

DESIGN AND DEVELOPMENT OF TRIFAROTENE-LOADED TRANSFERSOMAL GEL USING BOX-BEHNKEN DESIGN

Prajakta Satanakar, Ruchi Jain*, Sumit Chaurasia, R.B. Goswami

Sagar Institute of Research Technology & Science – Pharmacy, Ayodhya Bypass Road, Bhopal (M.P.)

Corresponding author mail id: jainruchi02@gmail.com

DOI: [https://doi.org/10.63001/tbs.2026.v21.i04.S.I\(4\).pp98-114](https://doi.org/10.63001/tbs.2026.v21.i04.S.I(4).pp98-114)

KEYWORDS

*Transferosomes;
Design of Experiments (DoE);
Optimization;
Entrapment Efficiency;
Vesicle Size; Topical Gel;
In Vitro Drug Release;
Release Kinetics;
Stability Study;
Controlled Drug Delivery.*

Article Info

Received: 15/08 /2026

Accepted :21/09/2026

Published: 09/10/2026

Abstract

The present study aimed to develop and optimize a transferosomal formulation using Design of Experiments (DoE) and evaluate its suitability for incorporation into a topical gel delivery system. A three-factor, three-level experimental design was employed to investigate the effects of Soya Phosphatidylcholine (Soya PC) concentration, ethanol volume, and stirring speed on vesicle size and entrapment efficiency. Thirteen transferosomal formulations were prepared and evaluated. The entrapment efficiency ranged from 68.2% to 86.8%, while vesicle size varied between 133.4 and 190.5 nm. Formulation F8 was identified as the optimized formulation, exhibiting an entrapment efficiency of 86.8% and vesicle size of 180.2 nm, which closely matched the predicted values obtained from the optimization model.

The optimized transferosomes were incorporated into gel formulations and evaluated for viscosity, pH, drug content, extrudability, and spreadability. Among the developed gels, formulation FG2 exhibited desirable physicochemical characteristics with a pH of 6.78, viscosity of 9450 cP, drug content of 95.80%, and satisfactory spreadability and extrudability. In vitro drug release studies demonstrated sustained drug release over 8 hours, with FG2 showing 91.80% cumulative drug release. Release kinetic analysis revealed that the formulation followed first-order kinetics ($R^2 = 0.9947$) with diffusion-controlled drug release behavior. Stability studies conducted under refrigerated and room temperature conditions for three weeks indicated no significant changes in physical appearance or drug content. The findings suggest that the optimized transferosomal gel is a promising topical drug delivery system capable of enhancing drug entrapment, providing controlled release, and maintaining stability during storage.

Introduction

Acne vulgaris is one of the most common chronic inflammatory disorders of the pilosebaceous unit affecting adolescents and young adults worldwide. It is characterized by the formation of comedones, papules, pustules, nodules, and cystic lesions primarily on the face, chest, and back^[1].

The pathogenesis of acne involves multiple factors including excessive sebum production, follicular hyperkeratinization,

colonization of *Cutibacterium acnes*, inflammation, and hormonal imbalance. Persistent acne lesions may lead to pigmentation, scarring, psychological stress, and reduced quality of life. Therefore, effective topical therapy with improved patient compliance and enhanced skin penetration is essential for successful management of acne vulgaris^[2].

Topical retinoids are considered first-line agents in acne therapy due to their ability to normalize follicular epithelial desquamation, reduce comedone formation, and exert anti-inflammatory activity. Trifarotene is a fourth-generation selective retinoic acid receptor (RAR)- γ agonist recently approved for the treatment of acne vulgaris [3]. It demonstrates high selectivity toward RAR- γ receptors predominantly present in the skin, resulting in enhanced therapeutic efficacy with reduced systemic adverse effects. Trifarotene effectively reduces inflammatory and non-inflammatory acne lesions by regulating keratinocyte differentiation and minimizing inflammatory responses [4].

However, conventional topical formulations of Trifarotene are associated with certain limitations such as poor aqueous solubility, low skin retention, instability, irritation, and limited penetration through the stratum corneum [5].

Novel vesicular drug delivery systems such as transfersomes have gained considerable attention for improving topical and transdermal delivery of poorly permeable drugs. Transfersomes are ultra-deformable lipid vesicles composed of phospholipids and edge activators that possess high elasticity and flexibility [6]. These vesicles

can penetrate through narrow skin pores and enhance drug permeation across the stratum corneum while improving drug retention within deeper skin layers. Transfersomal systems offer several advantages including enhanced drug entrapment, controlled release, improved bioavailability, reduced skin irritation, and targeted delivery to the affected site [7].

Incorporation of transfersomes into a gel base further improves formulation stability, ease of application, spreadability, and patient acceptability. Transfersomal gel systems can provide prolonged residence time at the site of application and sustained drug release, thereby improving therapeutic effectiveness in acne management [8].

The selection and optimization of formulation variables play an essential role in achieving desired vesicle size, entrapment efficiency, deformability, and drug release characteristics [9]. Statistical optimization techniques such as Box–Behnken Design (BBD) are widely employed in pharmaceutical formulation development to evaluate the influence of formulation variables and their interactions systematically [10]. BBD is an efficient response surface methodology that reduces the number of experimental runs while providing reliable optimization data. It

enables the identification of optimal formulation conditions for achieving desired product characteristics with minimal experimental error and cost ^[11].

Therefore, the present study was undertaken to design and develop Trifarotene-loaded transfersomal gel using Box–Behnken Design for enhanced topical delivery and therapeutic efficacy in acne treatment. The study aims to optimize formulation variables affecting vesicle characteristics, evaluate physicochemical properties, and investigate the potential of transfersomal gel as an advanced topical drug delivery system for acne vulgaris management.

Material and Methods

Material

Soya phosphatidylcholine (Soya PC) was used as the primary phospholipid for the preparation of transfersomes. Ethanol was employed as an edge activator and vesicle-forming component, while distilled water was used as the aqueous phase. The active pharmaceutical ingredient (drug) used in the study was incorporated into the transfersomal vesicles during formulation. Carbopol 934 was utilized as the gelling agent for the preparation of transfersomal gel. Triethanolamine was used for pH adjustment and gel neutralization, whereas propylene glycol served as a humectant and

penetration enhancer. Methyl paraben was added as a preservative to improve formulation stability. Analytical-grade chemicals and reagents were used throughout the study. The formulation and evaluation processes involved the use of a magnetic stirrer, Brookfield viscometer, pH meter, UV–Visible spectrophotometer, centrifuge, particle size analyzer, and other standard laboratory equipment for characterization and analysis of the developed transfersomal gel.

Methods

Preparation of Trifarotene loaded Transfersomes

Preparation of Transfersomal Dispersion by DoE Approach

Transfersomal dispersions were prepared using a modified ethanol injection method based on Design of Experiments (DoE) to optimize formulation variables. Soya phosphatidylcholine (Soya PC) was dissolved in ethanol at varying concentrations (0.5–2% w/v) as per the experimental design, using 5–20 mL of ethanol in a closed vessel. The ethanolic lipid solution was maintained at $30 \pm 1^\circ\text{C}$ using a water bath ^[12].

Aqueous phase, consisting of distilled water or drug solution (1% w/v), was also preheated to the same temperature and added slowly in a fine stream to the lipid phase under continuous magnetic stirring. The stirring speed (700–1000 rpm) and addition rate were varied according to the DoE matrix to study their effect on vesicle characteristics. Mixing was continued for 5–10 minutes to ensure uniform dispersion.

After complete addition, the vesicular dispersion was allowed to cool at room temperature ($25 \pm 1^\circ\text{C}$) for 30–60 minutes, facilitating vesicle formation and stabilization. Different transferosomal formulations were prepared by systematically varying independent variables such as lipid concentration, ethanol volume, and stirring speed, as defined in the DoE design, and their compositions are presented in Table 1.

Table 1: Independent and Dependent Variables Used in DoE

Variables Type	Factors/Responses	Symbol	Levels		
			Low	Medium	High
Independent Variables (Factors)	Soya PC Concentration (% w/v)	A	0.5	1.25	2.0
	Ethanol Volume (mL)	B	5	12.5	20
	Stirring Speed (rpm)	C	700	850	1000
Dependent Variables (Responses)	Vesicle size (nm)	Y_1	To be minimized		
	Entrapment Efficiency (%)	Y_2	To be maximized		

Table 2: Experimental design matrix for transferosomal formulations using DoE

F. Code	Std	Run	Factor 1	Factor 2	Factor 3
			A:Soya PC	B:Ethanol	C:Stirring Speed
			% w/v	mL	rpm
F1	14	1	1.25	12.5	850
F2	6	2	2	12.5	700
F3	7	3	0.5	12.5	1000
F4	3	4	0.5	20	850

F5	12	5	1.25	20	1000
F6	10	6	1.25	20	700
F7	4	7	2	20	850
F8	2	8	2	5	850
F9	11	9	1.25	5	1000
F10	8	10	2	12.5	1000
F11	1	11	0.5	5	850
F12	9	12	1.25	5	700
F13	5	13	0.5	12.5	700

Final Equation in Terms of Coded Factors

$$\text{Entrapment Efficiency} = 78.84 + 8.0125A - 0.9125B + 0.25C - 0.25AB - 0.075AC - 0.425BC - 0.87A^2 - 0.72B^2 - 0.195C^2$$

Final Equation in Terms of Actual Factors

$$\begin{aligned} \text{Entrapment Efficiency} = & 49.495 + 15.6722(\text{Soya PC}) + 0.5750(\text{Ethanol}) + \\ & 0.02196(\text{Stirring Speed}) - 0.04444(\text{Soya PC} \times \text{Ethanol}) - 0.000667(\text{Soya PC} \times \text{Stirring Speed}) - \\ & 0.000378(\text{Ethanol} \times \text{Stirring Speed}) - 1.5467(\text{Soya PC})^2 - 0.0128(\text{Ethanol})^2 - 0.00000867(\text{Stirring Speed})^2 \end{aligned}$$

Final equation in terms of coded factors

$$\text{Vesicle size} = 165.38 + 3.75A - 7.9125B - 18.9375C - 1.175AB - 1.275AC - 1.6BC + 3.335A^2 - 2.19B^2 - 2.09C^2$$

Final equation in terms of actual factors

$$\begin{aligned} \text{Vesicle size} = & 185.2817 + 2.4222(\text{Soya PC}) \\ & + 1.3883(\text{Ethanol}) + 0.06361(\text{Stirring} \end{aligned}$$

$$\begin{aligned} \text{Speed}) - & 0.2089(\text{Soya PC} \times \text{Ethanol}) - \\ & 0.01133(\text{Soya PC} \times \text{Stirring Speed}) - \\ & 0.001422(\text{Ethanol} \times \text{Stirring Speed}) + \\ & 5.9289(\text{Soya PC})^2 - 0.03893(\text{Ethanol})^2 - \\ & 0.00009289(\text{Stirring Speed})^2 \end{aligned}$$

Characterization of Trifarotene loaded Transfersomes

Entrapment efficiency

One milliliter of Transfersomes suspension was centrifuged at 15.000 rpm for 1 h to allow the separation the entrapped drug from the un-entrapped drug ^[13]. After removal of the supernatant, the sediment was lysed using methanol and then analyzed spectrophotometrically at 270nm using a UV spectrophotometer (Labindia 3000+). The EE% of in prepared Transfersomes was calculated applying the following equation:

$$\% \text{ Entrapment Efficiency}$$

$$= \frac{\text{Terotical drug content} - \text{Practical drug content}}{\text{Terotical drug content}}$$

$$\times 100$$

Vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK) ^[14]. Zeta potential measurement of the Transfersomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a Zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 IS/cm.

Preparation of gel base

The gel base was prepared by dispersing accurately weighed Carbopol 934 (0.5 to 1.5%) w/v) in approximately 80 mL of double distilled water under continuous stirring at 800 rpm for 1 hour to ensure complete hydration and uniform dispersion. After proper swelling of the polymer, propylene glycol (10 mL) was added to enhance the viscosity and consistency of the gel. The final volume was then adjusted to 100 mL using double distilled water. The prepared gel base was subjected to sonication for 10 minutes to remove entrapped air bubbles and obtain a smooth, homogeneous system ^[15].

The pH of the gel was subsequently adjusted to 6.8 using a suitable neutralizing agent to ensure compatibility with skin and stability of the formulation. Further, the optimized transfersomal formulation (F8) containing Trifarotene was incorporated into the prepared gel base to obtain a final drug concentration of 0.005% w/w. The incorporation was carried out under gentle stirring to ensure uniform distribution of vesicles within the gel matrix, resulting in a transfersomal gel formulation suitable for topical application.

Characterization of Transfersomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical Transfersomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm; viscosity^[16].

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation

was measured and readings shown on display were noted^[17].

Drug content

Accurately weighed equivalent to 100 mg of topical transfersomal gel was taken in beaker and added 20 ml of methanol^[18]. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 270nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load^[19]. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadability

Spreadability of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response^[20]. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadability, placing 2-5 g of gel between two slide and

gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 6cm upon adding 20g of weight was noted. Good spreadability show lesser time to spread.

$$\text{Spreadability (g.cm/sec)} = \frac{\text{Weight added to Upper Slide} \times \text{Lenth moved on the glass slide}}{\text{Time taken to slide}}$$

In vitro drug diffusion study

The *In-vitro* diffusion study is carried by using Franz Diffusion Cell. Egg membrane is taken as semi permeable membrane for diffusion^[21]. The Franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm² size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4.

The receptor compartment is surrounded by water jacket so as to maintain the temperature at $32 \pm 0.5^\circ\text{C}$. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh

receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of drug 270nm.

Stability studies

Stability study was carried out for drug loaded Transfersomes at two different temperatures i.e. refrigeration temperature ($4.0 \pm 0.2^{\circ}\text{C}$) and at room temperature ($25-28 \pm 2^{\circ}\text{C}$) for 3 weeks ^[22]. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drug content.

Results and Discussion

The present study focused on the optimization of transferosomal formulation using Design of Experiments (DoE), followed by incorporation into a gel base and evaluation of its physicochemical characteristics, in vitro drug release behavior, release kinetics, and stability.

The effect of formulation variables including Soya Phosphatidylcholine (Soya PC) concentration, ethanol volume, and stirring speed on vesicle size and entrapment efficiency was systematically investigated using a three-factor experimental design (Table 1). Thirteen experimental

formulations were prepared according to the DoE matrix (Table 2), and their responses were evaluated.

The results demonstrated a significant influence of formulation variables on both entrapment efficiency and vesicle size (Table 3). Entrapment efficiency ranged from 68.2% to 86.8%, while vesicle size varied from 133.4 nm to 190.5 nm. Formulation F8 exhibited the highest entrapment efficiency (86.8%), whereas formulation F5 showed the smallest vesicle size (133.4 nm). The increase in phospholipid concentration contributed to improved entrapment efficiency due to the availability of a larger lipid bilayer for drug encapsulation. Ethanol concentration and stirring speed also affected vesicle formation and particle size distribution.

The statistical analysis generated through DoE indicated a good agreement between predicted and experimental values. The normal probability plots of residuals showed random distribution of residuals, indicating adequacy of the developed model. The predicted versus actual plots demonstrated a strong correlation between observed and predicted responses, confirming the reliability of the optimization process. The contour and three-dimensional response surface plots further illustrated the combined

effects of ethanol and Soya PC concentration on entrapment efficiency and vesicle size. Based on desirability criteria, formulation F8 was selected as the optimized formulation. The predicted entrapment efficiency and vesicle size were 86.42% and 179.36 nm, respectively, while the experimentally observed values were 86.80% and 180.20 nm (Table 4). The close agreement between predicted and observed values confirmed the validity and robustness of the optimization model.

The optimized transferosomes were incorporated into gel formulations and evaluated for viscosity, pH, drug content, extrudability, and spreadability (Table 5). Among the formulations, FG2 showed desirable characteristics with a viscosity of 9450 cP, pH of 6.78, drug content of 95.80%, extrudability of 185 g/cm², and spreadability of 15.8 g·cm/sec. The pH values of all formulations were within the acceptable range for topical application, suggesting good skin compatibility. The drug content values indicated uniform distribution of the drug within the gel matrix.

The in vitro drug release study revealed sustained release behavior from all gel formulations (Table 6). FG1 exhibited the highest cumulative drug release (98.36%) at

the end of 8 hours, followed by FG2 (91.80%) and FG3 (85.60%). The slower release observed in FG3 may be attributed to its higher viscosity, which could retard drug diffusion through the gel network. FG2 demonstrated a controlled and prolonged drug release profile suitable for topical delivery.

To understand the mechanism of drug release, the release data of the optimized formulation (FG2) were fitted to various kinetic models (Tables 7 and 8). Among the tested models, the first-order kinetic model exhibited the highest correlation coefficient ($R^2 = 0.9947$), indicating that drug release was concentration dependent. The Higuchi model also showed a high R^2 value (0.9861), suggesting diffusion-controlled release from the matrix system. The Korsmeyer–Peppas model demonstrated good fitting ($R^2 = 0.9880$), further supporting a diffusion-mediated release mechanism.

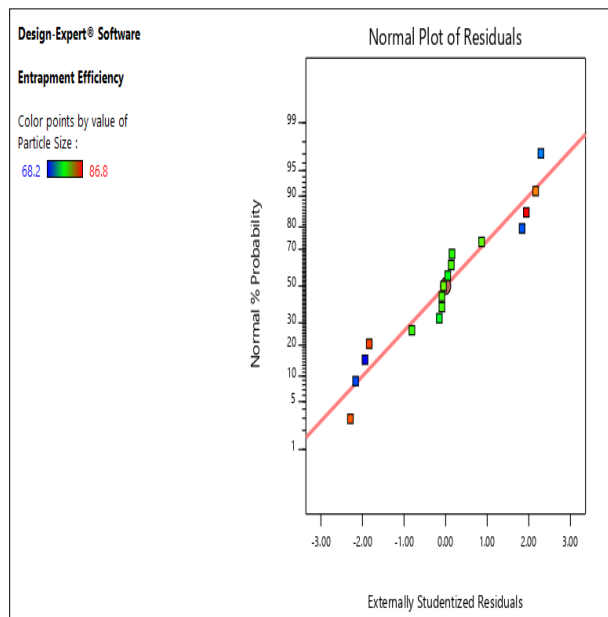
The stability study of the optimized gel formulation (FG2) demonstrated excellent stability under both refrigeration and room temperature conditions over a period of three weeks (Table 9). No noticeable changes in physical appearance were observed, and the drug content remained above 95% throughout the study period. The formulation retained 95.60% drug content

under refrigeration and 95.10% at room chemical stability.
 temperature, indicating good physical and

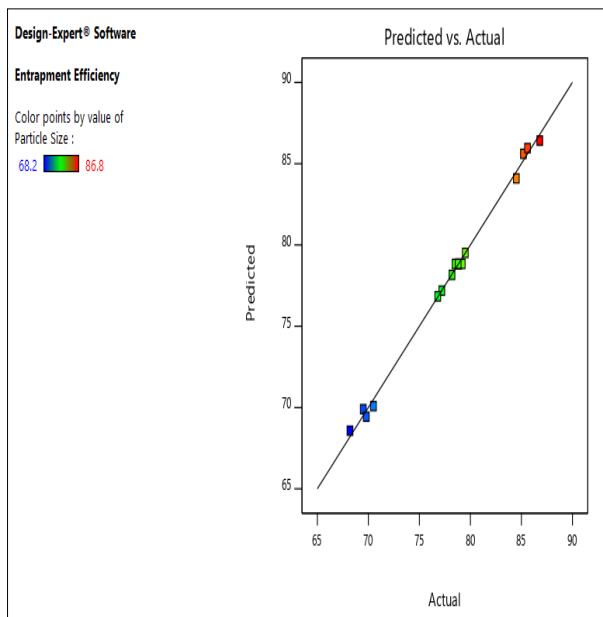
Table 3: Results of Entrapment Efficiency and Vesicle size

F. Code	Response 1	Response 2
	Entrapment Efficiency	Vesicle size
	(%)	(nm)
F1	78.5	165.2
F2	85.2	190.5
F3	70.5	145.3
F4	68.2	155.2
F5	76.8	133.4
F6	77.2	174.6
F7	84.5	160.4
F8	86.8	180.2
F9	79.5	150.8
F10	85.6	150.2
F11	69.5	170.3
F12	78.2	185.6
F13	69.8	180.5

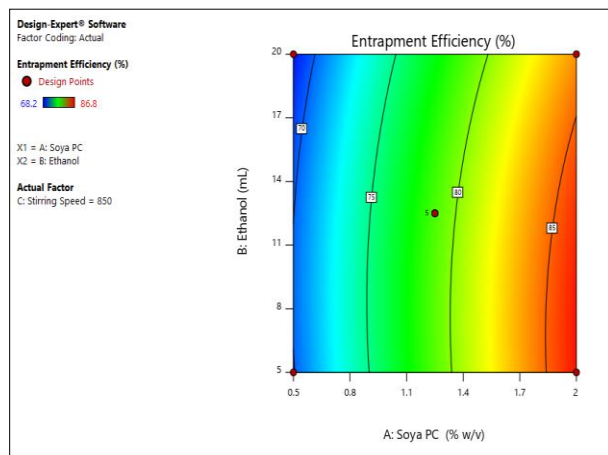
Graph of Entrapment efficiency obtained by DOE



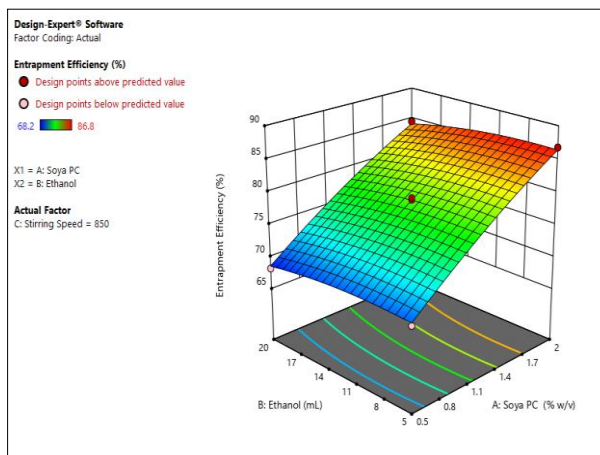
Graph of normal plots of residuals



Graph of predicted vs actual

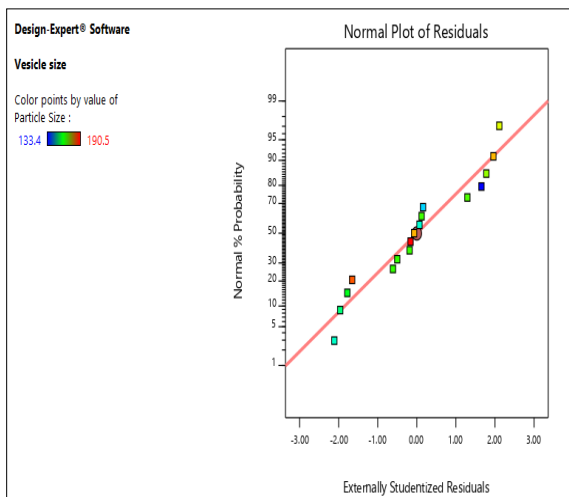
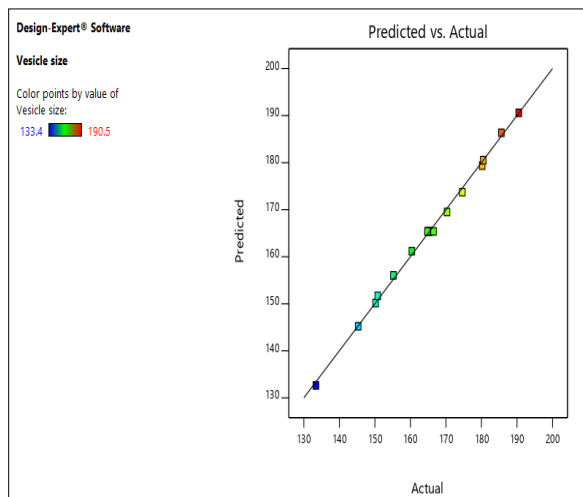
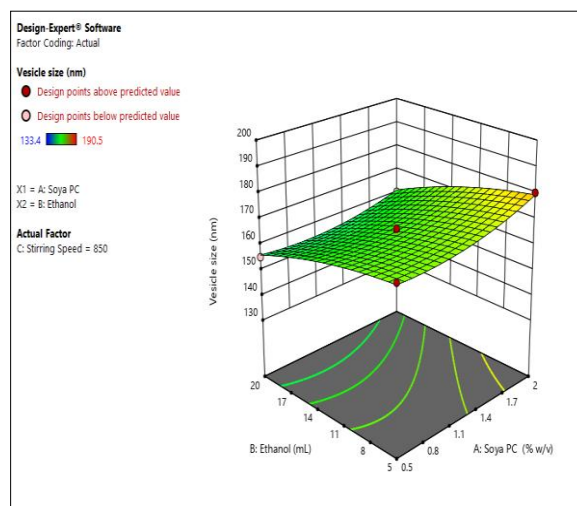
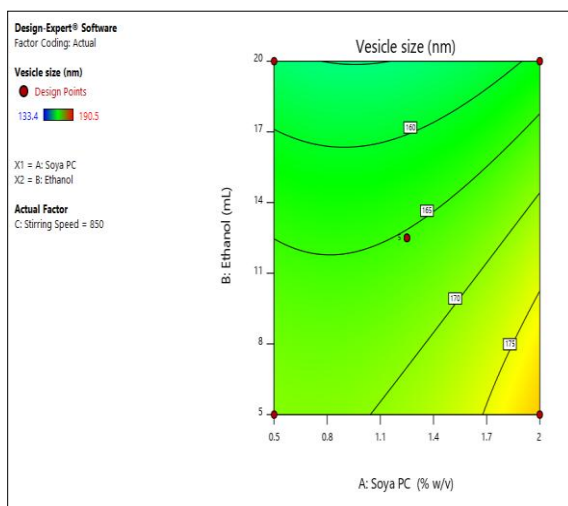


Contour plots between ethanol and soya PC



3D plots between ethanol and soya PC

Figure 1: Graphs of entrapment efficiency Obtained by DOE

**Graph of normal plots of residuals****Graph of predicted vs actual****Contour plots between ethanol and soya PC 3D plots between ethanol and soya PC****Figure 2: Graphs of vesicle size obtained by DOE****Table 4: Optimized formulation as per Predicted and Experimental Responses**

F. Code	Response	Predicted Value	Experimental Value
F8	Entrapment Efficiency (%)	86.42	86.80
	Vesicle size (nm)	179.36	180.20

Table 5: Results of Viscosity of Gel Formulation

Formulation	Viscosity (cP)	pH	Drug Content (%)	Extrudability (g/cm ²)	Spreadability (g·cm/sec)
FG1	6820	6.52	91.25	165	12.5
FG2	9450	6.78	95.80	185	15.8
FG3	12850	6.91	97.45	210	18.2

Table 6: Results of *in vitro* drug release study

Time (hrs)	% In vitro drug release		
	FG1	FG2	FG3
0.5	19.50	17.80	15.20
1	34.25	31.60	28.40
2	50.60	46.80	42.75
3	62.45	58.90	54.10
4	72.80	68.40	63.25
5	81.20	76.50	70.80
6	96.60	83.10	76.95
7	98.10	88.75	82.40
8	98.36	91.80	85.60

Table 7: Results of *in vitro* drug release kinetics study

Time (hrs)	% Drug Release	Log Time	Log % Drug Release	√Time	Cumulative % Drug Remaining
0.5	17.80	-0.301	1.250	0.707	82.20
1	31.60	0.000	1.499	1.000	68.40
2	46.80	0.301	1.670	1.414	53.20
3	58.90	0.477	1.770	1.732	41.10
4	68.40	0.602	1.835	2.000	31.60
5	76.50	0.699	1.883	2.236	23.50
6	83.10	0.778	1.919	2.449	16.90
7	88.75	0.845	1.948	2.646	11.25

8	91.80	0.903	1.963	2.828	8.20
---	-------	-------	-------	-------	------

Table 8: Kinetic model fitting of optimized formulation (FG2)

Kinetic Model	R ² Value
Zero Order	0.9410
First Order	0.9947
Higuchi Model	0.9861
Korsmeyer–Peppas Model	0.9880

Table 9: Results of stability study of formulation (FG2)

Storage Condition	Duration	Physical Appearance	Drug Content (%)
Refrigeration (4.0 ± 0.2°C)	3 weeks	No change	95.60
Room Temperature (25–28 ± 2°C)	3 weeks	No change	95.10

Conclusion

The present study successfully developed and optimized a transferosomal formulation using a Design of Experiments (DoE) approach. The optimized formulation (F8) exhibited high entrapment efficiency (86.8%) and nanosized vesicles (180.2 nm), indicating efficient drug encapsulation and suitable vesicular characteristics. Incorporation of the optimized transferosomes into a gel base resulted in a formulation with acceptable pH, viscosity, spreadability, extrudability, and drug content. The transferosomal gel demonstrated sustained drug release over 8 hours and followed first-order release kinetics with a diffusion-controlled mechanism. Stability studies confirmed the physical and chemical stability of the

formulation under different storage conditions. The developed transferosomal gel represents a promising and effective topical drug delivery system with potential for enhanced therapeutic performance and patient compliance.

References

1. Alsaadoon NS, Al-Refaie AM, Habashy AY. Acne vulgaris in adolescents: a comprehensive review. *Benha Journal of Applied Sciences*. 2024;9(12):5-13.
2. Mawardi P, Ardiani I, Primisawitri PP, Nareswari A. Dual role of *Cutibacterium acnes* in acne vulgaris pathophysiology. *Bali Medical Journal*. 2021;10(2):486-490.
3. Tajdari M, Abolghasemi S, Khanniri E, Bayanati M, Hakimimofrad R,

- Mahboubi-Rabbani M. A comprehensive review of acne treatments: unpacking the chemical structures and effective bioactive compounds. *Health Science Reports*. 2026;9(2):e71803.
4. Kang S, Lee H, Jun SH, Park SG, Kang NG. Enhancement of efficacy of retinoids through enhancing retinoid-induced RAR activity and inhibiting hydroxylation of retinoic acid, and its clinical efficacy on photo-aging. *Pharmaceutics*. 2022;14(11):2412.
 5. Cosio T, Di Prete M, Gaziano R, Lanna C, Orlandi A, Di Francesco P, Bianchi L, Campione E. Trifarotene: a current review and perspectives in dermatology. *Biomedicines*. 2021;9(3):237.
 6. Sharma U, Verma P, Jain NK. A review on novel vesicular drug delivery system: transfersomes. *International Journal of Pharmacy and Life Sciences*. 2020;11(7):6710-6718.
 7. Yu YQ, Yang X, Wu XF, Fan YB. Enhancing permeation of drug molecules across the skin via delivery in nanocarriers: novel strategies for effective transdermal applications. *Frontiers in Bioengineering and Biotechnology*. 2021;9:646554.
 8. Shukla KV, Meshram R, Yadav M. Formulation, development and evaluation of transfersomal gel. *Journal of Drug Delivery and Therapeutics*. 2019;9(4-A):642-645.
 9. Pande S. Liposomes for drug delivery: review of vesicular composition, factors affecting drug release and drug loading in liposomes. *Artificial Cells, Nanomedicine, and Biotechnology*. 2023;51(1):428-440.
 10. Beg S, Akhter S. Box–Behnken designs and their applications in pharmaceutical product development. In: Beg S, Hasnain MS, editors. *Design of Experiments for Pharmaceutical Product Development: Volume I - Basics and Fundamental Principles*. Singapore: Springer; 2021. p. 77-85.
 11. Youn BD, Choi KK. A new response surface methodology for reliability-based design optimization. *Computers and Structures*. 2004;82(2-3):241-256.
 12. Gayathri H, Dr. S.Sangeetha. Pharmaceutical Development of

- Methotrexate loaded Transfersosomal Gel For Skin Cancer By DOE Approach. Journal of Pharmaceutical Negative Results. Volume 13 (Special Issue 5): 2022;2456-2468.
13. Guggila Niharika, Mekala Pallavi and Arumugam Yasodha. Formulation and evaluation of Ebastine transfersomes. World Journal of Biology Pharmacy and Health Sciences, 2024, 19(01), 393–400.
 14. Ghanbarzadeh S, Arami S. Enhanced transdermal delivery of diclofenac sodium via conventional liposomes, ethosomes, and transfersomes. BioMed research international. 2013 Oct;2013.
 15. Arjumand Begum Nooreen, Syed Abdul Azeez Basha, Abdul Mannan, Arshiya Begum. Formulation and Evaluation of Gentamicin-Loaded Transfersosomal Gel. Int J Health Sci Res. 2023; 13(12):307-321.
 16. Qushawy M, Nasr A, Abd-Alhaseeb M, Swidan S. Design, optimization and characterization of a transfersosomal gel using miconazole nitrate for the treatment of candida skin infections. Pharmaceutics. 2018 Feb 23;10(1):26.
 17. Mishra M. and Biswal P., Complexation, Optimization, Formulation development and characterization of clindamycin phosphate gel using zinc acetate dehydrate, international journal of pharmacy, 2012; 2(3):472-486.
 18. Madhulika et al, Formulation and Development of Transfersosomal gel incorporating with Chrysin for Topical Application., Indo Am. J. P. Sci, 2023; 10 (06):170-176.
 19. Malakar J, Sen SO, Nayak AK, Sen KK. Formulation, optimization and evaluation of transfersosomal gel for transdermal insulin delivery. Saudi pharmaceutical journal. 2012 Oct 1;20(4):355-63.
 20. Thakur N, Jain P, Jain V. Formulation development and evaluation of transfersosomal gel. Journal of Drug Delivery and Therapeutics. 2018 Sep 15;8(5):168-77.
 21. Omar MM, Hasan OA, El Sisi AM. Preparation and optimization of lidocaine transfersosomal gel containing permeation enhancers: a promising approach for enhancement of skin permeation. Universal

Journal of Pharmaceutical
Research.2019; 14:155.

22. ICH. 2003. Q1A(R2): stability testing of new drug substances and products. ICH Harmonised Tripartite Guideline, International Conference on Harmonisation.