Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : http://ijpsdronline.com/index.php/journal

Research Article

Formulation and Characterization of Invasomes Containing Adapalene Using Box-Behnken Design

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ARTICLE INFO

Article history:

Received: 29 November, 2024

Revised: 23 April, 2025

Accepted: 29 April, 2025

Published: 30 May, 2025

Keywords:

Adapalene, Invasomes, Box-Behnken design, Transdermal drug delivery, Limonene, Phosphatidylcholine, Entrapment efficiency, Vesicle size, Drug release, Stability study.

DOI:

10.25004/IJPSDR.2025.170303

ABSTRACT

This study concentrated on the creation and description of invasomes loaded with adapalene for improved topical delivery using a Box-Behnken experimental design. Invasomes, flexible vesicular carriers, were formulated with varying concentrations of limonene and ethanol as penetration enhancers and different levels of phosphatidylcholines and sonication time. The formulation parameters were changed to decrease the size of the vesicle as well as improve the efficiency of entrapment (EE). A size of vesicle 96.65 nm, an EE of 73.32%, as well as potential zeta of -42.23 mV were all shown by the optimized formulation (F6). *In-vitro* drug release studies indicated continued release of adapalene over 12 hours, with enhanced permeation observed with both limonene and ethanol as enhancers. Research on stability at 4 and 25°C demonstrated good stability, with only slight changes in vesicle size and encapsulation efficiency (EE) over a 3-month period. The results suggested that the optimized adapalene-loaded invasions offered a promising strategy for improving the transdermal delivery and therapeutic efficacy of adapalene for treating dermatological disorders.

Introduction

The creation of innovative drug delivery systems has garnered considerable interest lately due to their potential to enhance patient compliance, minimize side effects, and improve therapeutic efficacy. Using invasomes, which are specific liposomal formulations designed to facilitate the transdermal distribution of active pharmaceutical ingredients (APIs), is one such method. Compared to traditional liposomes, invasomes, a subclass of liposomes, include penetration enhancers built into their structure that improve medication penetration across the skin barrier. Because of their increased ability to penetrate the skin, invasomes are especially useful for delivering lipophilic medications like adapalene, which is frequently used to treat acne vulgaris and other dermatological diseases.

Adapalene (ADP) belongs to the class of retinoids that is third-generation, which exhibits a number of therapeutic benefits, such as keratolytic, anti-inflammatory, and antiseborrheic properties. By striking a balance between skin differentiation and oil production, ADP has been proven to be helpful in treating acne. It is also effective in treating keratosis pilaris and other skin conditions. 6-[3-(1-adamantyl)-4-methoxyphenyl] naphthalene-2-carboxylic acid chemical formula is ADP. The most common form of ADP is crystalline, has a color that ranges from white to off-white and is practically insoluble in aqueous solutions.[1] However, because it is lipophilic, its low permeability across the skin barrier makes it difficult to provide the best possible therapeutic results. To overcome this limitation, invasomes offer a promising solution by

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

enhancing the transdermal delivery of adapalene and increasing its bioavailability at the site of action.

Utilizing a response surface method (RSM), often employed to enhance the formulation of complex systems, the present study produced and studied adapalene-loaded invasomes using a Box-Behnken design (BBD). The type and concentration of surfactants, the concentration of penetration enhancers, and other formulation components are among the most important parameters influencing the properties of invasome formulations, and this design aids in identifying them. It is anticipated that the optimized invasomes will release adapalene in a controlled and targeted manner, thereby increasing its therapeutic potential and reducing adverse effects.

Invasome formulation development greatly increased the drug's permeability and retention in the skin, according to several studies that explored its potential in drug delivery. Lakshmi *et al*. (2014)[2] developed curcumin-filled invasomes for enhanced skin delivery, demonstrating improved permeation and therapeutic efficacy compared to free curcumin. Similarly, Babaie *et al*. (2015)[3] formulated lidocaine-loaded Nanoethosomes and observed superior skin penetration in contrast to traditional liposomes. Additionally, Arooj *et al*. (2023)[4] reported the use of adapalene-loaded lipid-based nanocarriers for acne treatment, emphasizing the potential of lipid-based systems for improving the stability and release profile of adapalene.

The pharmaceutical advantage of the study in the present scenario lay in the enhanced transdermal delivery of adapalene, a key treatment for acne and other skin disorders. By utilizing invasomes as a delivery system, the study addressed common limitations of conventional topical formulations, such as poor skin penetration and low bioavailability. By adding penetration enhancers like ethanol and limonene, the drug's absorption was further refined, increasing therapeutic results and lowering systemic adverse effects.

The objective of the current study was to formulate and assess invasomes loaded with adapalene while optimizing the formulation parameters using the Box-Behnken design. The objective of this study was to create and enhance invasomes filled with adapalene for improved transdermal delivery, addressing the limitations of conventional formulations that hinder effective skin penetration. The research aimed to optimize entrapment efficiency (EE) and reduce vesicle size by using a Box-Behnken design and adding ethanol and limonene as penetration enhancers, thereby improving drug stability, release profile, and therapeutic efficacy. This approach was scientifically justified, as it enhanced adapalene's skin permeability, ensuring better localized treatment of dermatological conditions.

Materials And Methods

Materials

Adapalene, the active component, had been provided with Bioplus Life Science, Bangalore, as a gift sample. To prepare invasomal vesicles, phosphatidylcholines from Thomas Baker, Mumbai, were an essential component of the formulation. Buffering agents, such as dipotassium hydrogen orthophosphate and disodium hydrogen phosphate, were sourced from S. D. Fine Chem. Ltd., Mumbai, to keep the pH of the formulation at the required level. Solvents such as methanol, ethanol, and chloroform were sourced from Qualigens Fine Chemicals in Mumbai and used to create the invasomes as well as other components.

Methods

Formulation of invasomes containing adapalene using Box Behnken design

- *Effect of permeation enhancer*

A Franz diffusion cell was used to conduct drug penetration tests through the skin of the rat abdomen (cell capacity of 10 mL and area was 2.5 cm^2). After being kept in a saline solution at 4 to 5°C, the skin was thawed to room temperature and then cleaned with distilled water. The skin was sliced into the appropriate size after its thickness (140 ± 7.86 µm) was determined. With the dermal side of the skin toward the receptor compartment of the diffusion cell, it was positioned between the donor and receptor compartments. In order to adjust the pH of the diffusion cell's receptor compartment, 22 mL of phosphate buffer were supplied. 7.5 mL of a drug solution (1 percent w/v) made with distilled water and alcohol (9:1) were put into the donor compartment. The experimental temperature of the diffusion medium was maintained at 37 ± 0.5°C. Throughout the experiment, a magnetic bead was used to continually swirl the receptor media at 200 rpm. A constant amount of phosphate buffer pH 7.4 was added each time a sample (1 mL) was removed at a different time. Using a blank of phosphate buffer that has a 7.4 pH, the absorbance of each sample was measured spectrophotometrically at 238 nm for adapalene after it had been appropriately diluted. The drug penetration rate per unit area for each time interval was computed and displayed against time. The experiments were performed in triplicate, and the mean result was recorded.

- *Permeation enhancement technique*

Penetration's effect enhancer for permeation of drug across full-thickness rat skin was studied in presence of, e.g., terpenes (limonene) and, terpenes ethanolic solution (limonene). A solution containing 1% w/v drug and different concentrations penetration enhancer, i.e.,

Table 1: Composition of solution containing adapalene for preliminary studies

S. No	*Formulation Code*	*Penetration enhancer*	*% (v/v) of Penetration enhancer*
1	PELF1	Limonene	0
2	PELF2	Limonene	1
3	PELF3	Limonene	1.5
4	PELF4	Limonene	2
1	PEELF1	Ethanol + Limonene	0
2	PEELF2	Ethanol + Limonene	1
3	PEELF3	Ethanol + Limonene	1.5
4	PEELF4	Ethanol + Limonene	2

1, 1.5 and 2% w/v for terpenes in water, and 1, 1.5 and 2% v/v for ethanol and terpenes limonene (Table 1). The drug solution containing no penetration enhancer was considered as a control group. We tracked the drug's ability to pass through rat skin as its concentration of a single permeation enhancer increased linearly.

Experimental Design

Drug permeation studies from solutions containing 1% w/v drug have been done in presence and absence of penetration enhancers. The data from these preliminary studies were useful in determining the minimum and maximum concentrations of penetration enhancers to incorporate into a drug-loaded invasomal gel for transdermal drug delivery systems.

The drug-loaded invasomes system was formulated and evaluated for its entrapment efficiency and vesicle size. The invasomes, which showed maximum entrapment efficiency and minimum vesicle size, were selected for the incorporation of different polymers at varying concentrations using a three-factor three-level Box-Behnken factorial design.

Adapalene-containing invasomes were optimized using a Box Behnken design (BBD) with three layers and three components. Phosphatidylcholine (PC), limonene (penetration enhancers), and sonication time (min.) were chosen as independent variables. Three levels of these independent variables (factors) were chosen: low (-1), medium (0), and high (+1).

Table 2 displays variables' levels as well as replies that were received. These levels selected were based on initial trials. The independent variables X_1 limonene (% v/v), X_2 phosphatidylcholines (mg) and X_3 sonication time (min) and efficiency of entrapment (Y1, % EE) and size of vesicle (Y2, nm) were the dependent variables (response) examined in this study. A total of seventeen experiment runs were designed (Table 3). All formulations include the same quantity of medication, and the amount of ethanol used was determined by the experimental design.

Table 2: Levels of formulation variables in Box-Behnken design

Independent variables	*Level*		
	Low (-)	*Medium (0)*	*High (+)*
X_1: Limonene (% v/v)	1	2	3
X_2: Phosphatidylcholine (mg)	100	150	200
X_3: Sonication Time (min)	5	7.5	10
Response variables			
Y_1: Percentage Entrapment Efficiency (% EE)	Maximizing		
Y_2: Vesicle Size (nm)	Minimizing		

You may find the instructions for creating the polynomial equations in the Design-Expert® software here.

Y = Amounts of β0, β1X1, β2X2, β3X3, β12X1X2, β13X1X3, β23X2X3, β11X12, β22X22, and β33X32

Here, X1, X2, and X3 operate as independent variables, Y as the dependent variable, β0 as the intercept, and β1-β3 as the regression coefficients.

In order to determine the compositions of optimum formulations, the quadratic response surface plots, contour plots, and polynomial equations acquired from BBD were created and examined for statistical validity.

Technique for Formulation of Invasomes Containing Adapalene

The mechanical dispersion method was used to create invasomes loaded with adapalene.[5] A clear solution was obtained by briefly adding adapalene to an ethanolic combination of soy phosphatidylcholine and then vortexing the mixture for five minutes. 5 to 10 minutes later, the mixture was subjected to sonication. The mixture was continuously vortexed while a fine stream of distilled water was added using a syringe. To make sure the final invasomal formulation formed, vortexing was carried out for a further five to ten minutes.

Characterization of Adapalene Loaded Invasomes

Vesicle size, zeta potential and PDI

Using a Zetasizer (Malvern Zetasizer Nano series, Worcestershire, UK), the appropriately diluted aqueous drug-loaded dispersions were assessed for vesicle size, zeta potential, and PDI at 25 ± 0.5°C. [6] Utilizing the dynamic light scattering (DLS) method, the variation in light scattering over time induced by the particles' Brownian motion at a 90º angle is reviewed.

Entrapment efficiency

Quantity of unentrapped drug recovered in the supernatant after centrifugation of the resulting nanosuspension was

Table 3: Adapalene invasomes' coded values are represented by a design matrix in the Box-Behnken style

Std	*Run*	*Factors*		
		Coded values		
		Factor A	*Factor B*	*Factor C*
12	1	0	1	1
4	2	1	1	0
7	3	-1	0	1
1	4	-1	-1	0
17	5	0	0	0
8	6	1	0	1
3	7	-1	1	0
6	8	1	0	-1
14	9	0	0	0
13	10	0	0	0
11	11	0	-1	1
2	12	1	-1	0
9	13	0	-1	-1
10	14	0	1	-1
5	15	-1	0	-1
15	16	0	0	0
16	17	0	0	0

used to quantify the quantity of adapalene entrapped in the adapalene-loaded Invasomes.[7] Using UV-vis spectroscopy at 238 nm, the unentrapped medication was calculated in the supernatant after the nanosuspension was spun using a cooling centrifuge set to 15000 RPM for 10 minutes at 10°C. Additionally, The following formula was used to ascertain the percentage of entrapment efficiency by substituting the total quantity of the drug and the unentrapped drug in the supernatant.

$$Drug\ entrapment(\%) = \frac{Concentration\ of\ total\ drug - Concentration\ of\ unentrapped\ drug}{Concentration\ of\ unentrapped\ drug} x100$$

Study In-vitro drug diffusion

The *in-vitro* diffusion experiments are carried out using a Franz diffusion cell. The egg membrane is considered to be semi-permeable when it comes to diffusion. [8] About 3.14 cm^2 is the effective surface area of penetration in the receptor compartment of the Franz diffusion cell and an effective volume of around 60 mL. The donor and receptor compartments are separated by the egg membrane. A 2 cm^2 patch that has been weighed and measured is put on the donor compartment's membrane-facing side. Phosphate buffer with a pH of 7.4 is the receptor media. The receptor chamber is covered with a water jacket to maintain a temperature of 32 ± 0.5°C. To provide heat, a thermostatic hot plate and a magnetic stirrer are used. To stir up the receptor fluid, a magnetic bead coated with teflon is introduced into the diffusion cell.[8]

Stability studies

Invasomes filled with drugs were tested for stability for a period of three months at two different temperatures: room temperature (25-28 ± 2°C) and refrigerator temperature (4.0 ± 0.2°C). A borosilicate container was used to keep the formulation under stability testing so that it wouldn't come into touch with the glass container. Vesicle size and any trapping efficacy were examined in the formulations. [9]

Results And Discussion

The study's primary objective was to formulate and characterize adapalene-loaded invasomes using a Box-Behnken experimental design. This approach enabled the systematic evaluation of various formulation variables, including limonene concentration, phosphatidylcholine concentration, and sonication time. The findings showed that these factors had a major impact on important characteristics such as *in-vitro* drug release, vesicle size, and entrapment efficiency (EE), all of which are critical for maximizing adapalene delivery.

The method of mechanical dispersion used to create invasomes has been proven effective in previous studies for preparing drug-loaded liposomal systems. In this case, adapalene was successfully incorporated into the lipid bilayer of soy phosphatidylcholine-based invasomes. This technique, which involved vortexing followed by sonication, ensured the formation of stable invasomes. The final formulations demonstrated uniformity in terms of size and encapsulation, which is essential for their application in transdermal drug delivery.

Drug Permeation Studies

Drug permeation studies from solutions containing 1% w/v drug have been done in the presence and absence of penetration enhancers. The data from these preliminary studies were useful in determining the minimum and maximum concentrations of penetration enhancers to incorporate into drug-loaded invasomal gel for transdermal drug delivery systems (Tables 4 and 5). Additionally, the research looked at how permeation enhancers affected the drug release profile. Two groups of formulations were prepared: one containing only limonene and the other containing a combination of limonene and ethanol. The limonene-only formulations (PELF1–PELF4) exhibited a gradual increase in the cumulative release of adapalene, reaching a maximum of 49.98% at 10 hours for the 2% limonene formulation (Table 4). The formulations containing both limonene and ethanol (PEELF1–PEELF4) showed even higher cumulative drug release, with the highest release of 60.68% at 12 hours for the 2% combination (Table 5), indicating that ethanol significantly enhanced the permeation of adapalene.

Table 4: *In-vitro* permeation of adapalene (ADP) from solution containing limonene as penetration enhancer

S. No.	*Time (hr)*	*Percentage cumulative released (%)*			
		PELF1 SD (±)	*PELF2 SD (±) (1%)*	*PELF3 SD (±) (1.5%)*	*PELF4 SD (±) (2%)*
1	0	0	0	0	0
2	1	3.65 ± 0.11	4.65 ± 0.41	5.58 ± 0.44	8.85 ± 0.22
3	2	5.58 ± 0.25	6.98 ± 0.74	7.12 ± 0.25	10.32 ± 0.32
4	3	6.96 ± 0.36	7.85 ± 0.32	8.98 ± 0.33	14.45 ± 0.14
5	4	10.32 ± 0.32	12.25 ± 0.41	13.36 ± 0.14	20.22 ± 0.25
6	5	12.23 ± 0.14	16.65 ± 0.36	18.85 ± 0.22	26.65 ± 0.23
7	6	14.56 ± 0.25	18.85 ± 0.22	22.23 ± 0.36	32.23 ± 0.32
8	7	16.85 ± 0.36	22.32 ± 0.14	24.45 ± 0.45	39.98 ± 0.18
9	8	18.85 ± 0.22	28.85 ± 0.12	30.32 ± 0.32	45.56 ± 0.33
10	9	22.23 ± 0.16	32.21 ± 0.33	34.45 ± 0.95	49.98 ± 0.25
11	10	26.32 ± 0.32	36.65 ± 0.25	39.98 ± 0.14	51.12 ± 0.14
12	11	27.74 ± 0.25	42.23 ± 0.31	43.32 ± 0.33	53.32 ± 0.32
13	12	28.12 ± 0.11	44.85 ± 0.21	48.85 ± 0.25	55.56 ± 0.12

Table 5: *In-vitro* permeation of adapalene (ADP) from solution containing limonene and ethanol as a penetration enhancer

S. No.	*Time (hr)*	*Percentage cumulative released (%)*			
		PEELF1 SD (±)	*PEELF2 SD (±) (1%)*	*PEELF3 SD (±) (1.5%)*	*PEELF4 SD (±) (2.0%)*
1	0	0	0	0	0
2	1	3.65 ± 0.25	5.85 ± 0.22	6.85 ± 0.11	9.98 ± 0.33
3	2	5.58 ± 0.32	7.74 ± 0.32	9.95 ± 0.25	12.25 ± 0.25
4	3	6.96 ± 0.15	8.95 ± 0.15	10.32 ± 0.36	16.65 ± 0.14
5	4	10.32 ± 0.32	13.65 ± 0.25	14.65 ± 0.14	22.32 ± 0.22
6	5	12.23 ± 0.65	14.99 ± 0.33	19.95 ± 0.25	28.85 ± 0.32
7	6	14.56 ± 0.15	16.65 ± 0.15	26.65 ± 0.35	33.36 ± 0.15
8	7	16.85 ± 0.25	20.25 ± 0.32	28.87 ± 0.41	49.99 ± 0.22
9	8	18.85 ± 0.23	23.32 ± 0.25	31.12 ± 0.32	46.65 ± 0.32
10	9	22.23 ± 0.32	26.65 ± 0.11	36.66 ± 0.33	50.23 ± 0.15
11	10	26.32 ± 0.22	36.65 ± 0.15	42.25 ± 0.14	53.32 ± 0.23
12	11	27.74 ± 0.18	44.58 ± 0.18	45.52 ± 0.22	55.45 ± 0.32
13	12	28.12 ± 0.19	48.98 ± 0.16	53.32 ± 0.25	60.68 ± 0.15

Factorial Design

To optimize invasomes containing adapalene, a Box-Behnken design (BBD) with three components and three levels was used. As independent variables, limonene (penetration enhancers), phosphatidylcholine (PC), and sonication duration (min.) were selected. The runs and responses of the Box-Behnken design of adapalene invasomes formulations were presented in Table 5. The three factors (Limonene concentration, phosphatidylcholines concentration, and sonication time) were systematically altered in order to evaluate their impact on vesicle size and entrapment efficiency (EE). The results showed that the highest entrapment efficiency of 75.65% was achieved at the 3% limonene concentration, with a corresponding increased vesicle size of 126.65 nm (Table 6). Increasing the sonication time and phosphatidylcholines concentration resulted in smaller vesicles and higher encapsulation, demonstrating the effectiveness of this experimental design in optimizing invasomal formulations.

Coded factors-final equation

Entrapment Efficiency (Y_1) = $+65.86+3.08X_1-0.1175X_2-0.6775X_3+2.23X_1X_2+1.64X_1X_3+0.1325X_2X_3+4.08X_1^2+0.6508X_2^2-0.5242X_3^2$

Actual factors-final equation

Entrapment Efficiency (Y_1) = +103.96500-24.84800 Limonene-0.177690 Phosphatidylcholines-0.485800

Table 6: Runs and responses of Box-Behnken design of adapalene invasomes formulations

Run	*Independent variables*			*Dependent variables*	
	X_1: Limonene (% v/v)	*X_2: Ethanol (mL)*	*X_3: Phosphatidylcholine (% v/v)*	*Y_1: Entrapment Efficiency (%)*	*Y_2: Vesicle Size (nm)*
F1	2	200	10	65.58	115.65
F2	3	200	7.5	75.65	126.65
F3	1	150	10	63.32	93.32
F4	1	100	7.5	69.98	140.65
F5	2	150	7.5	67.74	132.54
F6	3	150	10	73.32	96.65
F7	1	200	7.5	65.58	150.23
F8	3	150	5	72.21	115.65
F9	2	150	7.5	63.23	130.25
F10	2	150	7.5	67.74	125.47
F11	2	100	10	65.85	105.65
F12	3	100	7.5	71.12	120.36
F13	2	100	5	66.65	148.85
F14	2	200	5	65.85	153.32
F15	1	150	5	68.78	150.12
F16	2	150	7.5	66.45	125.45
F17	2	150	7.5	64.12	130.36

Sonication Time + 0.044650 Limonene * Phosphatidylcholines + 0.657000 Limonene * Sonication time+0.001060 Phosphatidylcholines* Sonication time+4.07575 Limonene^2 +0.000260 Phosphatidylcholines^2-0.083880 Sonication Time^2

Coded factors-final equation

Vesicle Size (Y_2) = $+128.81-9.38X_1+3.79X_2-19.58X_3-0.8225X_1X_2+9.45X_1X_3+1.38X_2X_3-5.64X_1^2+11.30X_2^2-9.24X_3^2$

Actual factors-final equation

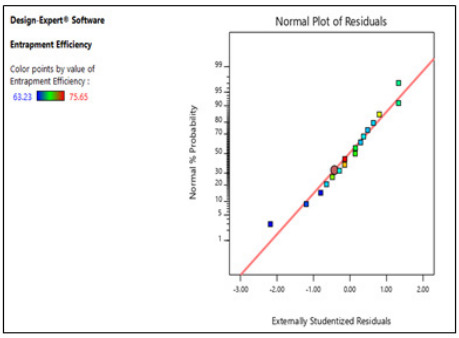
Vesicle Size (Y_2) = +255.08125-12.71075 Limonene -1.32966 Phosphatidylcholines+5.12830 Sonication Time + -0.016450 Limonene * Phosphatidylcholines+3.78000 Limonene * Sonication Time +0.011060 Phosphatidylcholines*Sonication Time -5.63700 Limonene^2 +0.004518 Phosphatidylcholines^2 -1.47872 Sonication Time^2

Different Graphs Obtained by DOE for Entrapment Efficiency

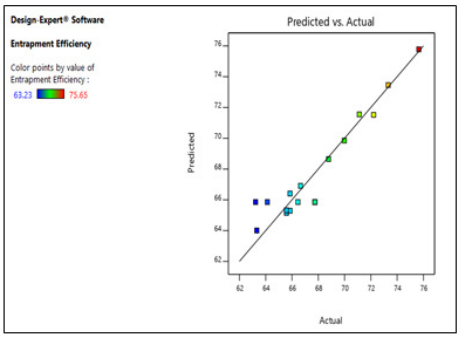
The graphical analysis (Fig. 2) from the Box-Behnken Design confirms the model's adequacy and reliability in predicting vesicle size. Phosphatidylcholine and limonene concentrations significantly influenced vesicle size. Formulation F6 showed a vesicle size of 96.65 nm (Fig. 3), closely aligning with the predicted 94.43 nm, demonstrating strong model predictability and consistent experimental outcomes. Fig. 4 presents the zeta potential analysis of the optimized formulation F6. The zeta potential was found to be -42.23 mV, indicating a high degree of electrostatic repulsion between vesicles, which contributes to colloidal stability by preventing aggregation. A zeta potential value greater than ±30 mV is generally considered stable; hence, the obtained result suggests that the invasomal formulation is physically stable and suitable for long-term storage.

Table 7: Predicted response for experimental data

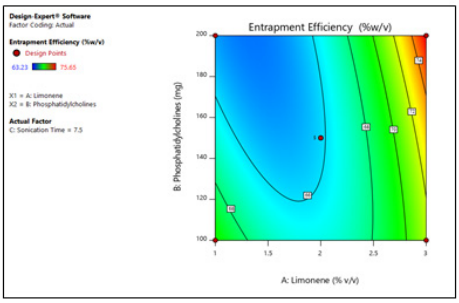
Formulation code	*Limonene (% v/v)*	*Ethanol (mL)*	*Phosphatidylcholine (% v/v)*	*Parameters*	*Actual value*	*Predicted value*
F6	3	150	10	Entrapment efficiency (Y_1, %)	73.32	73.45
				Vesicle size (Y_2, nm)	96.65	94.43



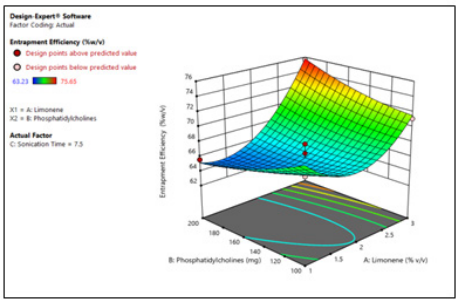
a) Graph for Entrapment Efficiency
(Normal Plots for Residuals)



b) Graph for Entrapment Efficiency
(Predicted vs. Actual)

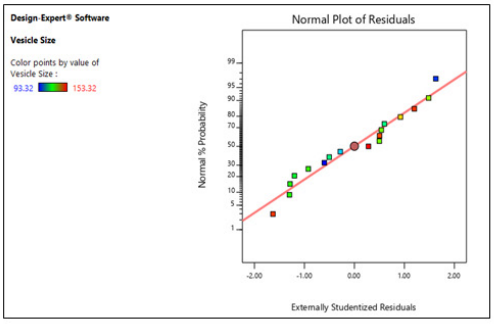


c) Contour plots for Entrapment Efficiency
(between Limonene vs. Phosphatidylcholines)

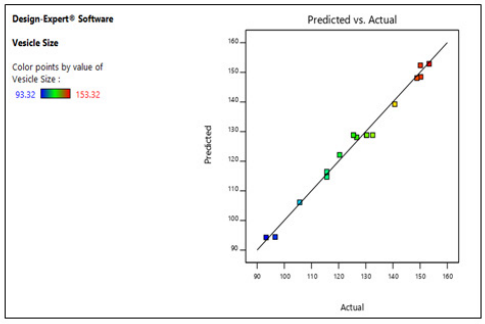


d) 3D surface plats for Entrapment Efficiency
(between Limonene vs. Phosphatidylcholines)

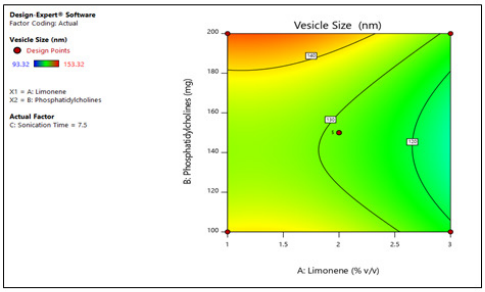
Fig. 1: Different graphs obtained by DoE for entrapment efficiency



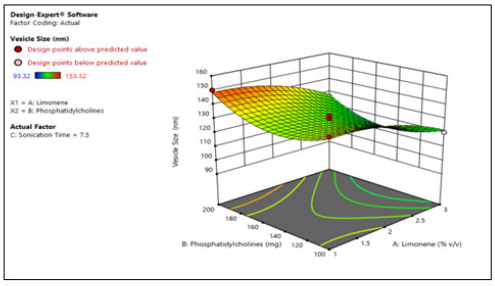
a) Graph for Vesicle size
(Normal Plots for Residuals)



b) Graph for Vesicle size
(Predicted vs. Actual)



c) Contour plots for Vesicle size
(between Limonene vs. Phosphatidylcholines)



d) 3D surface plots for Vesicle size
(between Limonene vs. Phosphatidylcholines)

Fig. 2: Different graphs obtained by DoE for vesicle size

Table 8: Results of *in-vitro* drug release of optimized formulation F6

Time	*%Cumulative drug release*
0.5	9.65
1	16.65
2	26.65
4	38.85
6	49.99
8	56.65
10	78.85
12	89.98

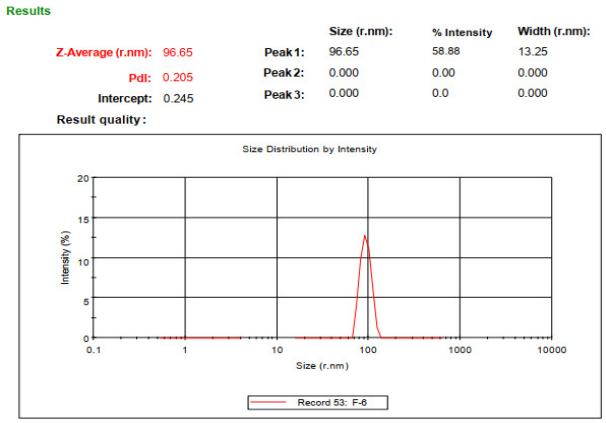


Fig. 3: Graph of vesicle size of optimized formulation F6

Table 9: Stability study of optimized formulation of invasomes F6

Characteristics	*Time (Month)*					
	1 Month		*2 Months*		*3 Months*	
Temperature	4.0 ± 0. 2°C	25.28 ± 2°C	4.0 ± 0. 2°C	25.28 ± 2°C	4.0 ± 0.2°C	25-28 ± 2°C
Average vesicle size (nm)	96.22 ± 0.25	98.32 ± 0.15	97.78 ± 0.32	102.23 ± 0.22	78.95 ± 0.74	110.25 ± 0.63
%EE	73.05 ± 0.33	72.22 ± 0.28	72.32 ± 0.45	69.98 ± 0.15	71.15 ± 0.22	65.58 ± 0.32

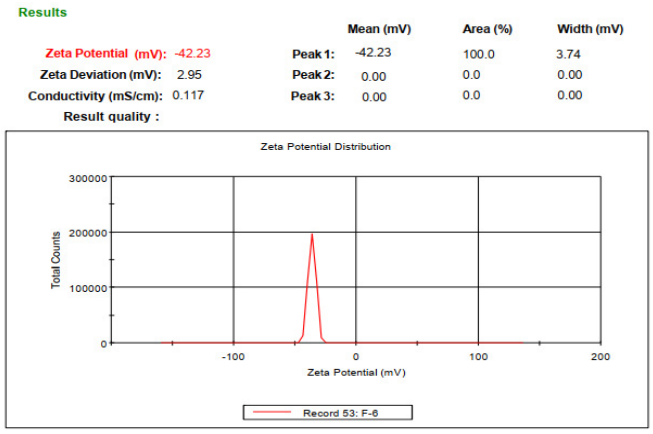


Fig 4: Graph of zeta potential of optimized formulation F6

Different Graphs Obtained by DoE for Vesicle Size

Formulation F6 was identified as the optimal formulation, which contained 3% limonene, 150 mg of phosphatidylcholines, and a 7.5-minute sonication time. This formulation exhibited an entrapment efficiency of 73.32% and a vesicle size of 96.65 nm, which, according to Table 7, was very congruent with the expected values. In addition, F6's controlled release *in-vitro* drug release was 56.65% within 8 hours and 89.98% after 12 hours (Table 8), indicating that the formulation is appropriate for long-term drug administration.

The stability study of F6 revealed some interesting findings. At 4°C, the formulation remained relatively stable with only slight changes in vesicle size and entrapment efficiency over the course of 3 months. However, at room temperature (25–28°C), a noticeable increase in vesicle size and a slight decrease in entrapment efficiency were observed, which is a common issue with lipid-based formulations due to vesicle aggregation and drug leakage (Table 9). These results underscore the importance of maintaining storage conditions to ensure the stability and efficacy of the formulation.

Conclusion

Using the Box-Behnken statistical methodology, the research successfully created and described adapalene-loaded invasomes while optimizing formulation parameters, including vesicle size, *in-vitro* drug release profile, and entrapment efficiency (EE). The findings showed that limonene and phosphatidylcholine significantly improved adapalene's transdermal penetration, with higher limonene concentrations enhancing both drug release and penetration. At 12 hours, the improved formulation (F6) showed a promising total drug release of 89.98%, a vesicle size of 96.65 nm, and an entrapment effectiveness of 73.32%. These attributes suggest that the formulation could provide sustained and effective delivery of adapalene, which is essential for treating acne vulgaris while minimizing side effects. The stability tests confirmed that the formulation remained intact for three months under a range of storage conditions. The slight increase in vesicle size and decrease in entrapment efficiency were within acceptable limits, indicating that the formulation remains stable even at ambient temperatures.

Acknowledgment

For his essential advice, unwavering support, and encouragement throughout this study, the author would like to sincerely thank Dr. Mohan L. Kori, study Guide. Additionally, we would like to express our sincere gratitude to the Pharmacy Faculty at RKDF University in Bhopal, Madhya Pradesh, India, for providing the necessary research facilities and a favorable setting for conducting this study. Their support and knowledge have been crucial to the accomplishment of this study.

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HOW TO CITE THIS ARTICLE: Singhai M, Kori ML. Formulation and Characterization of Invasomes Containing Adapalene Using Box-Behnken Design. Int. J. Pharm. Sci. Drug Res. 2025;17(3):234-242. **DOI:** 10.25004/IJPSDR.2025.170303