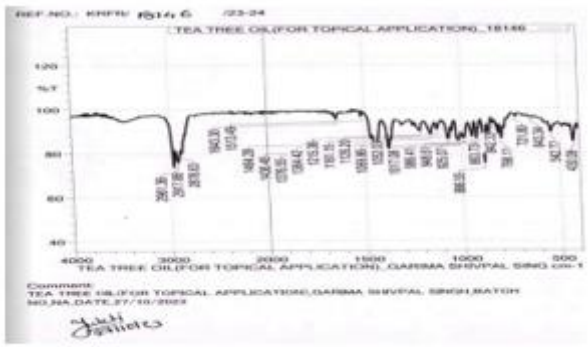

Figure 5: FTIR of Tea Tree Oi

Table 11: Characteristic FTIR Peaks of Tea Tree Oil

Bond Type	Observed Value (cm^{-1})	Std. Value (cm^{-1})
-CH_2	1465	1464.28
-CH_3	1376	1450-1375
-CN	1643	1640-1550

Analytical method development

calibration curve of adapalene

Table 12: Concentration and Absorbance of Adapalene

Concentration (ppm)	Absorbance at 235 nm
3	0.193
5	0.326
7.5	0.505
10	0.64
12	0.741

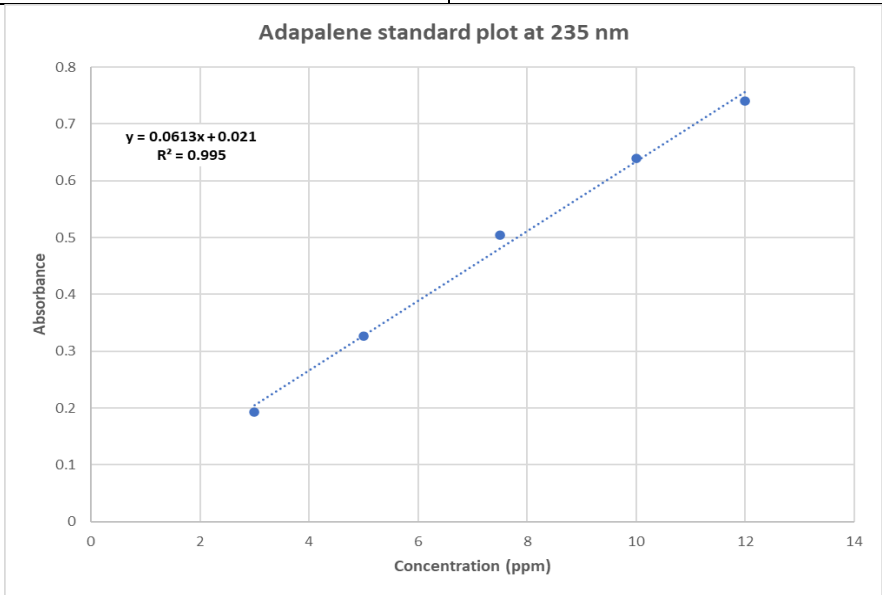

Figure 6: Adapalene Standard Plot

Discussion

The calibration curve for absorbance at 235 nm shows a strong linear relationship ($R^2 = 0.995$) with the equation $Y = 0.0613x + 0.021$, indicating high accuracy and reliability. The absorbance values increase proportionally with concentration, confirming adherence to Beer-Lambert's law. This linearity ensures the method's suitability for quantitative analysis of the compound.

Drug and Excipient Compatibility Study [7]

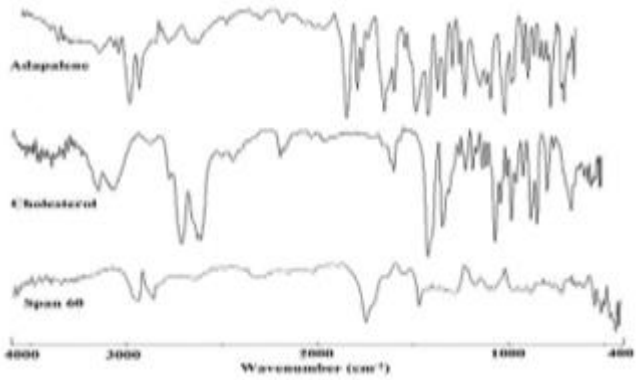

Figure 7: FTIR of API and Excipient

Discussion:

The FTIR spectra of adapalene, cholesterol, and Span 60 were analyzed for compatibility studies, showing no significant shifts or disappearance of characteristic peaks. This indicates no major interactions between the components, confirming their compatibility for niosomal gel formulation

Selection of Excipients

Span 60 Optimization [8]

Analysis of entrapment efficiency was carried out by centrifuging 1 ml of the formulation in a microcentrifuge tube at 10000 rpm for 10 min. The supernatants of the samples were separated from the pellet. The pellets were dissolved in 200ul THF and volume was made up to 1 ml with distilled water. The diluted samples were analyzed for drug content by UV spectrophotometry at 235 nm as the λ_{max}.

Out of all 40, 60, 80 mg concentration of span 60 were giving optimum result for PDI and % EE, So, these concentrations were used for further analysis.

Carbopol Optimization [9]

Table 13: Preliminary Batch

Ingredient	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Adapalene	10 mg	10 mg	10 mg	10 mg	10 mg
Span 60	40 mg	40 mg	40 mg	40 mg	40 mg
Tween 80	131mg	131mg	131mg	131mg	131mg
Cholesterol	60 mg	60 mg	60 mg	60 mg	60 mg
Ethanol	6 ml	6 ml	6 ml	6 ml	6 ml
Carbopol	0.30%	0.5 %	0.75 %	0.9 %	1 %
Water	4 ml	4 ml	4 ml	4 ml	4 ml

In order to determine the optimal Carbopol concentration, we played around with different amounts and saw how the formulation's texture, stability, extrudability, and ease of application changed.

Table 14: Extrudability of Preliminary Batches

Batches	Extrudability
T1	+
T2	++
T3	+++
T4	++++
T5	++++

Out of all 5 batches, batch 3 was selected having concentration of 0.75% Carbopol as it was providing the optimum result for texture and extrudability.

Discussion:

The Carbopol optimization study aimed to determine the ideal concentration for achieving the best texture, stability, and extrudability in the formulation. Five batches were prepared with varying Carbopol concentrations (0.30% to 1%), and their extrudability was assessed. Batch T3 (0.75% Carbopol) was selected as the optimal formulation, as it provided the best balance of smooth texture and ease of extrusion, making it suitable for further development.

Optimization of Formulation Using 3^2 Full Factorial Design [10]

Table 15: 3^2 Design Layout with Respective Observed Response

	REAL VALUES		TRAMSFORMED VALUES		DEPENDENT VARIABLE	
BATCH CODE	Span 60 Concentration (mg)	Cholesterol Concentration (mg)	X1	X2	Y1 Particle Size (nm)	Y2 Entrapment Efficiency (%)
F1	40	10	-1	-1	172.3	71
F2	60	10	0	-1	400.9	57
F3	80	10	1	-1	581.3	43
F4	40	20	-1	0	166.2	80
F5	60	20	0	0	477.2	62
F6	80	20	1	0	512.7	54
F7	40	30	-1	1	254.2	75
F8	60	30	0	1	361.6	68
F9	80	30	1	1	641.7	58

Discussion:

The formulations show great potential for niosomal gel preparation, with promising particle size and entrapment efficiency. While particle size increases with higher Span 60 concentrations, it can be optimized for enhanced bioavailability. The entrapment efficiency

(%EE) values demonstrate the formulation's capability to effectively encapsulate the active ingredient.

Evaluation of Factorial Batches [11]

Table 16: Evaluation of Factorial Batches

Batches	Particle Size(nm)	% EE	PDI	Viscosity(cp)	Spread ability ($gm.cm/s^2$)	Extrudability
F1	172.3	71	0.301	45000	12.2	+
F2	400.9	57	0.324	22000	13.18	+
F3	581.3	43	0.312	13000	13.32	++
F4	166.2	80	0.324	8000	18.4	+++
F5	477.2	62	0.322	7800	19.2	+++
F6	512.7	54	0.458	13200	21.4	++
F7	254.2	75	0.591	22000	22.5	++
F8	361.6	68	0.761	46000	20.3	++
F9	641.7	58	0.790	48000	20.1	++

Discussion:

The evaluation of nine formulations (F1-F9) showed varying particle sizes (166.2–641.7 nm), entrapment efficiency (43–80%), PDI, viscosity, spreadability, and extrudability. F4 (166.2 nm, 80% EE, good spreadability, and extrudability) was the most optimal, balancing stability and application properties, making it the best candidate for further formulation studies.

Diffusion Study of Factorial Batches [12]:

Table 17: Diffusion Study of Factorial Batches

Time(hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	2.4	3.5	4.8	0.77	4.3	3.6	5.47	0.74	1.8
1	12.2	13.2	13.3	6.00	14.2	16.1	14.03	5.2	9.4
2	21.4	21.3	20.5	15.34	20.0	21.2	19.4	14.2	15.01

3	27.81	30.2	28.9	24.30	29.2	27.2	27.24	22.20	23.10
4	29.8	30.2	31.9	30.2	29.9	30.0	30.55	31.02	32.01
5	35.2	34.8	34.7	44.56	33.25	33.2	33.24	48.20	47.1
6	40.12	39.2	40.4	50.82	41.0	38.2	36.16	51.7	53.9
7	47.20	48.1	48.0	63.55	47.1	46.2	46.10	61.4	63.2
8	53.70	52.8	55.9	70.81	60.21	69.2	52.69	73.2	76.2

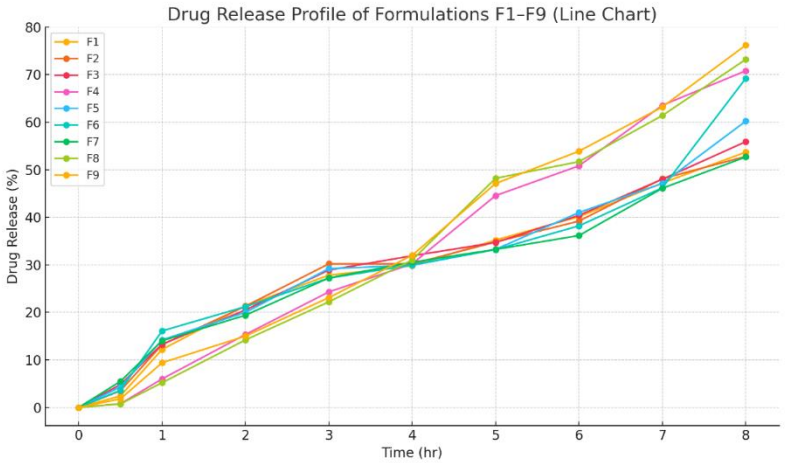

Figure 8: Time V/s drug Diffusion

Discussion:

Formulation F4 demonstrates a favourable release profile, with a steady increase in drug release over time. At 8 hours, it reaches 70.81%, indicating a controlled and sustained release. This gradual release suggests that F4 effectively balances both immediate and prolonged therapeutic action. Compared to other formulations, F4 provides a reliable and consistent release, making it an ideal choice for sustained drug delivery. The results highlight its potential for optimal performance in niosomal gel preparations. Thus, batch F4 has been selected for further development due to its promising release characteristics.

Statistical Analysis [13]:

Using the design Expert program, a three-level factorial was conducted with three independent variables. Particle size and percent energy efficiency were chosen as the dependent variables, whereas cholesterol content and Span 60 were chosen as the independent variables.

ANOVA for Particle size

Table 18: ANOVA for Particle size

Source	Sum of Squares	Df	Mean square	F-value	p-value	
Model	2.213E+05	4	1.098E+05	12.47	0.0157	significant
A-span	2.177E+05	1	2.177E+05	49.07	0.0002	
B-cholesterol	1768.17	1	1768.17	0.3985	0.5622	
A^2	1266.72	1	1266.72	0.2855	0.6215	
B^2	553.34	1	553.34	0.1247	0.7418	
Residual	19569.72	6	3261.62			
Cor Total	2.391E+05	8				

Discussion:

The ANOVA results confirm that the developed model is statistically significant, as indicated by a Model F-value of 12.47 and a p-value of 0.0157. This suggests that the likelihood of obtaining these results purely by chance is only 1.57%, demonstrating the model's reliability in predicting the response variable.

Assessment of Model Parameters

- A-span is identified as a highly influential factor with a p-value of 0.0002, indicating a strong impact on the response.
- B-cholesterol shows a higher p-value (0.5622), implying that its contribution to the model is relatively minor.
- The quadratic terms (A^2 and B^2) have p-values of 0.6215 and 0.7418, respectively, suggesting that their effect on the response is minimal.

Model Fit and Variability

- The Residual Sum of Squares (19,569.72) represents the unexplained variation in the response, while the Total Sum of Squares (2.391E+05) accounts for the overall

variability in the dataset.

- A significant portion of the total variation is explained by the model, confirming its validity.

CONCLUSION

The statistical evaluation supports that the model is well-structured and effectively represents the observed data. A-span emerges as the most impactful factor, whereas B-cholesterol, A^2, and B^2 contribute less significantly.

Fit Static

Table 19: Fit Static

Std. Dev.	60.50	**R^2**	0.9234
Mean	396.46	**Adjusted R^2**	0.8775
C.V %	15.26	**Predicted R^2**	0.7627
		Adeq Precision	10.2970

Coefficient Table

Table 20: Coefficient Table

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	402.14	1	57.15	220.28	584.01	1.0000
A-Span	190.50	1	31.30	90.89	290.11	1.0000
B-Cholesterol	17.17	1	31.30	-82.44	116.78	1.0000
AB	-5.38	1	38.33	-127.37	116.62	1.0000
A^2	-25.17	1	54.21	-197.70	147.36	1.0000
B^2	16.63	1	54.21	-155.90	189.16	1.0000

$$Y = -395.90556 + 17.61250X_1 - 3.32417X_2 - 0.062917X_1^2 + 0.166333X_2^2 - 0.026875X_1X_2 + \varepsilon$$

ANOVA for %EE

Table 21: ANOVA for %EE

Source	Sum of Squares	Df	Mean square	F-value	p-value	
Model	1045.36	2	534.83	32.12	0.0083	significant
A-span	840.17	1	888.17	129.07	0.0015	
B-cholesterol	150.00	1	181.50	23.04	0.0172	
AB	30.25	1	30.25	4.65	0.1201	
A^2	2.72	1	2.72	0.4182	0.5639	
B^2	553.34	1	22.22	3.41	0.1618	
Residual	19.22	6	6.51			
Cor Total	1088.89	8				

Discussion:

Interpretation of ANOVA Results

The results from the analysis of variance (ANOVA) confirm that the developed model is statistically significant, as demonstrated by a Model F-value of 32.12 and a p-value of 0.0083.

Assessment of Model Parameters

- A-span plays a dominant role in influencing the response, as indicated by a p-value of 0.0015 and a high F-value of 129.07, confirming its significant contribution.
- B-cholesterol also shows a notable effect, with a p-value of 0.0172 and an F-value of 23.04, suggesting that it has a meaningful impact on the model.
- The interaction term (AB) presents a p-value of 0.1201, indicating that its effect is not statistically significant at the chosen confidence level.
- The quadratic terms (A^2 and B^2) have p-values of 0.5639 and 0.1618, respectively, implying that their contributions to the model are relatively minor.

Model Fit and Variability Analysis

- The Residual Sum of Squares (19.22) represents the unexplained variability in the dataset, while the Total Sum of Squares (1088.89) accounts for the overall variation.
- Since the model captures a substantial portion of the total variability, it confirms its effectiveness in representing the data accurately.

Conclusion

The statistical analysis indicates that the model is well-developed and suitable for explaining the observed results. Among the factors examined, A-span has the most substantial influence, followed by B-cholesterol, while interaction and quadratic terms contribute less significantly.

Fit Static

Table 22: Fit Static

Std. Dev.	2.55	**R^2**	0.9817
Mean	63.11	**Adjusted R^2**	0.9511
C.V %	4.04	**Predicted R^2**	0.8420
		Adeq Precision	16.6815

Coefficient Table

Table 23: Coefficient Table

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	64.56	1	1.90	58.50	70.61	1.0000
A-Span	-11.83	1	1.04	-15.15	-8.52	1.0000
B-Cholesterol	5.00	1	1.04	1.69	8.31	1.0000
AB	2.75	1	1.28	-1.31	6.81	1.0000
A^2	1.17	1	1.80	-4.57	6.91	1.0000
B^2	-3.33	1	1.80	-9.07	2.41	1.0000

$Y=64.56-11.83X_1+5.00X_2+2.75X_1X_2+1.17X_1^2-3.33X_2^2+\varepsilon$

Contour Plot and Surface Plot of Design

Contour Plot for Particle Size

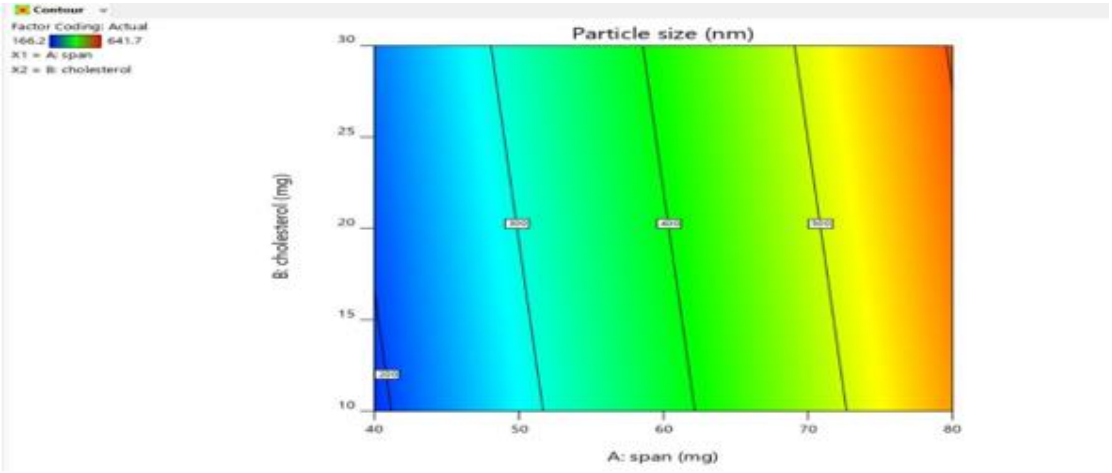

Figure 9: Contour Plot for Particle Size

Surface Plot for Particle Size

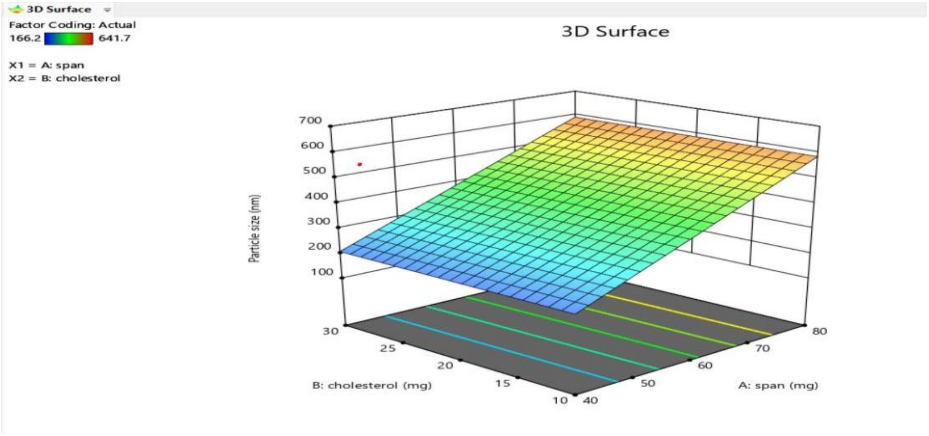

Figure 10: 3D Surface Graph for Particle Size

Contour Plot for %EE

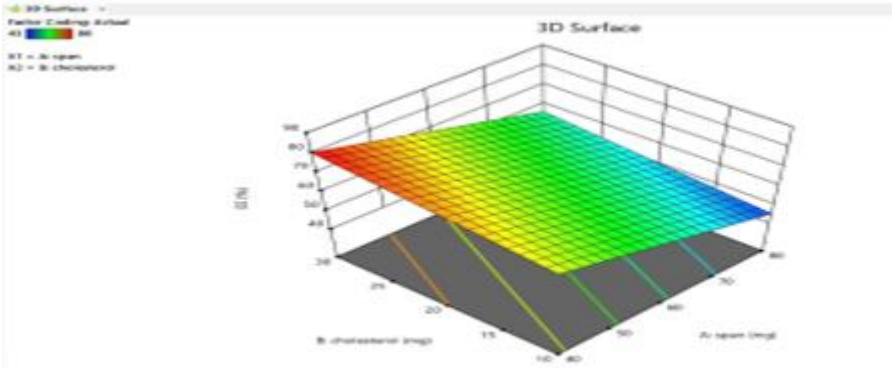

Figure 11: Contour Plot for %EE

Surface Plot for % EE

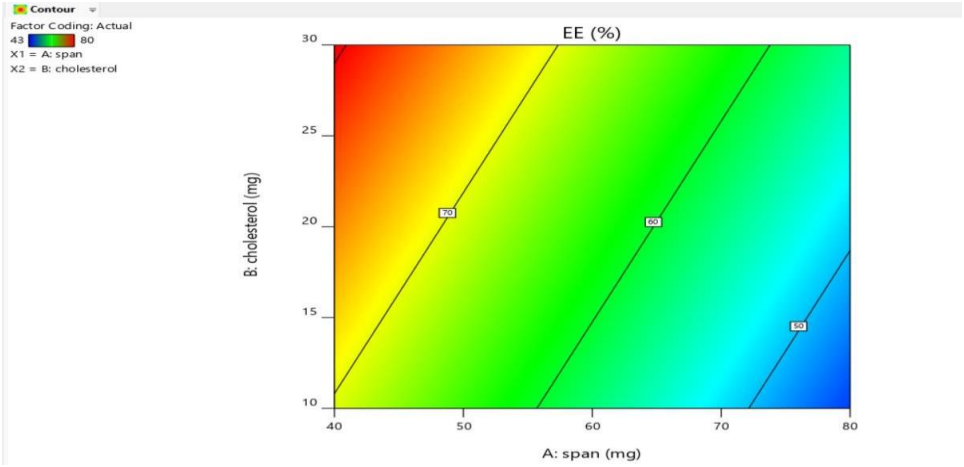

Figure 12: 3D Surface Graph of %EE

Discussion

Effect of Span 60 Concentration (x_1):

- **Particle Size (y1)**: The coefficient for x_1 is positive and statistically significant, indicating that as Span 60 concentration increases, particle size increases. This reflects a trend where higher x_1 leads to larger niosomal vesicles.
- **% Entrapment Efficiency (y_2)**: The coefficient for x_1 is negative and significant, suggesting that increasing Span 60 concentration decreases % EE. This indicates that higher x_1 may cause reduced drug entrapment efficiency, possibly due to larger vesicle size or other encapsulation challenges.

Effect of Cholesterol Concentration (x_2):

- **Particle Size (y_1)**: The coefficient for x_2 is negative and significant, showing that increasing cholesterol concentration results in smaller particle sizes. This implies that cholesterol stabilizes the niosomal membrane, leading to smaller, more uniform vesicles.
- **% Entrapment Efficiency (y_2)**: The positive coefficient for x_2 indicates that higher cholesterol concentration improves % EE. This suggests that increased cholesterol enhances drug retention within the niosomes.

Interaction Effects ($x_1.x_2$):

- The interaction term $x_1.x_2$ is statistically significant, demonstrating that the effects of Span 60 and cholesterol on particle size and % EE are interdependent. The interaction term shows that high levels of both x_1 and x_2 produce complex changes in these properties. Specifically, while increasing x_1 tends to increase particle size and decrease % EE, increasing x_2 can mitigate these effects, improving both particle size and % EE.

Overlay Plot

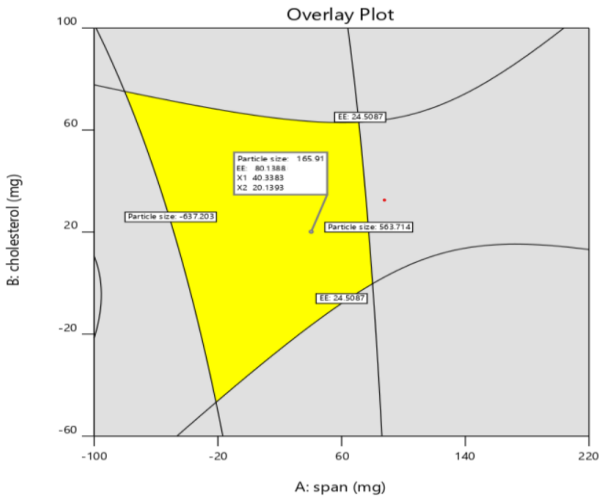

Figure 13: Overlay Plot

Optimized Batch:

Among all the experimental batches, Batch 4 emerged as the optimal formulation based on the combined performance metrics. This batch exhibited:

- **Entrapment Efficiency (EE)**: 80%
- **Polydispersity Index (PDI)**: 0.324
- **Viscosity**: 8000 cP
- **Spread ability**: 18.4

Batch 4's high % EE indicates effective drug entrapment, while the low PDI suggests a uniform particle size distribution. The viscosity of 8000 cP and spread ability of 18.4 are favourable for practical application, balancing ease of use and formulation stability.

Conclusion:

The factorial design analysis, including statistical coefficients and interaction effects, provides insights into how Span 60 and cholesterol concentrations influence particle size, % EE, and other key characteristics of the niosomal gel. Batch 4 represents the optimized formulation, achieving desirable characteristics and demonstrating the effectiveness of the factorial approach in formulation optimization.

Optimized Batch Formula and Evaluation Table [10]

Table 24: Optimized Batch

Formulation Ingredient	Observation
Adapalene	10 mg
Span 60	40 mg
Tween 80	130 mg
Cholesterol	20 mg
Carbopol	67 mg
Water	4 ml
Tea Tree Oil	0.5 ml
% EE	80
Particle size	166.2
PDI	0.324
Viscosity	8000 cp

Spread ability	18.4
Extrusion	+++
Diffusion (24 hr)	87.8

Validation Using Checkpoint Batch

To further validate the predictive strength of the factorial design model, a checkpoint batch was formulated for the Tea Tree Oil-based niosomal gel. This batch was carefully chosen within the optimized design space but with slight variations in key excipient concentrations to assess the robustness and accuracy of the optimization model.

Checkpoint Batch Composition:

Table 25: Checkpoint Batch Composition

Component	Optimized Batch (Batch 4)	Checkpoint Batch
Adapalene	10 mg	10 mg
Span 60	40 mg	40 mg
Tween 80	130 mg	130 mg
Cholesterol	20 mg	20 mg
Carbopol	67 mg	67 mg
Water	4 ml	4 ml
Tea Tree Oil	0.5 ml	0.5 ml

Comparison of Predicted vs. Experimental Results:

Table 26: Checkpoint Batch Composition

Parameter	Predicted Range	Experimental Results
Particle Size (nm)	166.2	168
Entrapment Efficiency (%)	80	81
Polydispersity Index (PDI)	0.324	0.310

Viscosity (cP)	8000	8250
Spread ability (g.cm/s²)	18.4	18.0

Evaluation and Interpretation: The checkpoint batch for Tea Tree Oil formulation closely matched the predicted values, demonstrating the consistency and accuracy of the factorial design model. This confirms that the factorial model is a reliable tool for formulation design and supports reproducibility in large-scale manufacturing.

Discussion of Characterization:

The niosomal gel formulation containing Adapalene was successfully optimized and evaluated for various parameters. The formulation demonstrated a **% Entrapment Efficiency (EE) of 81%**, which is within the desired range of 70-90%, indicating efficient encapsulation of the active ingredient.

The **particle size** was observed to be **168 nm**, which falls within the acceptable range for topical applications (100-200 nm). This size is optimal for enhanced skin penetration and stability of the niosomal gel.

The **Polydispersity Index (PDI)** was found to be **0.310**, which is below the critical value of 0.5, indicating a uniform particle size distribution and good formulation stability.

The **viscosity** of the formulation was measured at **8250 cP**, aligning with the desired range of 4000-10,000 cP for topical gels, ensuring that the formulation is neither too thick nor too fluid, providing ease of application.

The **spread ability** was recorded at **18.0 cm**, which is within the desired range of 15-20 cm, ensuring that the gel spreads easily and evenly over the skin.

Lastly, the **extrusion ability** was rated as **+++**, indicating excellent extrudability, which is crucial for user-friendly packaging and application.

In conclusion, the formulation met all the desired criteria for a stable, effective, and user-friendly niosomal gel. These characteristics suggest that the gel is well-suited for topical application, providing the necessary therapeutic benefits of Adapalene while ensuring patient compliance.

FTIR of optimized batch

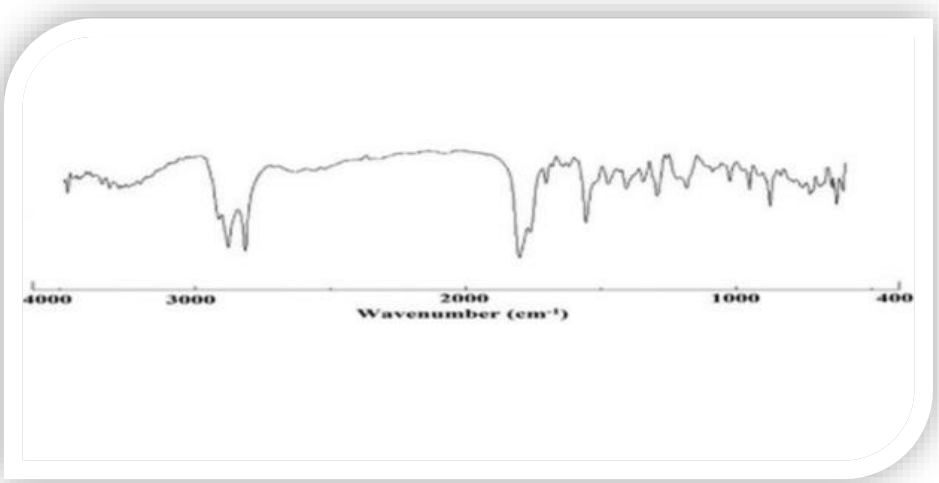

Figure 14: Optimized Batch FTIR

Discussion

The FTIR analysis of the optimized niosomal gel batch reveals the presence of characteristic peaks for adapalene, span 60, and cholesterol, with observed shifts and intensity changes indicating successful interaction between the components. These interactions confirm that adapalene is effectively encapsulated within the niosomal structure, and that Span 60 and cholesterol contribute to the stability of the formulation. The observed peak shifts and the absence of certain peaks suggest strong compatibility among the components, ensuring the formulation's stability and therapeutic efficacy.

Zeta Potential

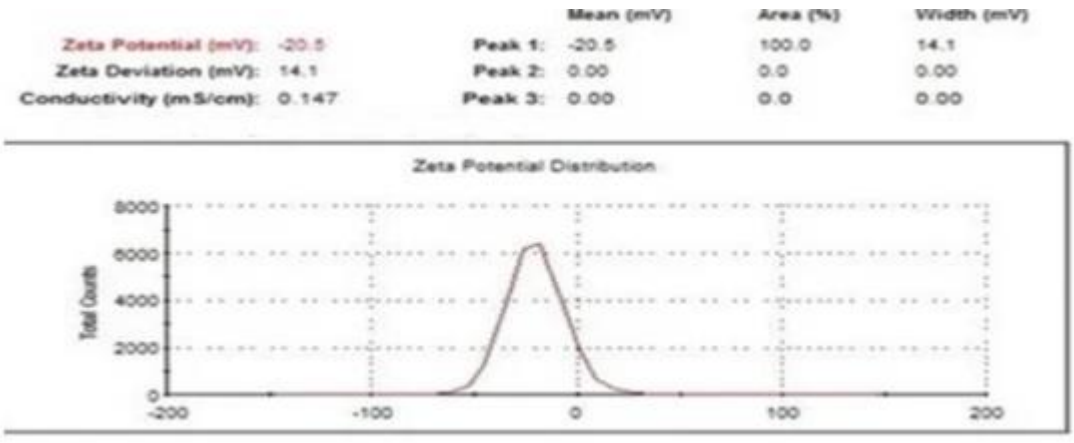

Figure 15: Zeta Potential of Optimized batch

Discussion

The zeta potential measurement indicates a high absolute value, which supports good colloidal stability and reduces the likelihood of aggregation. Together, these characteristics confirm the stability and quality of the niosomal gel formulation.

Kinetic Modelling and Mechanism of Drug Release

Zero Order Kinetics

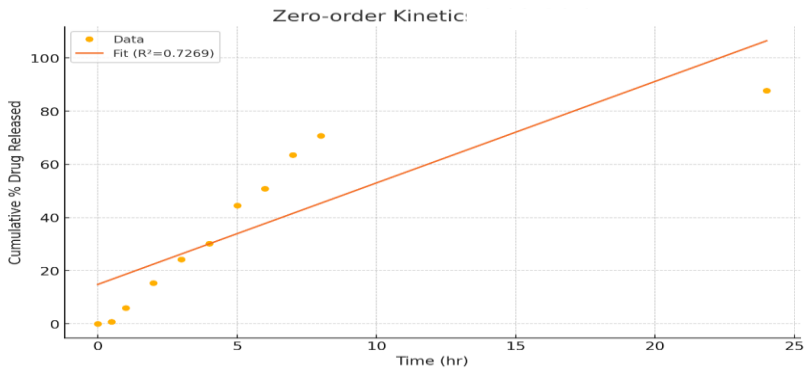

Figure 16: Zero order kinetics

First Order Kinetics

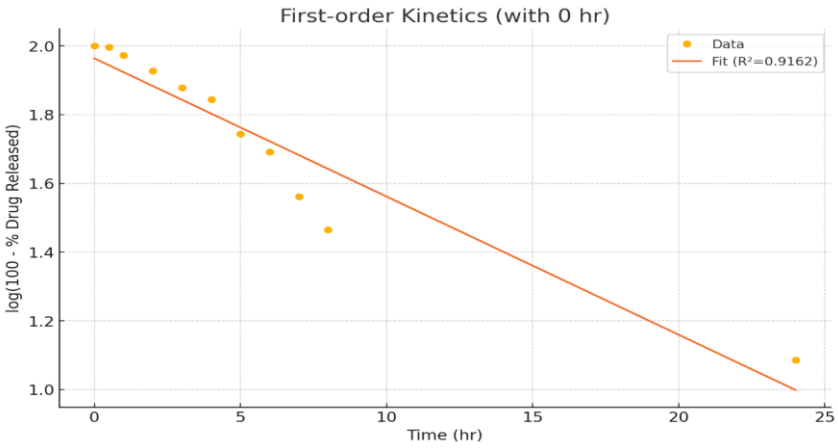

Figure 17: First Order Kinetics

Higuchi Model

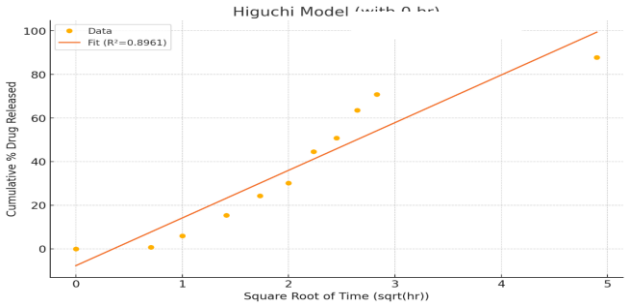

Figure 18: Higuchi Model

Korsmeyer Peppas Model

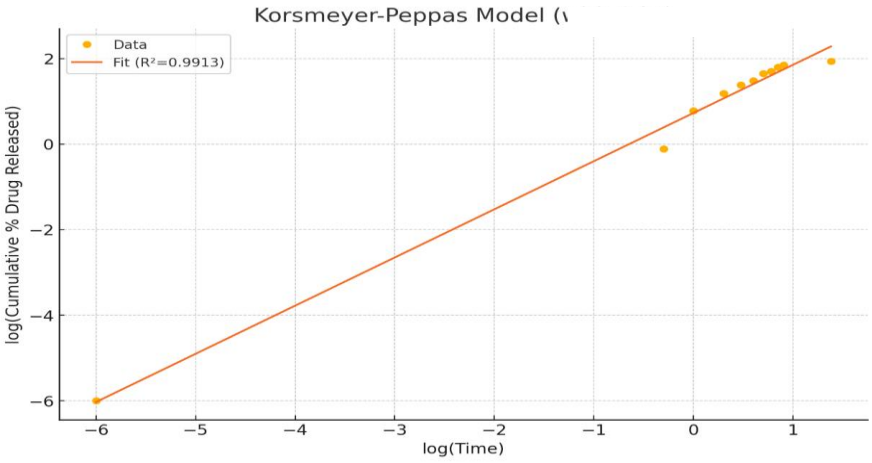

Figure 19: Korsmeyer-Peppas Model

Zero-order: $R^2 = 0.7269$

First-order: $R^2 = 0.9162$

Higuchi: $R^2 = 0.8961$

Korsmeyer-Peppas: $R^2 = 0.9913$

With the inclusion of zero-hour data, the **Korsmeyer-Peppas model** now shows the best fit ($R^2 = 0.9913$), suggesting that the drug release mechanism involves a combination of diffusion and erosion processes.

Discussion:

The model comparison shows that with the inclusion of zero-hour data, the Korsmeyer-Peppas model provides the best fit (R^2 = 0.9913). This suggests that the drug release mechanism follows a combination of diffusion and erosion processes, which is consistent with the high R^2 value, indicating strong predictive accuracy.

- Zero-order model (R^2 = 0.7269) shows a moderate fit, suggesting the release is not purely zero-order.
- First-order model (R^2 = 0.9162) fits better, indicating some concentration-dependent release.
- Higuchi model (R^2 = 0.8961) suggests that diffusion plays a role in the release, but not as dominantly as in the Korsmeyer-Peppas model.

Overall, the Korsmeyer-Peppas model's high R^2 value confirms a complex release mechanism, likely combining both diffusion and erosion, which is ideal for sustained drug delivery.

DSC

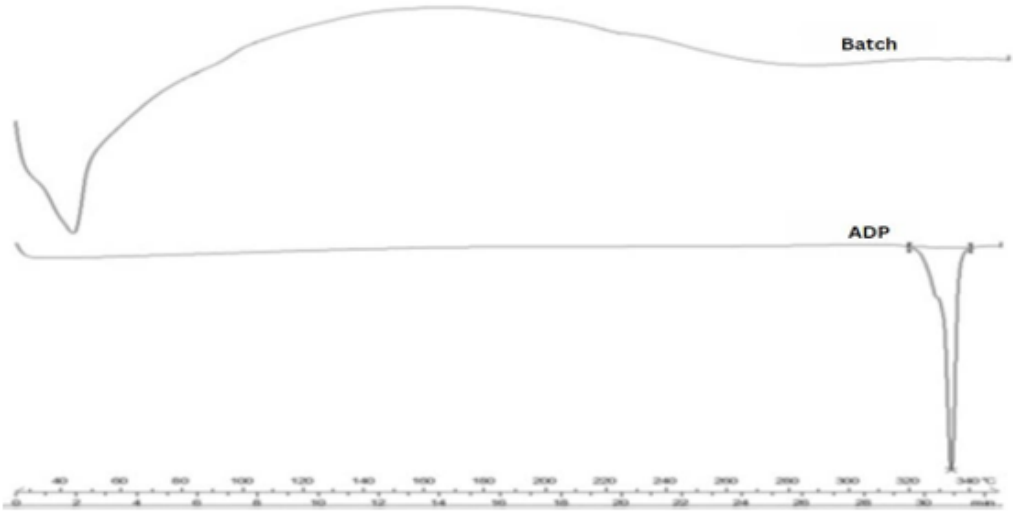

Figure 20: DSC of Optimized batch

Discussion

The DSC analysis reveals that the melting point of adapalene in the optimized niosomal gel batch is significantly altered compared to its pure form, indicating successful encapsulation within the niosomes. The shift or broadening of the melting peak for adapalene suggests it is

no longer in its crystalline state but is interacting with the other components of the formulation. This interaction supports the effective integration of adapalene into the niosomal matrix, ensuring the stability and potential efficacy of the gel.

Surface morphology (TEM)

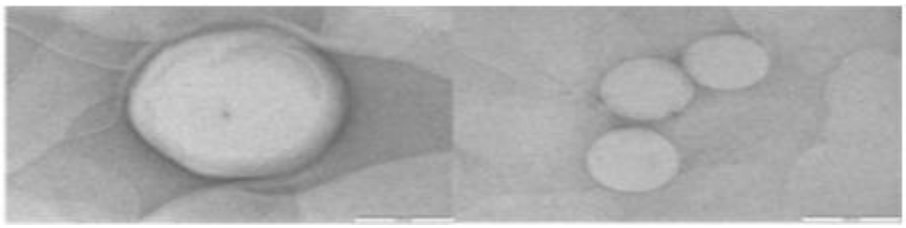

Figure 21: TEM of Optimized Batch

Discussion

The TEM analysis of the optimized adapalene niosomal gel with tea tree herbal oil reveals well-formed, spherical niosomes with a uniform size distribution and smooth surface morphology. The images confirm that the niosomes are consistently encapsulating the adapalene and tea tree herbal oil, with no significant aggregation or irregularities. The observed morphology supports the effectiveness of the formulation in stabilizing the drug and herbal oil within the niosomal system, indicating a successful encapsulation process and a stable, high-quality niosomal gel.

Stability Study [14]

Table 27: Stability Study

Condition	Appearance	Particle Size (nm)	% EE	Assay %	Microbial Growth	pH
Initial	Clear	166.2	80	100	No Growth	5.5
15 Days	Clear	169	80	100	No Growth	5.5
1 month	Clear	184.1	78	99	No Growth	5.4

Discussion

The stability testing data indicate that the niosomal gel formulation remained stable over a one-month period. Initially, the gel was clear with a particle size of 166.2 nm, an entrapment

efficiency (EE) of 80%, and an assay of 100%, with a pH of 5.5 and no microbial growth. After one month, the appearance remained clear, the particle size increased slightly to 184.1 nm, EE decreased to 78%, and the assay was 99%, with the pH adjusting slightly to 5.4. The absence of microbial growth throughout indicates that the formulation retained its stability and efficacy, suggesting good preservation of its physical and chemical properties over time.

Marketed formulation comparison [15]

Marketed Adapalene Gel

Table 28: Marketed Formulation % CDR

Tims (hrs)	Marketed formulation Diffusion Study (%)
0	0
0.5	2.4
1	12.2
2	27.81
3	40.12
4	53.7
5	69.51
6	80.2
7	89.63
8	90.8
9	94.6
10	97.3
11	98.9
12	100

Comparison of %Drug Diffusion of Marketed formulation with In-house Prepared Formulations of adapalene

Table 29: Comparative % CDR

Tims (hrs)	Adapalene+ Tea Tree oil Diffusion Study (%)	Marketed Adapalene Gel
0	0	0
0.5	0.77	2.4
1	3.00	12.2
2	15.34	27.81
3	24.30	40.12
4	30.2	53.7
5	44.56	69.51
6	50.82	80.2
7	63.55	89.63
8	70.81	90.8
24	87.81	-

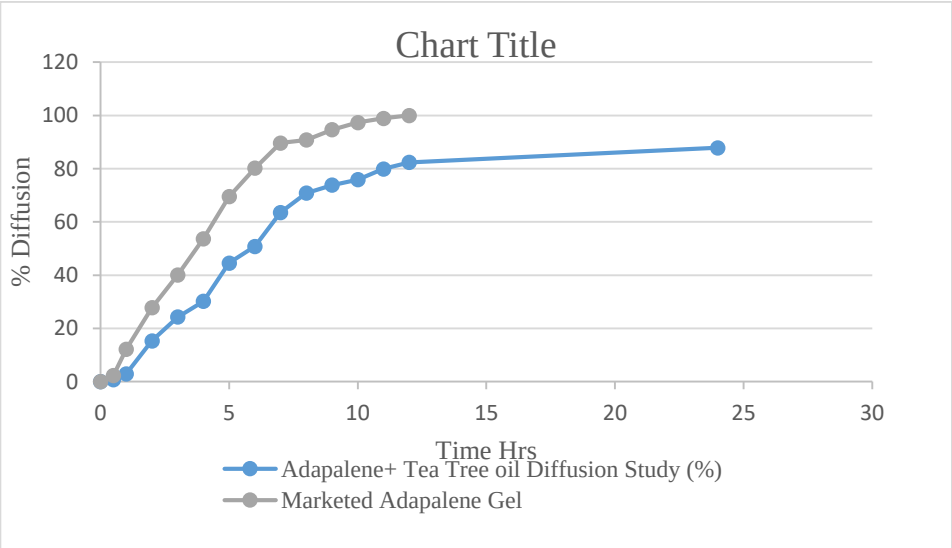

Figure 22: Comparative % CDR

Discussion:

The marketed formulation, lacking the niosomal delivery system, shows a less controlled release. Its quicker initial burst leads to lower overall diffusion, as much of the drug is lost before it can achieve sustained delivery.

This underscores the limitations of conventional gel formulations, which do not benefit from the protective and controlled-release characteristics of niosomes.

Summary

This study aimed to optimize a niosomal gel formulation containing Adapalene through a factorial design approach. The initial characterization of Adapalene confirmed it as a suitable active pharmaceutical ingredient (API), with an off-white powder appearance, solubility in ethanol and DMSO, and a melting point between 319-322°C. Tea Tree Oil and Lemongrass Oil were also characterized, showing Tea Tree Oil to be a pale yellow essential oil with a pH of 5.5 and Lemongrass Oil as having a pH of 6.5.

The factorial design investigated the effects of Span 60 and cholesterol concentrations on the niosomal gel properties. The evaluation parameters for the formulation included particle size, entrapment efficiency (EE), polydispersity index (PDI), viscosity, and spreadability. The design incorporated Span 60 at concentrations of 40, 60, and 80 mg and cholesterol at 10, 20, and 30 mg.

The evaluation parameters had the following ranges and desirability:

- **Particle Size**: Ranged from 166.2 nm (Batch 4) to 641.7 nm (Batch 9), with the desirable range being between 100-200 nm for optimal topical application.
- **Entrapment Efficiency (EE)**: Ranged from 43% (Batch 3) to 80% (Batch 4), with a desirable range of 70-90% indicating effective drug encapsulation.
- **Polydispersity Index (PDI)**: Ranged from 0.301 (Batch 1) to 0.790 (Batch 9), with a desirable value below 0.5 indicating uniform particle size distribution.
- **Viscosity**: Ranged from 7800 cP (Batch 5) to 46000 cP (Batch 8), with the desirable range being between 4000-10,000 cP for ease of application.
- **Spreadability**: Ranged from 12.2 cm (Batch 1) to 22.5 cm (Batch 7), with a desirable range

of 15-20 cm.

Batch 4 emerged as the optimal formulation on basis of which checkpoint batch was prepared, exhibiting the following final results:

- **Entrapment Efficiency (EE)**: 81%
- **Particle Size**: 168 nm
- **Polydispersity Index (PDI)**: 0.310
- **Viscosity**: 8250 cP
- **Spreadability**: 18.0 cm

Characterization analyses, including FTIR, zeta potential, DSC, and TEM, confirmed the successful encapsulation of Adapalene and the stability of the formulation. FTIR analysis showed characteristic peaks for Adapalene, Span 60, and cholesterol, indicating effective interaction. Zeta potential measurements indicated good colloidal stability, while DSC revealed changes in Adapalene's melting point, supporting its integration into the niosomal matrix. TEM images displayed well-formed, spherical niosomes with a uniform size distribution.

Stability studies showed that after one month, the formulation maintained a clear appearance, with a particle size of 184.1 nm, an EE of 78%, and a pH of 5.4. No microbial growth was observed, confirming the formulation's stability over time.

Overall, the factorial design approach effectively optimized the niosomal gel formulation, resulting in Batch 4 with desirable characteristics for topical application and demonstrating the efficacy of the design in improving formulation performance and stability.

CONCLUSION

The comprehensive evaluation and optimization of the niosomal gel formulation containing Adapalene have demonstrated a successful outcome across multiple parameters. The factorial design approach effectively identified Batch 4 as the optimal formulation, then checkpoint batch was prepared achieving a high entrapment efficiency of 81%, which is within the desired range of 70-90%. This batch also exhibited a particle size of 168 nm, falling within the optimal

range of 100-200 nm for enhanced skin penetration and stability. The low polydispersity index of 0.310 indicated a uniform particle size distribution, contributing to formulation stability.

Further, the viscosity of 8250 cP and spreadability of 18.0 cm were both within the preferred ranges, ensuring the gel's practical usability and ease of application. Stability testing revealed that the formulation maintained its clarity, with a slight increase in particle size to 184.1 nm and a minor decrease in entrapment efficiency to 78% after one month. The assay remained close to 100%, and the pH slightly adjusted to 5.4, with no microbial growth detected throughout the study. These results confirm that the niosomal gel formulation remains stable and effective over time.

Overall, the successful optimization and stability of the niosomal gel underscore the effectiveness of the factorial design in refining the formulation. The final product demonstrates desirable characteristics for topical application, highlighting its potential for therapeutic efficacy and patient compliance.

ACKNOWLEDGEMENT

Ms. Garima Singh, gratefully acknowledges the research guidance and encouragement received throughout this study. Sincere thanks are extended to the technical personnel and collaborators who contributed their time and support. The author also appreciates Vishal Chem, Mumbai, for supplying the necessary materials. This work would not have been possible without the insightful academic input and encouragement from mentors and fellow researchers.

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