

Formulation, Optimization and Evaluation of topical Niosomal Gel for Oxiconazole

Ms. Akshata Sanjay Patil^{1*}, Dr. Ravindra B. Patil²

^{1*}Department of Pharmaceutics, DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra-424005, India,

²Department of Pharmacology, DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra-424005, India.

***Corresponding Author:**

Ms. Akshata Sanjay Patil

Department of Pharmaceutics, DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra-424005, India.

Email: akshata1996patil@gmail.com

ORCID: 0000-0002-2332-8741

ABSTRACT

Niosomes are novel vesicular drug-delivery systems that can improve drug entrapment, provide controlled drug release, and prolong drug residence at the site of application. Oxiconazole nitrate is a broad-spectrum imidazole antifungal agent used for the treatment of superficial fungal infections. However, conventional topical formulations may have limited residence time. The objectives of the present study were to develop an ideal drug-delivery system for the effective treatment of fungal infections, maintain the therapeutic drug concentration for an appropriate period, enhance patient compliance, and improve the therapeutic effectiveness of the formulation. The study also aimed to reduce adverse effects and enhance the bioavailability of the drug. Furthermore, the study focused on formulating a novel drug-delivery system using an appropriate formulation technique and evaluating the developed formulation through SEM analysis, drug content, entrapment efficiency, and in-vitro drug-release studies. Finally, stability studies were performed to assess the physical and pharmaceutical stability of the optimized formulation. Oxiconazole-loaded niosomes were prepared by the ether injection method using Tween 80 and cholesterol. A Box–Behnken design was employed using cholesterol concentration, Tween 80 concentration, and stirring speed as independent variables. Particle size, zeta potential, and entrapment efficiency were selected as responses. The optimized niosomes were incorporated into a Carbopol 937-based gel and further optimized using Box–Behnken design, with Carbopol 937 concentration, Tween 80 concentration, and stirring speed as independent variables. The optimized formulations were evaluated for drug content, pH, viscosity, spreadability, extrudability, gel strength, gelling capacity, in-vitro drug release, FTIR, DSC, XRD, SEM, and stability. The optimized niosomal formulation (NO10) exhibited a particle size of 224.7 nm, zeta potential of –26.5 mV, and drug content of 86.42%. NO10 demonstrated 96.82% cumulative drug release at 8 h. The optimized niosomes were successfully incorporated into the topical gel. The optimized gel formulation (OG9) showed a pH of 4.96, spreadability of 6.64 g·cm/s, and 94.96% cumulative drug release at 8 h. XRD analysis indicated predominantly amorphous characteristics of the encapsulated drug. During three months of stability evaluation, only minor changes were observed in pH, spreadability, and drug release. The developed oxiconazole-loaded niosomal gel exhibited favorable physicochemical characteristics and sustained drug-release behavior. The optimized formulation demonstrated satisfactory stability and may serve as a promising novel topical drug-delivery system for oxiconazole.

KEYWORDS

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1. INTRODUCTION

Superficial fungal infections are common dermatological disorders caused by pathogenic fungi and may affect different areas of the skin. Common infections include tinea corporis, tinea cruris, tinea pedis, and cutaneous candidiasis. These infections may cause itching, redness, irritation, inflammation, and discomfort and often require prolonged antifungal therapy. The effectiveness of

topical treatment depends on maintaining an adequate concentration of the drug at the site of infection for a sufficient period. However, conventional topical formulations may be removed from the skin due to washing, sweating, or rubbing, which can reduce drug residence time and therapeutic effectiveness.¹⁻⁵

Oxiconazole nitrate is a synthetic imidazole antifungal agent possessing broad-spectrum activity

against several pathogenic fungi responsible for superficial skin infections. It acts mainly by inhibiting the biosynthesis of ergosterol, an essential component of the fungal cell membrane. Depletion of ergosterol alters fungal membrane structure and function, ultimately inhibiting fungal growth. Oxiconazole is commonly administered as a topical formulation; however, conventional creams, lotions, and gels may not always provide prolonged drug retention or controlled delivery at the site of infection. Therefore, an advanced delivery approach may improve the local availability and performance of oxiconazole.⁶⁻⁹

Niosomes are vesicular drug-delivery systems composed primarily of non-ionic surfactants, generally stabilized with cholesterol. Their vesicular structure allows incorporation of drug molecules and can provide advantages such as improved drug entrapment, protection of the drug, controlled release, and enhanced interaction with the skin. Niosomes are particularly attractive for topical delivery because they may improve drug deposition within the skin and prolong the residence time of the incorporated drug at the application site.¹⁰⁻¹³

The incorporation of drug-loaded niosomes into a topical gel can further enhance the practicality and performance of the delivery system. The gel provides suitable viscosity, spreadability, and prolonged contact with the skin, while the niosomal vesicles can act as carriers for controlled and localized drug delivery. Thus, a niosomal gel can combine the advantages of a conventional topical gel with those of a novel vesicular delivery system, potentially improving the therapeutic performance of oxiconazole.¹⁴⁻¹⁵

The characteristics of niosomes are strongly affected by formulation and process variables such as cholesterol concentration, surfactant concentration, and stirring speed. These variables can influence particle size, surface charge, drug entrapment, vesicle stability, and drug-release behavior. Therefore, systematic optimization is essential to obtain a formulation with desirable characteristics. Response Surface Methodology (RSM) provides an efficient statistical approach for studying the effects of multiple formulation variables and their interactions. In the present study, a Box–Behnken Design was employed using Design-Expert® software for formulation optimization.

In the present investigation, cholesterol, Tween 80, and stirring speed were selected as independent variables, while particle size, zeta potential, and entrapment efficiency were selected as dependent variables. The optimized oxiconazole-loaded niosomal formulation was subsequently incorporated into a suitable topical gel and evaluated for pH, viscosity, spreadability, gel strength, gelling capacity, drug content, and in-vitro drug release. The release data were further subjected to kinetic

analysis to determine the probable drug-release mechanism.

2. MATERIAL AND METHODS

2.1 MATERIALS

Oxiconazole was generous sample from..... Cholesterol, Tween 80 Carbopol 937, glycerin, and triethanolamine (TEA) were purchased from Cosmos Chem Pune. All other materials and solvents used in this study were of analytical grade.

2.2 Formulation of Niosomes (Ether Injection Method)

Oxiconazole-loaded niosomes were prepared using the ether injection method. Tween 80 and cholesterol were dissolved in 10 mL of diethyl ether to form the organic phase. Oxiconazole was dissolved in 10 mL of phosphate buffer (pH 7.4) to prepare the aqueous phase. The organic phase was slowly injected into the aqueous drug solution at a rate of 0.25 mL/min using a 23-gauge needle. The aqueous phase was continuously magnetically stirred during injection. The temperature was maintained at 60 °C throughout the process. Stirring was continued for 1 hour to allow complete evaporation of diethyl ether. A uniform niosomal suspension was obtained after solvent removal.¹⁶

2.2.1 Experimental design

In the present study, Response Surface Methodology (RSM) employing a Box–Behnken Design (BBD) was utilized for the systematic optimization of the formulation using Design-Expert® software (Version 13.0). Three independent variables were selected, namely cholesterol concentration (mg) (A), Tween 80 concentration (%) (B), and stirring speed (RPM) (C). Each factor was investigated at three levels: low (−1), medium (0), and high (+1). The cholesterol concentration was varied from 10 to 30 mg, Tween 80 concentration from 1 to 2%, and stirring speed from 1000 to 2000 RPM. The selected dependent variables were particle size (Y1), zeta potential (Y2) and entrapment efficiency (Y3). The formulation was optimized by applying the predefined constraints, with entrapment efficiency (%) and zeta potential (mV) being maximized, while particle size (nm) was minimized. The Box–Behnken experimental design consisted of factorial combinations and center-point runs, resulting in a total of 13 experimental runs. The experimental design was used to evaluate the individual and interactive effects of the selected formulation variables on the critical quality attributes and to identify the optimized formulation with desirable physicochemical characteristics. The details of the independent variables, their coded levels, dependent variables, and optimization constraints.

2.3 EVALUATION AND CHARACTERIZATION OF NIOSOMES

2.3.1 Particle Size, PDI and Zeta potential analysis

The particle size of Oxiconazole niosomes was determined in triplicate by using dynamic light

scattering with a scattering angle of 90° at 25°C. The particle size analysis data obtained were evaluated using the intensity profile. The formed niosomes were suitably diluted (1:100) with water before analysis.¹⁷

2.3.2 Drug content

For determining the drug content, 5 mg of formulation was weighed accurately and transferred to a 10 ml conical flask, followed by addition of 10 ml of methanol and sonication for 10 min. The following solution was filtered and analyzed spectrophotometrically at 231 nm for drug content.¹⁸

$$\text{Drug Content (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

2.3.3 Entrapment efficiency

The entrapment efficiency of Oxiconazole loaded niosomes formulation was determined by centrifugation method, in which 5 mg in 5 ml of methanol the prepared niosomes suspension was placed in the Eppendorf for centrifugation (RM-12) at 10000 rpm for 30min after centrifugation, the supernatant liquid was separated, containing the un-entrapped drug, diluted with methanol and analyzed using UV spectrophotometer at 231 nm.¹⁹

$$\text{Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \times 100 \quad (2)$$

2.3.4 In vitro drug release

The dialysis tube diffusion technique was used to determine the release profile of oxiconazole from niosomal dispersion. 5 ml of dispersion was placed in the dialysis tube, hermetically sealed and soaked in a tube containing 20 ml of phosphate buffer (pH 7.4). The entire system was kept at 32 °C ± 0.5 °C with continuous stirring at 100 rpm. 2 ml aliquots of the sample were withdrawn from the receiver solution at predetermined time intervals followed by filtering and analyzing the drug release using a spectrophotometer at 231nm. After each assay, the sample was returned to the receiver container. For all dissolution in vitro tests, the sink condition was maintained.²⁰

2.3.5 Fourier Transform Infrared Spectroscopy (FTIR) spectroscopy

The drug excipients compatibility study was performed by FTIR technique. The Optimized batch NO10 samples were scanned over wave number range of 500-4000 cm⁻¹ with diffraction reflectance scanning technique.²¹

2.3.6 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric (DSC) measurements were carried out on a modulated DSC (Mettler Toledo, SW STARE, and USA). The Optimized batch NO10 were weighed (2-8mg), the aluminum pans were used and hermetically covered with lead. The heating range was 0-250 °C for sample with constant increasing rate of temperature

at 10°C /min under nitrogen atmosphere (50-60ml/min). The resultant thermograms of formulation was obtained.²²

2.6.7 X-ray Diffraction Study

The data obtained from XRD was used to determine whether newly formed compounds are crystalline or amorphous, the following conditions were used for the measurement: target metals Cu, filter K, 40kV voltage, and 30 mA current. Optimized batch NO10 Samples were scanned over a two-degree range of 10–90°C with a 0.2° phase scale.²³

2.6.8 Scanning Electron Microscopy

The surface morphology of the optimized oxiconazole-loaded niosomal formulation (NO10) was examined using a Nova NanoSEM. A small quantity of the niosomal sample was placed on a suitable specimen stub, allowed to dry, and coated with a thin conductive layer. The sample was then analyzed by SEM at a magnification of 30,000× to evaluate the surface morphology and shape of the niosomal vesicles.²⁴

2.4 METHOD OF PREPARATION OF NIOSOMAL GEL

The preparation of niosomal gel the optimized niosomes formulation was used. Carbopol 937 and purified water was taken in a beaker and allowed to soak for several hrs. To this required amount of niosomal dispersion in water and then Carbopol 937 is neutralized with sufficient quantity of surfactant (Tween 80).Then glycerine was added slowly with continuous gently stirring until homogenous gel is formed. Then, triethanolamine (TEA) was utilized to cause gelling to adjust the pH of the prepared gel using a pH meter.²⁵

2.4.1 Experimental design

In the present study, Response Surface Methodology (RSM) employing a Box–Behnken Design (BBD) was used for the systematic optimization of the formulation using Design-Expert® software (Version 13.0). Three independent variables were selected, namely Carbopol 937 concentration (%) (A), Tween 80 concentration (%) (B), and stirring speed (rpm) (C). Each independent variable was studied at three levels: low (–1), medium (0), and high (+1). The concentration of Carbopol 937 was varied from 0.25 to 0.50%, Tween 80 concentration from 4 to 6%, and stirring speed from 1000 to 2000 rpm. The dependent variables selected for optimization were Ph(Y1), spreadability (Y2), and drug release (Y3). The Box–Behnken Design comprised a total of 13 experimental runs, including different combinations of the selected independent variables and center-point formulations. The effect of the formulation variables on the selected responses was evaluated using polynomial equations and statistical analysis. For optimization, the pH was kept within the specified acceptable range, while spreadability and drug release were set to maximum to obtain a formulation with desirable physicochemical and drug-release characteristics.

The details of the independent variables, their coded levels, dependent variables, and optimization constraints.

2.5. EVALUATION OF NIOSOMAL GEL

2.5.1 Visual evaluation of gel

The prepared gel was examined for appearance, clarity, color and homogeneity.²⁶

2.5.2 Extrudability

The formulated gel was filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weight of filled tubes was recorded and the tubes were sandwiched between two glass slides and were clamped. 500gm weight was placed over the slides and then the cap was removed to extrude. The amount of extruded gel was collected and weighed. Extrudability was determined by calculating the percentage of extruded gel. When it is greater than 90% then extrudability is excellent. When it is greater than 80% then extrudability is good. When it is 70% then extrudability is fair.²⁷

2.5.3 pH measurement

The pH of the optimized oxiconazole-loaded niosomal gel was determined using a calibrated digital pH meter. Approximately 1 g of gel was accurately weighed and dispersed in 10 mL of distilled water. The dispersion was allowed to equilibrate for approximately 30 min at room temperature. The pH meter was calibrated using standard buffer solutions of pH 4.0 and 7.0 before measurement. The electrode was then immersed into the gel dispersion, and the pH was recorded.²⁸

2.5.4 Viscosity

Brookfield viscometer (S-62, model LVDV-E) at 25 °C with a spindle speed of the viscometer rotated at 100 rpm was used to determine the viscosity of the niosomal gel.²⁹

2.5.6 Spreadability

These test important parameter for all Gel formulation. These tests were estimated by wooden block with scale and 2 glass slides having a pan mounted on a pulley. These formulations were applied 2 slide and 100g weight was placed on the upper glass slide for 5 min. for maintain uniform thickness formulation, 100gm weight added to the pan. After this process, time in second separated the slide and measure the Spreadability test. The results are show in table no. The Spreadability was calculated by using the following formula:³⁰

$$S = \frac{M \times L}{T} \dots (3)$$

Where, S= spreadability-weight tied to upper slides (20 g), L- length of the glass slide (5 cm), T- time taken in sec.

2.5.7 Gel strength

Sample (50 g) was placed in a 100 ml graduated cylinder. Gelation was carried out by placing the formulations in a thermostat at 37 °C. The strength of the gel was determined by measuring the time taken by a weight of 35 g to sink 5 cm in the gel.³¹

2.5.8 In -Vitro Gelation Studies

The gelling capacity of the prepared system was evaluated. It was performed by placing a drop of system in vials containing 1 ml of phosphate buffer pH 7.4, freshly prepared and equilibrated at 37°C, and visually assessing the gel formation and noting the time for gelation as well as time taken for the gel formed to dissolve.³²

2.5.9 In -vitro diffusion profile

In vitro diffusion rate of the Oxiconazole from the gel system for the topical drug availability was determined by the diffusion process. 1 ml of the formulation was kept in the donor compartment over a dialysis membrane (molecular weight cut off: 12000, pore size: 2.4 nm) which was rinsed & soaked for the 24 hours in the diffusion medium. The donor compartment was immersed in the receptor compartment which was calibrated containing 20 ml of the Phosphate buffer pH 7.4. The beaker containing diffusion medium (receptor compartment) was maintained at 37±0.5°C with the constant stirring at 50 rpm using the magnetic stirrer. 2 ml aliquots were withdrawn from the diffusion medium every hour for the 8 hours & same quantity of fresh, diffusion medium was replaced for the amount withdrawn. The samples withdrawn was analysed spectrophotometrically at 231 nm for the Oxiconazole using (Jasco V630 Double beam UV-Visible spectrophotometer). The cumulative percent release is calculated for each time (in h) interval.³³

2.5.10 Stability Study

Optimized OXZ-N gel formulations underwent short-term accelerated stability evaluation in line with the International Conference on Harmonization (ICH) Q1 A R2 guidelines. OXZ-N gel formulation was filled in suitable, well-closed containers in a stability chamber at 25 ± 2 °C and RH 60 ± 5%. The formulation along with the gel was then assessed for changes in pH, Spradability and Drug release for three months.³⁴

3. RESULTS AND DISCUSSION

3.1 Optimization of OXZ -Loaded Niosomes (OXZ-Ns)

Table 1: Experimental runs of the Box–Behnken design (BBD) for the development and optimization of niosomal formulations, showing the observed responses: particle size (Y₁), zeta potential (Y₂), and entrapment efficiency (Y₃), as generated by Design-Expert® software.

Table 2: Results of regression analysis for the responses Y₁, Y₂, and Y₃.

Formulation code	Cholesterol (mg)	Temperature 80 (%)	Stirring speed (RPM)	Particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
NO1	10	1.5	2000	272.5	-18.3	81.4 ± 0.125
NO2	30	1.5	2000	287.4	-21.4	73.29 ± 0.325
NO3	30	2	1500	251.6	-25.3	75.83 ± 0.025
NO4	10	1	1500	278.7	-20.8	81.62 ± 0.004
NO5	10	1.5	1000	258.8	-17.2	71.65 ± 0.084
NO6	20	2	2000	267.4	-16.9	74.89 ± 0.001
NO7	20	1	2000	285.8	-20.6	80.46 ± 0.036
NO8	20	1.5	1500	264.9	-21.1	78.98 ± 0.051
NO9	10	2	1500	285.4	-15.8	78.04 ± 0.008
NO10	20	2	1000	224.7	-26.5	82.75 ± 0.003
NO11	20	1	1000	275.3	-19.6	75.43 ± 0.014
NO12	30	1	1500	278.6	-23.7	74.11 ± 0.235
NO13	30	1.5	1000	259.6	-22.8	80.39 ± 0.0036

Model	Response	R ²	Adjusted R ²	Predicted R ²	SD	% CV	p-Value	Linear model	Response (Y1)	0.5994	0.4659	0.3195	12.66	4.72	< 0.000
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							0.1
2FI model	Response(Y2)	0.8388	0.6776	0.5033	1.848	8.88	<0.0001
2FI model	Response(Y3)	0.8297	0.6594	0.5021	2.11	2.72	<0.0001

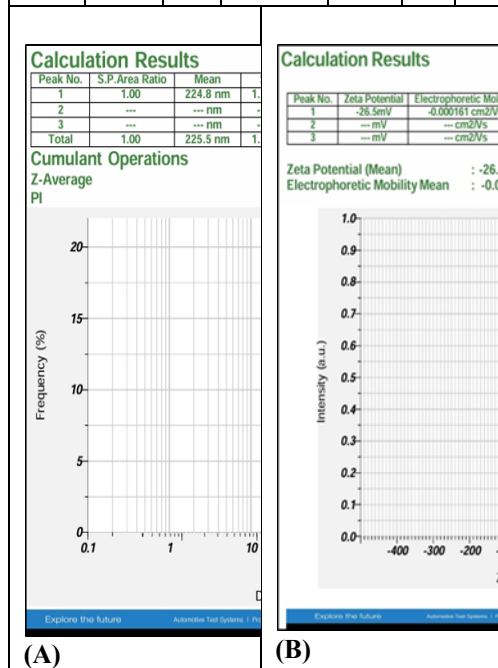


Figure 1: A- Particle size of NO10, B-Zeta potential of NO10

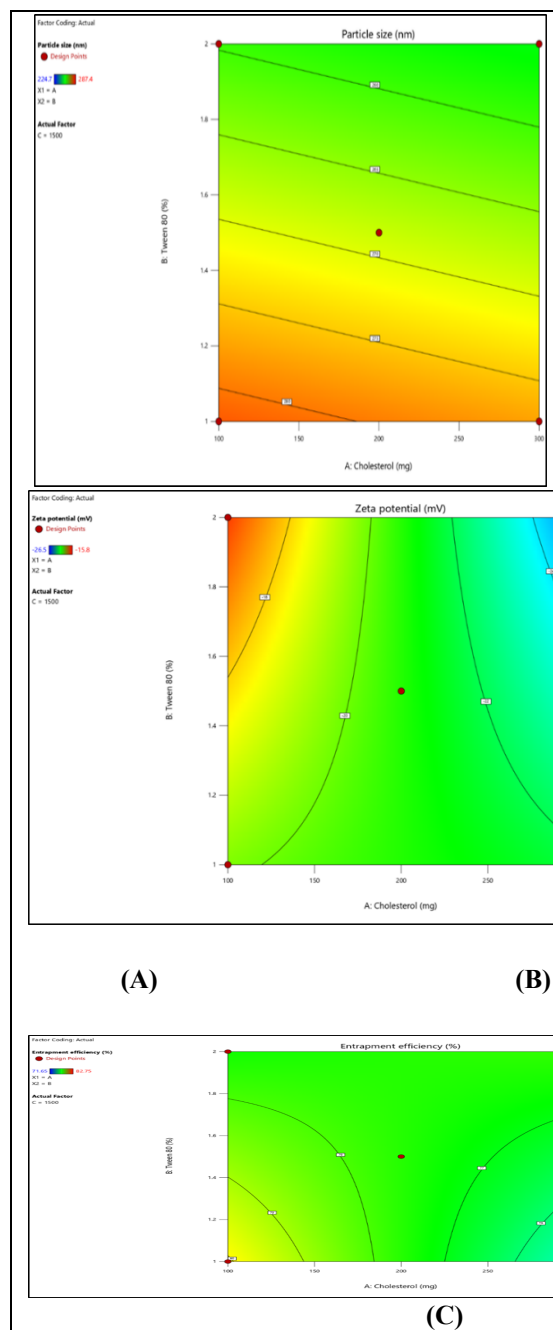
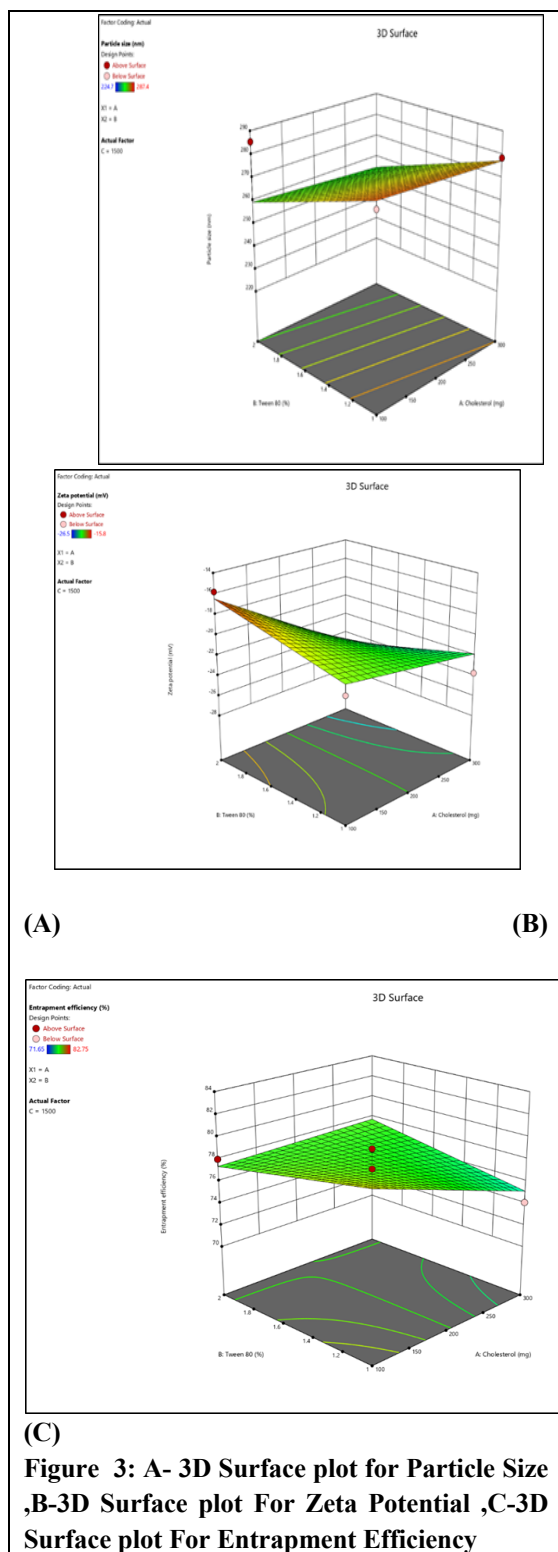


Figure 2: A- Counter plot for Particle Size ,B-Counter plot For Zeta Potential ,C-Counter Plot For Entrapment Efficiency



According to the polynomial equation obtained from the Box–Behnken design, the interaction effects of the independent variables on particle size (Y_1), Zeta Potential (Y_2) (and Entrapment efficiency (Y_3)) are represented by the following equation:

$$\text{Particle Size (nm)} = 268.52 - 2.28A - 11.17B + 11.83C$$

The coded equation shows that Tween 80 (B) has the greatest influence on particle size, followed by stirring speed (C) and cholesterol (A). The actual-

factor equation can be used to predict particle size using the original concentrations of cholesterol, Tween 80, and stirring speed. Overall, these equations are useful for predicting and optimizing particle size within the studied formulation range.

$$\text{Zeta Potential (mV)} = -20.77 - 2.64A + 0.0250B + 1.11C - 1.65AB + 0.6250AC + 2.65BC$$

The coded equation indicates that cholesterol (A), Tween 80 (B), and stirring speed (C), along with their interactions (AB, AC, and BC), influence the zeta potential. The actual-factor equation can be used to predict zeta potential using the original experimental units of the formulation variables.

$$\text{Entrapment Efficiency (\%)} = 77.60 - 1.14A - 0.0138B - 0.0225C + 1.33AB - 4.21AC - 3.22BC$$

The coded equation indicates that cholesterol (A), Tween 80 (B), and stirring speed (C) influence entrapment efficiency. The negative coefficients for A, B, and C indicate a decrease in entrapment efficiency with increasing factor levels. The AB interaction has a positive effect, whereas AC and BC interactions have negative effects. The equation can be used to predict entrapment efficiency at different factor levels.

3.1.1 Drug content

Table 3: Drug content of NO1-NO13

Formulation code	Drug content (%)
NO1	80.16 ± 0.12
NO2	83.62 ± 0.035
NO3	85.23 ± 0.025
NO4	76.67 ± 0.004
NO5	81.01 ± 0.041
NO6	84.60 ± 0.036
NO7	79.32 ± 0.045
NO8	80.73 ± 0.005
NO9	78.28 ± 0.002
NO10	86.42 ± 0.0142
NO11	78.09 ± 0.062
NO12	81.36 ± 0.054
NO13	82.52 ± 0.006

The drug content of all niosomal formulations (NO1–NO13) was within an acceptable range, indicating uniform drug distribution. NO10 showed the highest drug content (86.42%), followed by NO3 (85.23%) and NO6 (84.60%). Overall, the results confirmed good drug incorporation and reproducibility, with NO10 identified as the optimized formulation based on drug content.

3.1.2 In vitro diffusion study

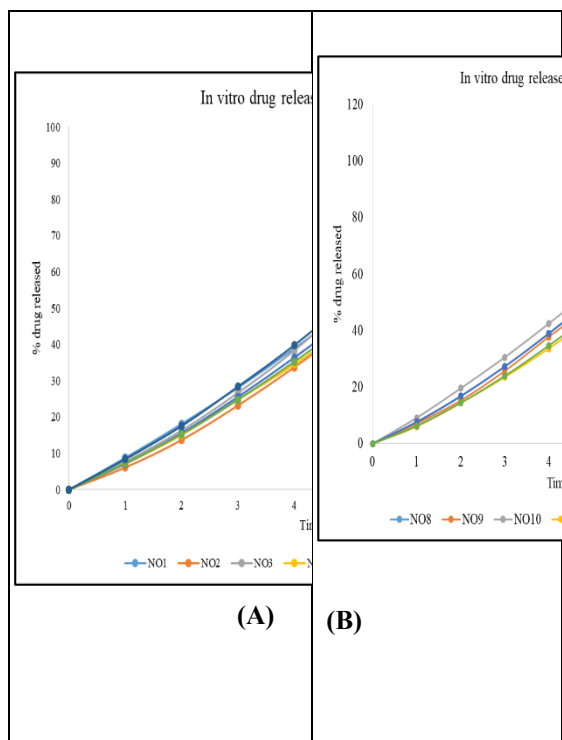


Figure 4: A-In vitro drug release of NO1-NO7, B-In vitro drug release of NO8-NO13

The in-vitro drug release study of formulations NO1–NO13 showed a gradual and sustained increase in drug release over the 8-hour period. All formulations exhibited controlled release behavior with cumulative drug release ranging from approximately 84–97% at 8 hours. Among the formulations, NO10 demonstrated the highest drug release (96.82%), followed by NO7 (94.84%) and NO1 (94.58%), indicating more efficient drug diffusion from these systems. In contrast, NO11 (84.63%) showed comparatively lower release, suggesting a slower drug release profile. Overall, the results indicate that the formulations successfully provided sustained and controlled drug release, with NO10 appearing to be the most promising formulation due to its higher cumulative release within the studied time period.

3.1.3 FTIR

Figure 5: FTIR Spectrum of OXZ: Physical Mixture (OXZ: Cholesterol: Tween 80) and NO10

The FTIR spectra of pure Oxiconazole, Oxiconazole physical mixture, and optimized niosomal formulation (NO10) were compared to evaluate drug–excipient compatibility. The characteristic spectral pattern of Oxiconazole was retained in the physical mixture, indicating the absence of major chemical interaction between the drug and formulation components. In the optimized NO10 formulation, the characteristic bands of Oxiconazole were largely retained, although some changes in intensity and slight shifts in the bands were

observed, which may be attributed to the incorporation of the drug into the niosomal system. No major disappearance or formation of new characteristic peaks was observed. Therefore, the FTIR study indicates good compatibility of Oxiconazole with the selected excipients and the absence of significant chemical interaction, supporting the successful formulation of Oxiconazole-loaded niosomes.

3.1.4 DSC

Figure 6: DSC thermogram of NO10

3.1.5 XRD

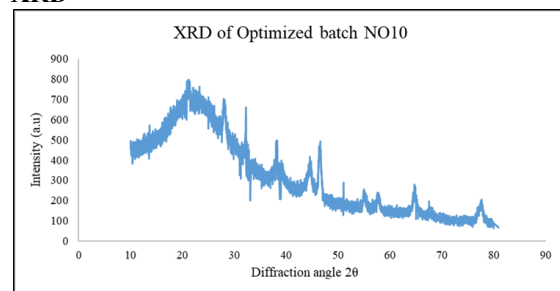


Figure 7: XRD of optimized batch NO10

The XRD pattern of the optimized niosomal batch NO10 shows a broad hump with a few low-intensity peaks, indicating a predominantly amorphous nature of the encapsulated drug. The absence of sharp, high-intensity crystalline peaks suggests that the drug is molecularly dispersed within the niosomal matrix. This amorphization can enhance drug solubility and dissolution rate, contributing to the sustained and improved release observed in the in vitro studies. Overall, the XRD results confirm successful encapsulation of the drug in a largely amorphous form, which is beneficial for enhanced bioavailability.

3.1.6 SEM

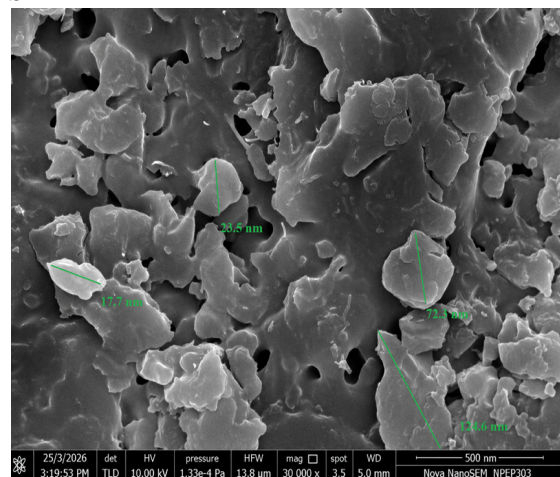


Figure 8: SEM of NO10

The SEM analysis of the optimized NO10 formulation confirmed the formation of nanosized particles with an irregular and rough surface morphology. The particles showed some degree of aggregation, with measured dimensions ranging from approximately 17.7 to 124.6 nm. The presence of nanoscale structures and the observed surface characteristics indicate successful development of the nanostructured formulation. Overall, the SEM findings support the suitability of NO10 as an optimized nanoformulation.

3.2 Optimization of Niosomal loaded Gel

Table 4: Experimental runs of the Box–Behnken design (BBD) for the development and optimization of Niosomal loaded Gel, showing the observed responses: pH (Y_1), Spreadability (Y_2), and Drug release (%) (Y_3), as generated by Design-Expert® software.

Table 5: Results of regression analysis for the responses Y₁, Y₂, and Y₃.

Formulation code	Carbopol 937 (%)	Tween 80 (%)	Stirring speed (rpm)	pH	Spreadability (gm·cm/se)	Drug release (%)
OG1	0.5	6	1500	4.98	6.52	86.02±0.0032
OG2	0.25	5	1000	4.86	5.97	87.93±0.000
OG3	0.5	5	1000	6.3	6.24	93.95±0.0035
OG4	0.375	4	2000	6.11	6.52	88.59±0.001
OG5	0.25	6	1500	5.37	6.14	81.87±0.0001
OG6	0.25	5	2000	6.23	6.42	90.56±0.0001
OG7	0.5	4	1500	5.89	6.22	84.76±0.0004
OG8	0.5	5	2000	5.5	7.14	83.55±0.0015
OG9	0.375	4	1000	4.96	6.64	94.96±0.0015
OG10	0.375	6	2000	4.98	7.41	85.21±0.0001
OG11	0.25	4	1500	5.12	5.3	89.64±0.0003
OG12	0.375	6	1000	5.36	5.92	88.25±0.0001
OG13	0.375	5	1500	6.22	6.85	91.45±0.0004

Model	Response	R ²	Adjusted R ²	Predicted R ²	SD	% CV	p-Value
Quadratic model	Response(Y1)	0.9947	0.9788	0.8452	0.0801	1.45	<0.001
Quadratic model	Response(Y2)	0.9641	0.8562	0.7564	0.2079	3.25	<0.001
Quadratic model	Response(Y3)	0.9894	0.9578	0.8752	0.0855	0.9132	<0.001

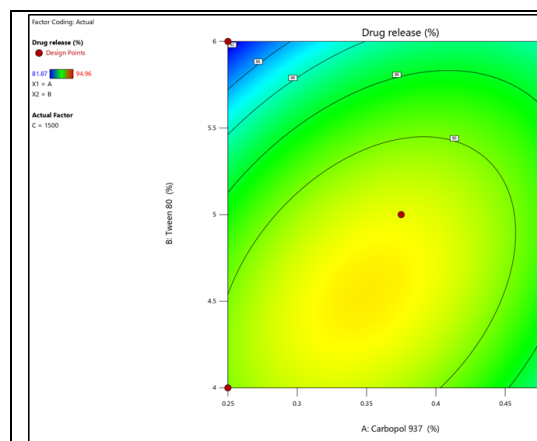
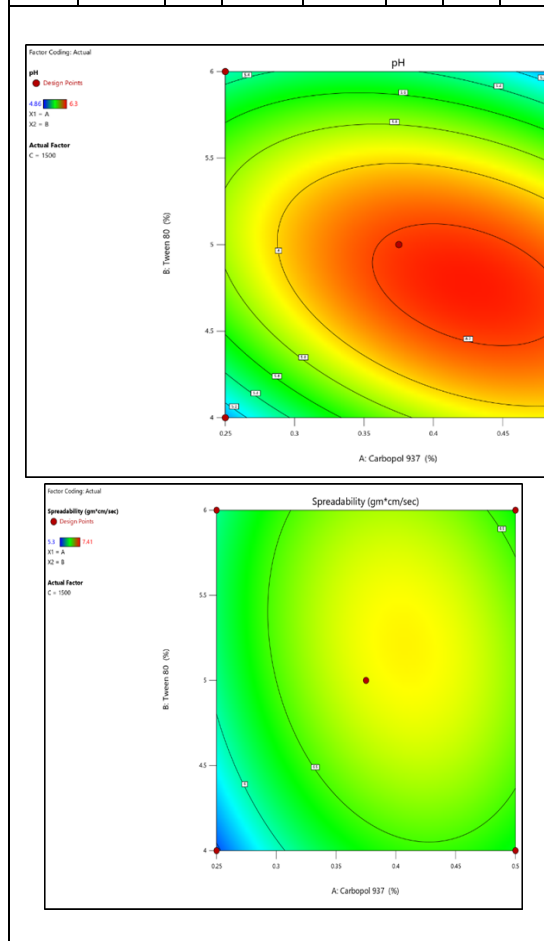
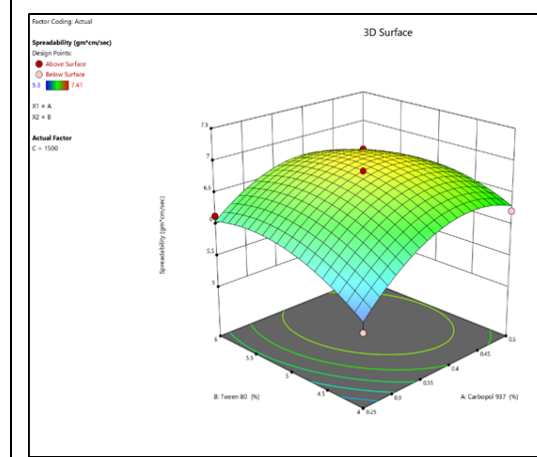
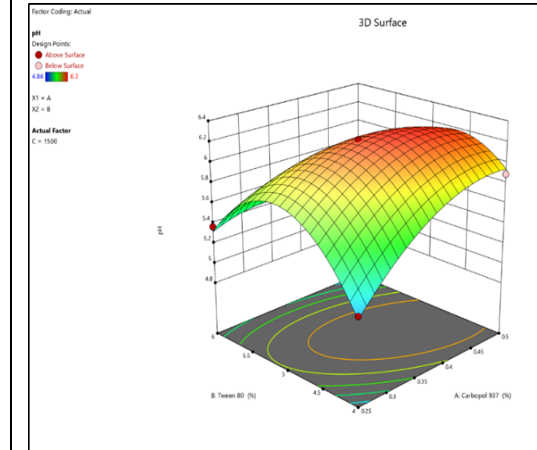


Figure 9: A- Counter plot for pH, B-Counter plot For Spradability, C-Counter Plot For In vitro diffusion (%)



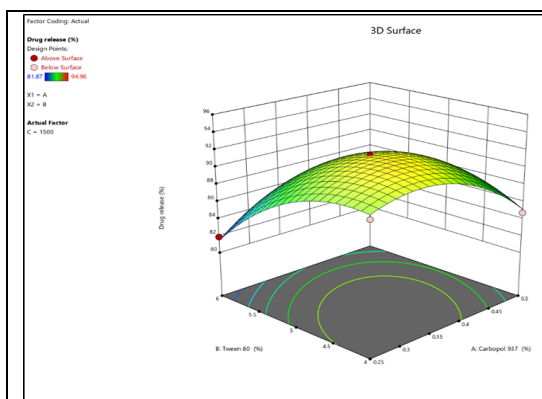


Figure 10: A- 3D Surface plot for pH, B-3D Surface plot For Spradability, C-3D Surface plot For In vitro diffusion (%)

According to the polynomial equation obtained from the Box–Behnken design, the interaction effects of the independent variables on pH (Y_1), Spradability (Y_2) and *In-vitro* Diffusion (Y_3) are represented by the following equation:

$$\text{pH} = 6.22 + 0.1362A - 0.1738B + 0.1675C - 0.2900AB - 0.5425AC - 0.3825BC - 0.2550A^2 - 0.6250B^2 - 0.2425C^2$$

The equation indicates that Carbopol 937 (A) and stirring speed (C) have positive effects on pH, whereas Tween 80 (B) has a negative effect. The interaction terms AB, AC, and BC show negative effects, indicating that their combined influence tends to decrease pH. The negative quadratic terms indicate a curved response within the studied factor ranges. The equation can be used to predict pH at different levels of the formulation variables.

$$\text{Spreadability} = 6.85 + 0.2862A + 0.1637B + 0.3400C - 0.1350AB + 0.1125AC + 0.4025BC - 0.4925A^2 - 0.3125B^2 + 0.0850C^2$$

The equation indicates that Carbopol 937 (A), Tween 80 (B), and stirring speed (C) have positive effects on spreadability. The AB interaction shows a negative effect, while AC and BC interactions show positive effects. The negative quadratic terms for A^2 and B^2 indicate a nonlinear effect of Carbopol 937 and Tween 80 on spreadability. Overall, the equation can be used to predict and optimize the spreadability of the formulation.

$$\text{Drug Release (\%)} = 91.45 - 0.2150A - 2.08B - 2.15C + 2.26AB - 3.26AC + 0.8325BC - 3.07A^2 - 2.81B^2 + 0.6137C^2$$

The equation indicates that Carbopol 937 (A), Tween 80 (B), and stirring speed (C) have negative individual effects on drug release. The AB and BC interactions show positive effects, whereas the AC interaction has a negative effect. The negative quadratic terms for A^2 and B^2 indicate a nonlinear effect on drug release. Overall, the equation can be used to predict and optimize drug release within the selected formulation range.

3.2.1 Visual evaluation of gel

Table 6: Visual evaluation of OG1-OG13

Formulation Code	Visual Appearance	Clarity	Color	Homogeneity
OG1	Smooth	Clear	Colorless	Excellent
OG2	Smooth	Clear	Colorless	Excellent
OG3	Smooth	Clear	Colorless	Excellent
OG4	Smooth	Clear	Colorless	Excellent
OG5	Smooth	Clear	Colorless	Excellent
OG6	Smooth	Clear	Colorless	Excellent
OG7	Smooth	Clear	Colorless	Excellent
OG8	Smooth	Clear	Colorless	Excellent
OG9	Smooth	Clear	Colorless	Excellent
OG10	Smooth	Clear	Colorless	Excellent
OG11	Smooth	Clear	Colorless	Excellent
OG12	Smooth	Clear	Colorless	Excellent
OG13	Smooth	Clear	Colorless	Excellent

All formulations (EG1–EG13) showed a smooth, clear, colorless, and homogeneous appearance, indicating good physical stability and uniformity. The viscosity ranged from 60 to 554 cP, demonstrating the effect of formulation composition on consistency. Overall, all formulations were visually acceptable and suitable for further evaluation.

3.2.2 Extrudability

Table 7: Extrudability of OG1-OG13

Formulation Code	Extrudability (%)	Conclusion
OG1	91.2	Excellent
OG2	86.5	Good
OG3	93.7	Excellent
OG4	92.0	Excellent
OG5	87.1	Good
OG6	86.9	Good
OG7	95.4	Excellent
OG8	88.6	Good

OG9	96.2	Excellent
OG10	92.8	Excellent
OG11	93.4	Excellent
OG12	89.2	Good
OG13	90.0	Good

The extrudability of all thirteen topical gel formulations (OG1–OG13) ranged from 86.5% to 96.2%, indicating that the gels can be easily expressed from a tube or container. Formulations OG7 (95.4%) and OG9 (96.2%) showed the highest extrudability, while OG2 (86.9%) and OG6 (86.9%) had the lowest, though still within an acceptable range. Overall, the results confirm that the formulations possess good mechanical properties, ensuring ease of application and patient compliance for topical use.

3.2.3 Viscosity

Table 8: Viscosity of OG1-OG13

Formulation Code	Viscosity (cP)
OG1	250
OG2	484
OG3	680
OG4	164
OG5	474
OG6	490
OG7	742
OG8	690
OG9	246
OG10	944
OG11	532
OG12	344
OG13	270

The viscosity of the 13 oxiconazole batches ranged from 164 cP (OG4) to 944 cP (OG10), indicating variation due to formulation composition. OG10 showed the highest viscosity, while OG4 showed the lowest. Most batches exhibited moderate viscosity, suggesting acceptable spreadability and consistency for topical application.

3.2.4 Gel strength

Table 9: Gel Strength of OG1-OG13

Formulation Code	Gel Strength (sec)
OG1	32.8
OG2	49.8
OG3	42.7

OG4	33.7
OG5	47
OG6	36.8
OG7	42
OG8	35.5
OG9	49.6
OG10	44.9
OG11	43.5
OG12	36.8
OG13	41

All formulations (OG1–OG13) showed acceptable gel strength, indicating adequate firmness and structural integrity. OG2 (49.8 sec) and OG9 (49.6 sec) exhibited the highest gel strength. Considering the overall evaluation parameters, OG9 was selected as the optimized formulation, providing a suitable balance between firmness and ease of application.

3.2.5 In –Vitro Gelation

Table 10: In Vitro Gelation Studies of OG1-OG13

Formulation Code	Gelling capacity
OG1	++
OG2	++
OG3	+
OG4	+++
OG5	++
OG6	+
OG7	++
OG8	+
OG9	+++
OG10	++
OG11	++
OG12	+
OG13	+

- No gelation, + Gelation after few minutes and lasts for few hours, ++ Gelation immediate and lasts for few hours, +++ Direct gelation, and last for extended time

3.2.6 In vitro diffusion profile

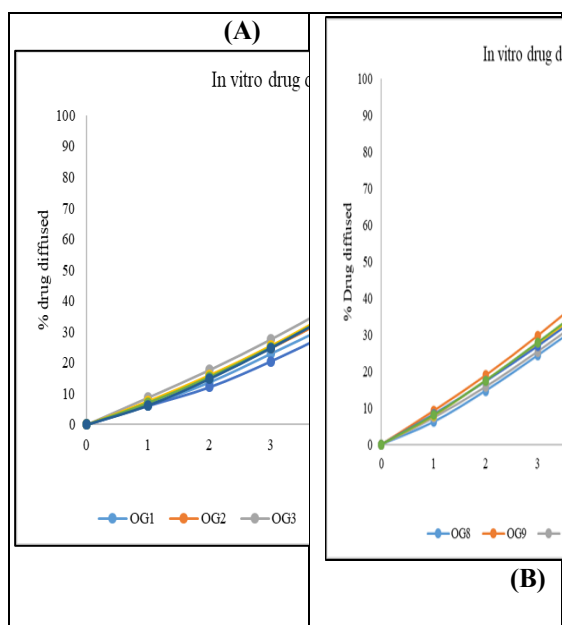


Figure 11: A-In vitro drug diffusion of OG1-OG7, B-In vitro drug diffusion of OG8-OG13

All formulations (OG1–OG13) showed a time-dependent increase in cumulative drug release over 8 hours, indicating a sustained and controlled release pattern from the topical gel matrix. The release profiles were uniform, suggesting consistent drug diffusion behavior among batches. Among all formulations, OG9 exhibited the highest cumulative drug release (94.96%) at 8 hours, demonstrating superior release performance. The enhanced release from OG9 may be attributed to its optimized gel structure, facilitating efficient drug diffusion. Overall, based on maximum cumulative drug release and controlled release behavior, OG9 was selected as the optimized formulation for topical application.

3.2.7 Stability Study

Table 11: Stability Study of Optimized Niosomal Gel Formulation (OG9)

Storage Period	pH	Spreadability (g·cm/s)	Drug Release (%)
Initial (0 Month)	4.98	7.41	85.21±0.0001
1 Month	4.97	7.38	85.18±0.0036
2 Months	4.95	7.34	85.14±0.047
3 Months	4.91	7.29	85.11±0.0089

The optimized niosomal gel formulation OG9 showed only slight changes in pH, spreadability, and drug release during the 3-month stability study. The pH decreased from 4.98 to 4.91, while spreadability decreased from 7.41 to 7.29 g·cm/s. Drug release showed only a minor reduction from 85.21% to

85.11%. Overall, the formulation maintained its physicochemical properties, indicating good stability during storage.

CONCLUSION

The present study successfully developed and optimized an oxiconazole-loaded niosomal gel using the ether injection method and Box–Behnken design. The optimized niosomes (NO10) showed a particle size of 224.7 nm, zeta potential of -26.5 mV, and 96.82% drug release at 8 h. The optimized niosomal gel (OG9) exhibited suitable pH, spreadability, extrudability, gel strength, and 94.96% drug release at 8 h. The formulation showed satisfactory stability over three months. Overall, the developed niosomal gel may be a promising topical delivery system for sustained and effective delivery of oxiconazole.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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