Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Design and Development of Minocycline Loaded Transfersomes Gel for Effective Management of ACNE

Manoj Patel, Neeraj Sharma

Madhyanchal Professional University (MPU) - Educational Institute Bhopal, Madhya Pradesh, India
Email: manojpharma86@yahoo.com

Acne vulgaris, a chronic inflammatory skin disorder, predominantly affects adolescents and young adults, causing significant cosmetic and psychological concerns. Traditional treatments, including oral antibiotics, have limitations such as systemic side effects and antibiotic resistance. Minocycline, an antibiotic effective against Propionibacterium acnes, is widely used for acne treatment but can cause systemic side effects when administered orally. To address these challenges, this study explores the development of minocycline-loaded transfersome gels for targeted topical delivery to the skin, aimed at reducing side effects and improving therapeutic efficacy. Transfersomes, lipid-based vesicular systems, offer enhanced skin penetration and sustained drug release. Various formulations (F1 to F17) were prepared and characterized for vesicle size, entrapment efficiency, drug content, spreadability, and viscosity. In vitro drug release studies demonstrated promising results for controlled release, with formulation F5 exhibiting optimal characteristics. Stability studies indicated that the formulation remained stable over a period of 28 days at different storage conditions. The results suggest that minocycline-loaded transfersome gels could be an effective, localized treatment option for acne, offering improved patient compliance and reduced systemic exposure.

Keywords: Acne vulgaris, minocycline, transfersomes, topical delivery, drug release, vesicle size, entrapment efficiency, controlled release, skin penetration, stability studies.

1. Introduction

Acne vulgaris is a common dermatological condition characterized by the inflammation of the sebaceous glands, resulting in pimples, blackheads, and cysts [1]. It affects individuals primarily during adolescence, though it can persist into adulthood, with consequences ranging

from scarring to psychological distress. The treatment of acne typically involves topical agents such as antibiotics, retinoids, and benzoyl peroxide, but these treatments often have limitations related to skin irritation, poor patient compliance, and inconsistent efficacy. Among these, minocycline, a tetracycline antibiotic, has proven effective in reducing Propionibacterium acnes and decreasing inflammation, making it a promising candidate for acne therapy [2]. Despite its efficacy, the oral administration of minocycline often leads to side effects like gastrointestinal disturbances and the potential for antibiotic resistance [3].

As a result, there has been increasing interest in developing topical formulations of minocycline to bypass systemic side effects and target the site of infection directly. Transfersomes, lipid-based vesicular systems, offer a promising solution for the topical delivery of drugs. These vesicles are composed of phospholipids and surfactants that can enhance drug penetration through the skin, offering a controlled and sustained release profile [4].

The use of transfersomes for drug delivery is beneficial because they possess unique properties such as deformability and elasticity, which allow them to pass through the skin's stratum corneum, an otherwise difficult barrier for many topical formulations [5].

In this context, minocycline-loaded transfersome gels have the potential to provide an efficient, localized treatment for acne, offering improved bioavailability and reduced systemic exposure.

This study focuses on the formulation and evaluation of minocycline-loaded transfersome gels for the effective management of acne. The gels were prepared with various formulations of transfersomes, and their physicochemical properties, including vesicle size, entrapment efficiency, drug release, and stability, were evaluated. The goal was to develop a stable, effective, and safe topical formulation that could enhance the therapeutic outcomes for acne treatment.

2. Material and Methods

Material

The Transfersomes gel was prepared using a variety of chemicals from trusted suppliers. Minocycline, an antibiotic, was obtained as a gift sample. Soya phosphatidylcholine, buffering agents (disodium hydrogen phosphate and dipotassium hydrogen orthophosphate), and sodium chloride were sourced from S. D. Fine Chem. Ltd. The solvents methanol, ethanol, and chloroform came from Qualigens Fine Chemicals. Carbopol 934P, used as a gelling agent, and preservatives (methyl and propyl paraben) were also obtained from S. D. Fine Chem. Ltd. Propylene glycol, a stabilizer, was included to enhance gel texture and skin penetration.

Methods

Statistical modelling for optimizing the Minocycline loaded Transfersomes formulation

One of the tools used in response surface methodology (RSM) was Box–Behnken Design (BBD) for the optimization of the formulated Minocycline loaded transfersomes. Span 80 (C), Ethanol (B), and Soya PC (A) were the three independent variables used to create the three

factors, three levels (3^3) Box–Behnken Design, with two levels being high (+1) and low (−1).

The study looked at two dependent variables: entrapment efficiency (%) (R2) and vehicle size (nm) (R1). The Software Design-Expert version 12.0 (Stat-Ease, Minneapolis, MN, USA) was utilized to assess how the formulation factors affected the dependent variables under investigation. In order to get the optimal formula with the necessary results, seventeen runs were created in accordance with the experimental design. In order to evaluate the model significance and substantiate the statistical analysis of the data, the analysis of variance (ANOVA) test was utilized [6].

Preparation of Minocycline loaded transferosomes

Formulations for transferosomes were created utilizing the Box-Behnken model and the Rotary Flask Evaporation Sonication approach, as previously reported by Shaji and Lal (2014) [7]. Ethanol was used to dissolve precise concentrations of Span 80, minocycline, and Soya PC (phospholipids). The rotary evaporator (Buchi rotavapor) was used to progressively evaporate the mixture of organic solvents at 60 °C while operating at lower pressure. In order to achieve a transfersomal dispersion, the dried thin lipid film was hydrated with 10 mL of phosphate buffer solution (PBS; pH 7.4) while being gently stirred in a water bath at 60°C for an hour. To allow for swelling, the transferosomes were kept at room temperature for an extra two hours. The transferosome vesicles were then subjected to a 20–30 minute bath sonication using a bath sonicator. Based on the experimental methodology, entrapment efficiency (EE%), and vescicle size of minocycline transferosomes, seventeen formulations were created and reported in Table 1.

Table 1: The independent variables used for optimizing different transfersomal formulations

F. Code	Std	Run	Factor A: Soya PC	Factor B:Ethanol	Factor C:Span 80
F1	1	1	100	10	25
F2	2	2	500	10	25
F3	8	3	500	15	30
F4	12	4	300	20	30
F5	4	5	500	20	25
F6	13	6	300	15	25
F7	7	7	100	15	30
F8	14	8	300	15	25
F9	16	9	300	15	25
F10	17	10	300	15	25
F11	6	11	500	15	20
F12	5	12	100	15	20
F13	15	13	300	15	25
F14	10	14	300	20	20
F15	3	15	100	20	25
F16	9	16	300	10	20

F17	11	17	300	10	30

Final Equation in Terms of Coded Factors

Average vesicle size = +237.97-28.19 A-6.42 B-31.04 C-1.31 AB-16.83 AC-6.49 BC-21.06 A^2-26.17 B^2+17.00 C^2

Final equation in terms of actual factors

Average vesicle size = +367.42356+0.615290 Soya PC+37.00020 Ethanol-31.26950 Span 80-0.001305 Soya PC * Ethanol-0.016832 Soya PC * Span 80-0.259600 Ethanol * Span 80-0.000526 Soya PC^2-1.04679 $Ethanol^2$+0.680110 Span 80^2

Final Equation in Terms of Coded Factors

Entrapment efficiency = +68.72+3.40 A+0.4912B+3.75 C+0.9025 AB-0.7000 AC+3.70 BC+3.02A^2+5.16 B^2-0.2912 C^2

Final equation in terms of actual factors

Entrapment efficiency = +143.62844-0.024350 Soya PC -10.06000 Ethanol-0.677500 Span 80+0.000903 Soya PC * Ethanol-0.000700 Soya PC * Span 80+0.148000 Ethanol * Span 80+0.000076 Soya PC^2+0.206250 $Ethanol^2$-0.011650 Span 80^2.

Characterization of Minocycline loaded transfersomes

Determination of vesicle size

Using the Zetasizer equipment (Mastersizer 2000 version 5.22, Malvern Instruments Ltd., Worcestershire, UK), the optimal transfersomal vesicle size was measured. The formulations' particle sizes were evaluated using the dynamic light scattering technique [8].

Encapsulation efficiency determination (EE%)

Centrifuging the dispersion for 60 minutes at 4°C at 6,000 rpm was used to measure the encapsulation effectiveness of transfersomal dispersions loaded with minocycline. Using a spectrophotometer (Labindia UV/VIS, 3000+) at λ_{max} 242 nm, the supernatant was obtained and diluted following centrifugation [9]. Finally, the absorbance was recorded.

Equation following was used to compute the % encapsulation efficiency:

Metric of Drug Encapsulation Efficiency: $(AT - AF)/AT \times 100$

In the above formula, AF is the free concentration of minocycline detected in the supernatants, and AT is the total quantity of minocycline in transfersomal dispersions.

Preparation of Minocycline Loaded Gel

A tiny particle size and good entrapment effectiveness of the invasive formulation F5 were added to the Carbopol 934 gel basis (1–3% w/v). In order to allow the gelling agent to fully expand, carbopol 934 was mixed with distilled water and left in the dark to create carbopol gel basis. Drop by drop, triethanolamine was added to the dispersion to form a translucent, viscous gel. Lastly, a mechanical stirrer was used to gently combine the optimized invasomal formulation with the Carbopol gel base while stirring it moderately [10].

Table 2: The composition of different minocycline loaded transfersomal gel

Composition	G1	G2	G3	G4	G5
Transfersomes eq to (0.5%)	1	1	1	1	1
Carbopol 934 (%)	1	1.5	2	2.5	3
Triethanolamine (%)	0.5%	0.5%	0.5%	0.5%	0.5%
Distilled water (Qs)	100 ml	100 ml	100 ml	100 ml	100 ml

Evaluation of Minocycline loaded transfersomal gel

Physical inspection

To evaluate the homogeneity of the created gel formulations loaded with minocycline, a visual inspection was conducted [11].

Estimation of pH Value

A calibrated digital pH meter (EI) was used to measure the pH of minocycline transfersomal gels at room temperature. After measuring the pH three times, the average value was determined [12].

Spreadability Test

This experiment was designed to evaluate the diameters of spreading when the developed gel was applied to the affected area and to examine the gel's spreadability. In short, a fixed weight was secured for one minute over the top slide while gel was held in place between the two slides. To gauge the spreadability, the spreading area diameter was measured [13].

Rheological Studies and Viscosity

The generated transfersomal gels' viscosity at 25 °C was measured using a Viscostar-R rotational viscometer (Fungilab S.A., Barcelona, Spain) and Spindle R5 at 2 rpm. Three measurements of the viscosity were made, and the mean was obtained [14].

Drug Content Determination

Phosphate buffer saline, pH 7.4, was precisely used to dilute 0.5 g of the produced gel formulations, or 5 mg of minocycline, to ten milliliters. The drug content was measured using spectrophotometry at λ_{max} 242 nm with a blank sample that had the same ingredients but no drug [15]. The following formula was used to get the percentage of drug content:

% Drug content = Actual amount of the drug in the formulation

Theoretical amount of the drug in the formulation × 100

In-vitro drug release from transfersomal gel

The created transfersomal gel formulations were subjected to the same approach to evaluate the release rate of Minocycline in comparison to free drug, transfersomes, and Minocycline gel preparation. Two milliliter samples were taken out at specific intervals (0.25, 0.5, 1, 2, 4, 6 to 12….hours) and replaced with new buffer. Using spectroscopy at λ_{max} 242 nm, the drug content of the samples was determined [16].

Physical stability studies of drug loaded transfersomal gel

Transfersomal gel stability investigations were carried out by analyzing their chemical or physical characteristics while being stored. The gel was placed within a borosilicate glass container and kept for six months under two distinct storage conditions: 4±2°C and 25±2°C with 60±5% relative humidity. During the stability research, the following parameters were examined at predetermined intervals of four weeks.

pH Evaluation

As was previously noted, the pH was assessed.

Physiochemical Evaluation

Through visual examination, the gel's organoleptic properties, washability, and clarity were investigated.

3. Results and Discussion

The study aimed at the design and development of minocycline-loaded transferosome gel for the effective management of acne. The optimization of transferosome formulations was evaluated based on vesicle size, entrapment efficiency, and release characteristics, followed by characterization of the gel formulations and stability studies.

From Table 3, the vesicle sizes of the formulations ranged from 152.23 nm (F5) to 278.85 nm (F12), with F5 showing the smallest vesicle size and the highest entrapment efficiency (83.35%). The vesicle size plays a significant role in the skin penetration and stability of the formulation, with smaller vesicles generally enhancing skin absorption. Formulations F1 to F17 demonstrated varying degrees of entrapment efficiency, with F5 showing the highest (83.35%), indicating that this formulation was capable of encapsulating a higher amount of minocycline, which is essential for ensuring an effective therapeutic dose in acne treatment.

Figures 1 and 2, showing the response surface plots for vesicle size and entrapment efficiency, highlight the relationships between formulation variables and the observed responses. The plots indicate that formulation F5, with a vesicle size of 152.23 nm and entrapment efficiency of 83.35%, was the most optimized formulation. This was further supported by the predicted vs. actual values in Table 4, where the actual and predicted vesicle size and entrapment efficiency for F5 were very close, confirming the accuracy of the predictive model. Additionally, the zeta potential of formulation F5 was found to be -39.85 mV (Figure 3), indicating good stability and repulsion between vesicles, which prevents aggregation.

The gel formulations (G1 to G5) were characterized for various parameters such as appearance, after-feel, consistency, pH, drug content, spreadability, and viscosity, as presented in Table 5. All formulations had a transparent appearance and smooth after-feel, which is desirable for topical applications. The pH values of the gels ranged between 6.3 and 6.8, which is compatible with the skin's natural pH. The drug content was high across all formulations, with formulation G4 showing the highest drug content (99.05%), ensuring adequate therapeutic dosage. Spreadability, an essential parameter for topical formulations, was highest in G1 (18.08 cm), while viscosity increased with the formulation from G1 (1555 cP) to G5

(1985 cP). This increase in viscosity suggests better gel consistency and the ability to stay on the skin for prolonged periods.

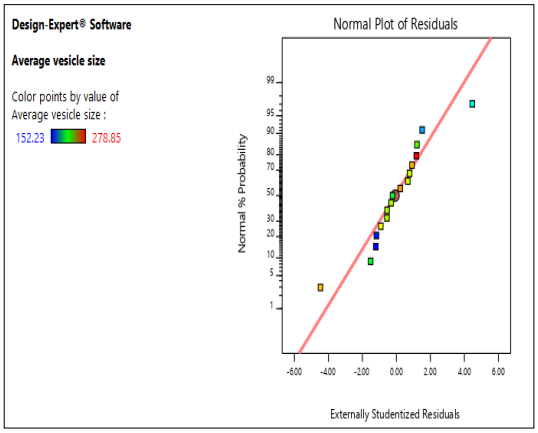
The cumulative drug release from minocycline-loaded transferosome gels is presented in Table 6. G1 showed the fastest release profile, achieving 97.78% release by hour 7, whereas G4, which showed a controlled release, reached 86.65% by hour 12. G4 exhibited a more gradual release pattern, suggesting its suitability for sustained drug delivery, which could help in the prolonged management of acne.

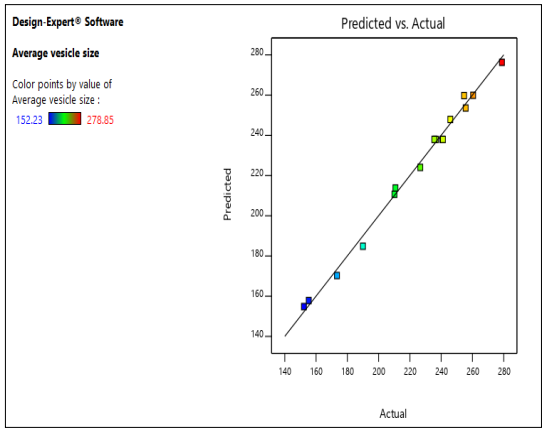
Table 7 presents the regression analysis for different drug release models. The Korsmeyer's Peppas equation demonstrated the best fit ($R^2 = 0.9816$) for formulation G4, suggesting that the drug release follows non-Fickian diffusion, a desirable characteristic for sustained-release formulations.

The stability studies for formulation G4, presented in Table 8, indicate that the gel maintained its properties well under different storage conditions. At 4°C and 25°C, the appearance remained smooth, and drug content was stable over the first 15 days. However, after 28 days, slight reductions in drug content and pH were observed, but the formulation still exhibited satisfactory homogeneity and washability.

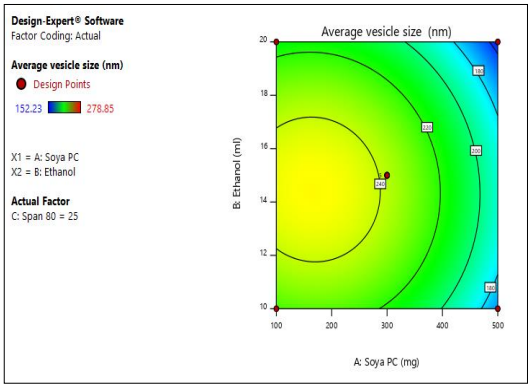
Table 3: Results of Vesicle size and Entrapment Efficiency of formulation F1 to F17

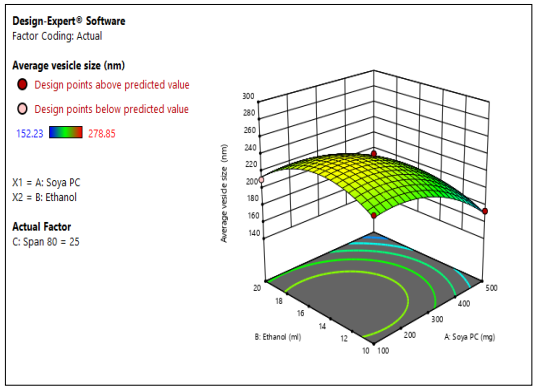
Formulation Code	Response 1: Vesicle size (nm)	Response 2: Entrapment Efficiency (%)
F1	226.65	72.25
F2	173.36	78.85
F3	155.32	76.65
F4	189.98	81.12
F5	152.23	83.35
F6	236.65	67.74
F7	245.85	72.85
F8	235.69	67.74
F9	240.74	68.12
F10	241.13	69.88
F11	255.65	71.45
F12	278.85	64.85
F13	235.65	70.12
F14	260.45	65.32
F15	210.74	73.14
F16	254.65	73.45
F17	210.14	74.45





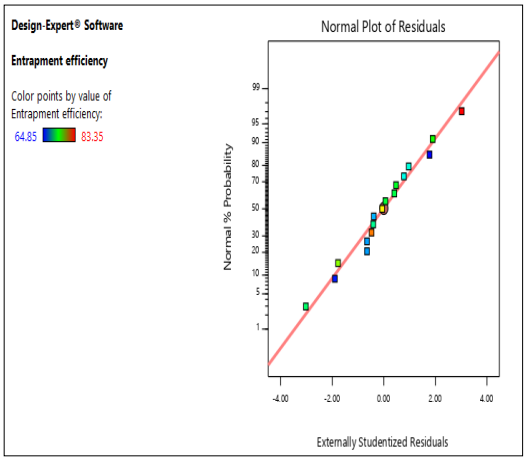
Normal Plots of Residuals Predicted vs. Actual

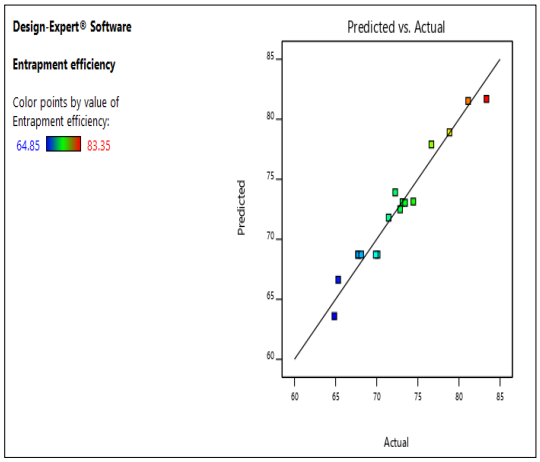




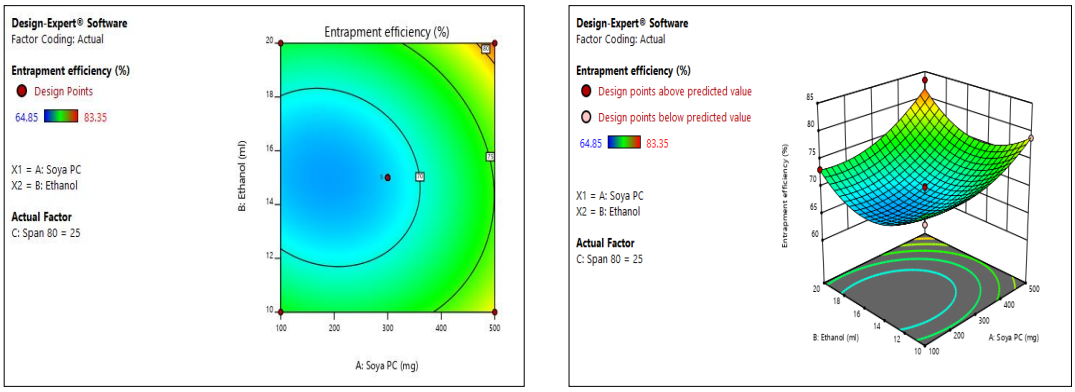
Contour graph 3D Surface graph

Figure 1: Figure of Response surface plots for average vesicle size





Normal Plots of Residuals Predicted vs. actual



Contour graph 3D Surface graph

Figure 2: Figure of Response surface plots for entrapment efficiency

Table 4: Experimental data with predicted response

Run Order	Formulation Code	Parameters	Actual Value	Predicted Value
5	F5	Vesicle size	152.23	154.82
		Entrapment Efficiency (%)	83.35	81.69
		Zeta potential	-39.85	

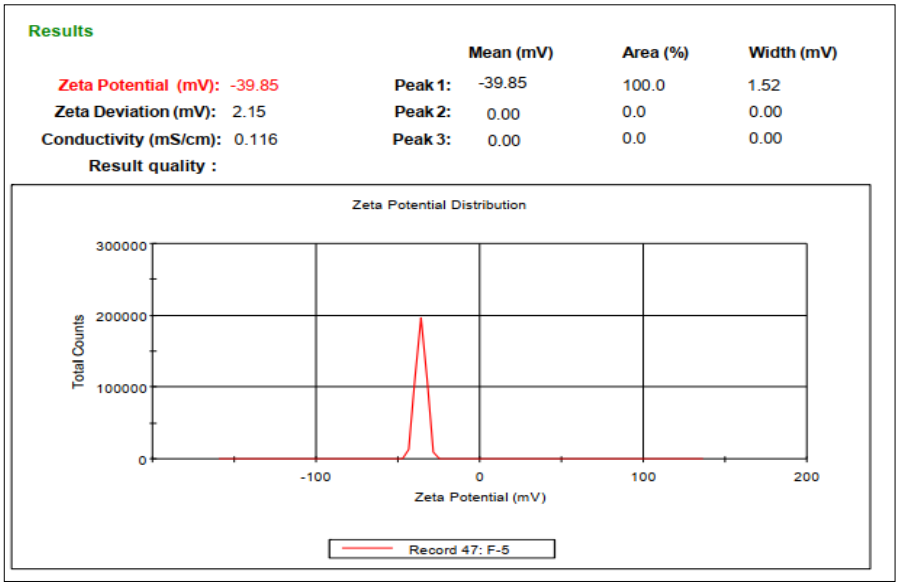


Figure 3: Graph of Zeta potential of optimized formulation F5

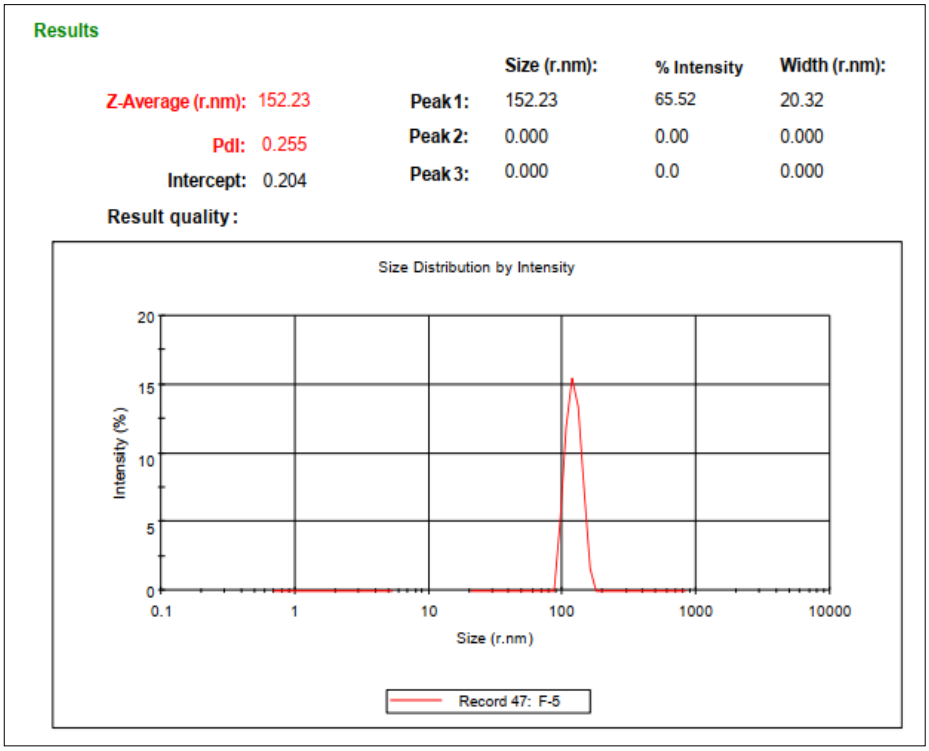


Figure 4: Graph of Vesicle size of optimized formulation F5

Table 5: Characterization of various gel preparations

Specifications	G1	G2	G3	G4	G5
Colour	Transparent	Transparent	Transparent	Transparent	Transparent
After feel effects	Smooth	Smooth	Smooth	Smooth	Smooth
Consistency	Easy pourable	Less	Good	Very good	High
Homogeneity	Good	Good	Good	Good	Good
pH	6.8±0.58	6.3±0.49	6.5±0.19	6.7±0.49	6.6±0.76
Drug Content (%)	95.04±0.58	94.59±0.73	96.84± 0.46	99.05± 0.12	96.28±0.23
Spreadability	18.08±3.42	17.75±3.59	16.36±5.25	13.53±3.27	12.28±4.23
Viscosity	1555±12.32	1645±10.45	1755±14.45	1885±12.74	1985±10.25

Table 6: Cumulative drug release from minocycline loaded transferosomes gel

Time in (Hr)	Cumulative percent release* (% CPR)				
	G1	G2	G3	G4	G5
1	23.65±0.15	22.32±0.15	20.23±0.32	15.65±0.74	10.23±0.15
2	35.45±0.32	33.45±0.22	32.12±0.15	22.58±0.32	22.21±0.32
3	48.78±0.25	45.85±0.32	42.05±0.25	38.78±0.56	36.65±0.22
4	56.65±0.36	54.45±0.15	53.32±0.36	46.65±0.44	45.58±0.41

5	68.85±0.14	63.32±0.26	60.14±0.56	55.65±0.52	53.32±0.32
6	85.45±0.22	82.25±0.32	80.23±0.33	64.47±0.32	68.85±0.14
7	97.78±0.30	95.65±0.45	96.65±0.14	76.85±0.41	72.26±0.36
8	-	97.78±0.32	98.85±0.32	79.98±0.33	78.98±0.32
10	-	-	99.85±0.15	83.32±0.41	82.25±0.11
12	-	-	-	98.14±0.36	86.65±0.74

*Average of Six determination

Table 7: Regression analysis data of minocycline loaded transferosomes gel

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
G4	0.9493	0.8528	0.9833	0.9816

Table 8: Results of stability studies of the minocycline loaded transferosomes gel formulation G4

Condition	Days	Appearance	% Drug content	pH	Homogeneity	Washability
4.0±0.5°C	7	Smooth	97.43±0.53	6.2	Good	Good
	15	Smooth	97.21±0.19	6.1	Good	Good
	28	Smooth	96.82±0.73	5.9	Satisfactory	Good
25±0.5ºC	7	Smooth	96.83±0.73	6.1	Good	Good
	15	Smooth	96.46±0.49	6.1	Satisfactory	Good
	28	Smooth	95.84±0.48	5.8	Satisfactory	Good

4. Conclusion

In conclusion, the developed minocycline-loaded transferosome gel formulations showed promising results for acne management. The optimized formulation (F5) demonstrated high entrapment efficiency, a suitable vesicle size for enhanced skin penetration, and good stability. The gel exhibited controlled drug release, particularly in formulation G4, which is ideal for sustained treatment. Stability studies confirmed the formulation's potential for commercial use, with minimal changes in drug content, pH, and other characteristics over time. These findings suggest that the minocycline-loaded transferosome gel is an effective and stable topical formulation for acne treatment.

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