

RESEARCH PAPER

Development, Optimization and Characterization of Ketoconazole-Loaded Niosomal Gel for Enhanced Topical Antifungal Drug Delivery

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Abstract

Ketoconazole is the widely used drug fungal infection on the upper part of skin but conventional topical formulations cannot permeate the skin easily and stay for a short period of time on the skin. The aim of this study is to overcome the limitations of conventional drug delivery system. Ketoconazole-loaded niosomes were prepared by the thin-film hydration technique using Span 60 and cholesterol, and then optimized by using Design of Experiments (DOE). Then the final formulation was added into Carbopol 934 gel. The optimized formulation was evaluated for vesicle size, zeta potential, entrapment efficiency, drug content, pH, viscosity, spreadability, in-vitro drug release, release kinetics, and antifungal activity against *Candida albicans*. The final results shown by gel are a pH of 5.9, viscosity of 4000 cps, and spreadability of 6 g-cm/s, with gradual drug release and a 16 mm zone of inhibition against *C. albicans*. Overall, the developed ketoconazole-loaded niosomal gel showed improved topical delivery and treatment to superficial fungal infections.

Keywords: Ketoconazole; Niosomes; Topical drug delivery; Thin-film hydration; Carbopol 934; Design of Experiments (DOE); Sustained drug release; Antifungal activity

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INTRODUCTION

Superficial fungal infections of the skin are common problems caused mainly by dermatophytes, *Candida* species, and *Malassezia* spp. These infections affect the skin, hair, and nails and also cause itching, redness, scaling, inflammation, and other problems to the skin sometimes. Dandruff, seborrheic dermatitis, tinea infections, cutaneous candidiasis, and pityriasis versicolor are some common examples. These infections do not cause any life-threatening harm to the patient but they affect their day-to-day comfort.

Topical antifungal medicines are commonly used for localized skin infections because they deliver the drug directly to the affected area and reduce systemic exposure of the drug. Conventional topical formulations have some problems such as poor penetration through the stratum corneum, limited drug retention, and removal of the formulation by washing, sweating, or friction. These limitations sometimes require frequent application and can affect patient compliance.

Ketoconazole is a broad-spectrum antifungal drug of imidazole class widely used for the treatment of several superficial fungal skin infections. It acts by inhibiting fungal ergosterol synthesis, which affects the integrity of the fungal cell membrane. However, its poor aqueous solubility and limited penetration through the skin can limit its effectiveness when used in conventional topical formulations.

Niosomes are vesicular drug delivery systems made mainly from non-ionic surfactants and cholesterol. They improve drug entrapment, protect the drug from GI fluids, provide controlled release, and increase drug retention at the skin surface. In this study Span 60 and cholesterol were used because of their ability to form stable vesicles and improve incorporation of drug. The niosomal dispersion was further incorporated into Carbopol 934

gel to obtain a niosomal gel with suitable viscosity and spreadability.

Design of Experiments (DoE) was used to optimize 3 formulation variables. Response Surface Methodology helped to study the effect of the selected variables and their interactions to identify the optimised formulation with desirable characteristics.

Therefore, the present study aimed to develop and optimize a ketoconazole-loaded niosomal gel using the thin-film hydration method and DoE. The optimized formulation was evaluated for vesicle size, zeta potential, entrapment efficiency, drug content, physicochemical properties, *in vitro* drug release, release kinetics, and antifungal activity to determine its potential as an improved topical drug delivery system.

MATERIALS AND METHODS

Materials

Ketoconazole was Purchased from S. M. Pharmaceuticals. Span 60 (Sorbitan monostearate), Tween 80 (Polysorbate 80), cholesterol, Carbopol 934, polyethylene glycol (PEG 400), methanol, chloroform, sodium hydroxide, and other analytical-grade chemicals and reagents were given by SVERI's College of Pharmacy. Double-distilled water was used throughout the study. All chemicals and solvents used were of analytical reagent (AR) grade and were used without further purification.

Experimental Design

The formulation was optimised using Response Surface Methodology (RSM) with an I-optimal experimental design generated using Design-Expert® software (Version 12).

Three Formulation variables Were selected and entrapment efficiency was optimised.

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Independent variables	Coded levels		
X1	-1	0	+1
X2	-1	0	+1
X3	-1	0	+1

Table no. 01 Code levels of Design Expert software

Formulation	X1 Cholesterol (mg)	X2 Tween 80 (mg)	X3 Span 60 (mg)
1	100	80	300
2	200	80	300
3	100	160	300
4	200	160	300
5	100	120	150
6	200	120	150
7	100	120	450
8	200	120	450
9	150	80	150
10	150	160	150
11	150	80	450
12	150	160	450
13	150	120	300
14	150	120	300
15	150	120	300

Table no. 02 Box-Behnken Design of 3³ factorial

Preparation of Ketoconazole-Loaded Niosomes

Ketoconazole loaded niosomes were prepared by the thin-film hydration method. Span 60, Tween 80, cholesterol, and ketoconazole (40 mg) were dissolved in chloroform: methanol (2:1, v/v). The solution was evaporated at $58 \pm 2^\circ\text{C}$ and 110 rpm using a rotary flash evaporator to form a thin film. After that, the film was kept under vacuum for 30 min and then hydrated with 25 mL phosphate buffer (pH 7.4) at $55 \pm 2^\circ\text{C}$ for 30 min to get a niosomal dispersion. The obtained Niosomal dispersion was sonicated using a Labman Pro-650 probe sonicator at 50% amplitude for 7 min with 30 s ON/15 s OFF cycles. The prepared niosomal dispersion was stored at 4°C .

Optimization of Niosomal Formulation

The formulations were optimized using Design-Expert® software based on their entrapment efficiency. The experimental data from 15 formulations were fitted to a model. Response surface plots, contour plots, and desirability analysis were used to identify the optimized formulation, which was then selected for gel preparation.

Preparation of Niosomal Gel

Carbopol 934 (1.65 g) was dispersed in distilled water and kept overnight to hydrate.

PEG 400 (2.5 g) and propyl paraben (0.1 g) were then added. The optimized niosomal dispersion was added gradually with continuous stirring. The pH was adjusted to 6.0 ± 0.2 using 0.2 N sodium hydroxide. The prepared gel was stored at 4°C .

Characterization of Niosomes

Vesicle size: The drop of niosomal dispersion was examined using an electronic microscope. The sizes of individual vesicles were measured from the captured images and expressed in nanometres.

Zeta potential: Zeta potential was measured after dilution of the niosomal dispersion. The obtained values were used to determine the surface charge which affected physical stability of the vesicles.

Entrapment efficiency: The niosomal dispersion was centrifuged at 15,000 rpm for 40 min at 2°C . The free drug present in the supernatant was measured at 243 nm using a UV-Visible spectrophotometer. Entrapment efficiency was calculated from the total and free drug amounts.

Drug content: An accurately weighed sample was dissolved in methanol and filtered through Whatman filter paper No. 1. After suitable dilution, the drug concentration was measured at 243 nm using the ketoconazole calibration curve.

Evaluation of Niosomal Gel

The appearance, texture, homogeneity, and phase separation of prepared gel was examined. The pH of 1 gram gel was determined by dispersing it in 10 ML distilled water. Viscosity was measured using a Brookfield viscometer. Spreadability was determined by the glass-slide method using 0.5 g of gel and a 20 g weight.

In Vitro Drug Release Study

Franz diffusion apparatus was used to study the release of drug. The Phosphate buffer having pH 7.4 was used as a medium for drug dissolution which was maintained at 37°C degree Celsius. With 100 rpm stirring on magnetic stirrer. Samples were

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withdrawn at specific intervals and replaced with an equal volume of fresh buffer. The samples were analysed at 243 nm, and cumulative drug release was calculated.

Drug Release Kinetics

The release data was fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The R^2 values obtained from each model were compared to determine the model that best described the drug-release behaviour.

Antifungal Activity

Agar-well diffusion method was used to determine the antifungal activity against *Candida albicans*. Wells of 8 mm diameter were prepared and Dextrose Agar medium was added then filled with the optimized niosomal gel, conventional ketoconazole gel, and blank gel. The plates were placed in incubation chamber at $37 \pm 1^\circ\text{C}$ for 48 h. The zones of inhibition were measured in mm and compared among the formulations

RESULTS AND DISCUSSION

Evaluation of drug and niosomes

1. Physical Evaluation

Parameter	Observation
Colour	White to slightly cream
Appearance	Crystalline powder
Physical State	Solid
Odour	Odourless
Texture	Fine crystalline powder

Table no. 03 Physical evaluation of ketoconazole

2. Solubility Study

Solvent	Solubility
Distilled Water	Practically insoluble
Methanol	Freely soluble
Chloroform	Soluble
Ethanol	Slightly soluble
Phosphate Buffer (pH 7.4)	Slightly soluble

Table no. 04 Solubilization properties of ketoconazole

3. Vesicle Size

The vesicle size of the optimized ketoconazole-loaded niosomes was determined using an electronic microscope and average vesicle size was found to be **81.71 nm**.

4. Zeta Potential

The optimized ketoconazole-loaded niosomes exhibited a zeta potential of -18.7 to -37.4 mV Which indicates that they have negatively charged vesicle surface. The ± 30 mV generally suggest good colloidal stability. The observed charge is attributed to surfactant orientation and hydroxyl ion adsorption, providing electrostatic repulsion and reducing vesicle aggregation.

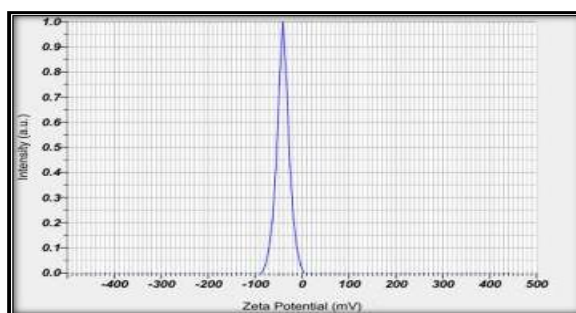


Fig.no. 1 Zeta Potential of Ketoconazole loaded Niosomes

Parameter	Observed Result
Zeta Potential	-18.7 to -37.4 mV

Table no. 05 Zeta potential of niosomes

5. ENTRAPMENT EFFICIENCY

The entrapment efficiency of ketoconazole loaded niosomes was 83%, indicating effective drug incorporation. This happened due to ketoconazole's lipophilic nature and its affinity with the Span 60-cholesterol bilayer. The low HLB and high phase transition temperature of Span 60, along with cholesterol stabilization reduced the drug leakage.

Evaluation of Ketoconazole-Loaded Niosomal Gel

1. Physical Appearance and Homogeneity

Parameter	Observation
Colour	White to off-white
Appearance	Smooth and homogeneous
Consistency	Uniform
Grittiness	Absent
Phase Separation	Absent
Homogeneity	Good

Table no. 06 Physical appearance of ketoconazole niosomal gel

2. pH Determination

The pH of the optimized ketoconazole-loaded niosomal gel was measured using a digital pH meter.

Parameter	Observed Result
pH	5.9

Table no. 07 pH of Ketoconazole Niosomal gel

3. Viscosity

Viscosity was measured using a Brookfield Viscometer.

Parameter	Observed Result
Viscosity	5887 cps

Table no. 08 Viscosity of Ketoconazole Niosomal gel

4. Spreadability

Spreadability was determined by the glass slide method.

Parameter	Observed Result
Spreadability	6 g·cm/sec

Table no. 09 Spreadability of Ketoconazole Niosomal gel

5. Antifungal Activity

The antifungal activity of the optimized ketoconazole-loaded niosomal gel was evaluated against *Candida albicans*. The niosomal gel showed a 16 mm zone of inhibition, and the conventional ketoconazole gel showed a 13 mm zone of inhibition.

6. DRUG CONTENT

The optimized ketoconazole-loaded niosomes showed a drug content of 87%, which means minimal drug lost during preparation. This high value suggests efficient drug retention and uniform distribution within the niosomal dispersion. The result also showed the suitability and reproducibility of the thin film hydration method for manufacturing of ketoconazole niosomal gel.

The blank gel did not show any inhibition, which clearly shows that the gel base had no antifungal effect on its own.

The better antifungal activity of the niosomal gel is due to its small vesicle size (81.7133 nm) and good entrapment efficiency (83%). The small niosomes provide better contact with the fungal

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cells and helps to improve the delivery of ketoconazole to the infected area.

Overall, the results showed that the optimized ketoconazole-loaded niosomal gel had better antifungal activity than the

conventional ketoconazole gel. The improved activity suggests that the niosomal system can enhance local delivery and its useful for treating superficial fungal infections.

Formulation	Zone of Inhibition (cm)	Zone of Inhibition (mm)
Blank Gel	0.0	0
Conventional Ketoconazole Gel	1.3	13
Ketoconazole-Loaded Niosomal Gel	1.6	16

Table no. 10 Spreadability of Ketoconazole Niosomal gel



Fig.no. 2 Petri plates of microbial test

6. Response Surface Analysis

The 3D response surface graph shows how cholesterol (A) and Tween 80 (B) affect the entrapment efficiency (%) of the ketoconazole-loaded niosomes. The vertical axis represents the entrapment efficiency, while the two horizontal axes show the amounts of cholesterol and Tween 80 used in the formulation. From the graph, it can be seen that the entrapment efficiency changes with different amounts of cholesterol and Tween 80. When the levels of these ingredients are increased towards the middle range, the entrapment efficiency also increases. The highest response is seen near the central region of the graph, where the entrapment efficiency reaches approximately 84–85%. However, further changes in the concentration of cholesterol or Tween 80 do not continue to increase the entrapment efficiency. At higher or lower levels, the response decreases slightly. This shows that both ingredients

need to be present in a suitable proportion to obtain better drug entrapment. The curved shape of the surface also indicates that cholesterol and Tween 80 have a combined effect on entrapment efficiency. Overall, the graph suggests that an appropriate combination of both components can produce higher entrapment of ketoconazole in the niosomal formulation. Thus, the response surface is helpful in identifying the optimum formulation conditions for achieving good entrapment efficiency. Y_1 (Entrapment Efficacy) = $3.47577 + 0.0284371 * \text{Cholesterol} + 0.0383626 * \text{Tween 80} + 0.00712056 * \text{Span 60} + -3.53634e-05 * \text{Cholesterol} * \text{Tween 80} + 2.05086e-06 * \text{Cholesterol} * \text{Span 60} + -4.80956e-06 * \text{Tween 80} * \text{Span 60} + 7.57471e-05 * \text{Cholesterol}^2 + -0.000126553 * \text{Tween 80}^2 + -9.05572e-06 * \text{Span 60}^2$

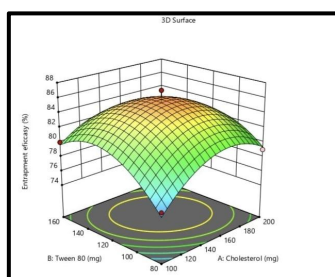


Fig.no. 3 RSM of Entrapment efficiency

7. In Vitro Drug Release Study

The optimized ketoconazole-loaded niosomal gel showed sustained drug release, increasing from 14.77% at 30 minutes to 75.69% at 240 minute.

Time (min)	Absorbance	Drug Concentration (µg/mL)	Cumulative Drug Permeated	Cumulative Drug Release (%)

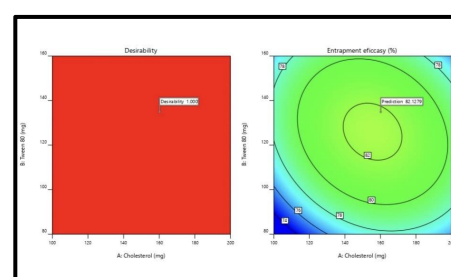


Fig.no. 4 Contour Plot and Desirability Plot for Optimization of Entrapment Efficiency

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			(mg)	
0	0.000	0.00	0.000	0.00
30	0.032	24.62	1.477	14.77
60	0.027	20.77	2.723	27.23
120	0.029	22.31	4.062	40.62
180	0.034	26.15	5.631	56.31
240	0.042	32.31	7.569	75.69

Table no. 11 In vitro Drug Release Data of Ketoconazole Niosomal gel

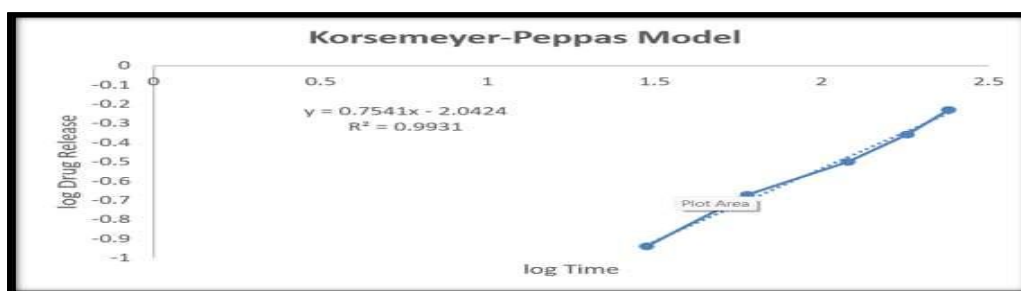


Fig.no. 5 Invitro release of ketoconazole From Niosomal gel

The optimized gel showed sustained drug release, with approximately 80% of ketoconazole released over 240 min. Release kinetic analysis showed that the Korsmeyer–Peppas model ($R^2 = 0.9931$) best described the release profile, suggesting that drug release was very good by diffusion from the niosomal bilayer and gel matrix.

8. Stability study:

The optimized ketoconazole-loaded niosomal gel was subjected to stability studies under long-term and accelerated

conditions. The formulation was stored at $25 \pm 2^\circ\text{C}/60 \pm 5\%$ RH and $40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH for 3 months in its final container. Samples were withdrawn at specific intervals and evaluated for physical appearance, pH, viscosity, spreadability, vesicle size, zeta potential, entrapment efficiency, drug content, and in-vitro drug release. The results obtained during storage were compared with the initial values to find out the stability of the formulation.

Parameters	Initial	1 month	2 months	3 months
Appearance	Smooth & homogeneous	Smooth & homogeneous	Smooth & homogeneous	Smooth & homogeneous
pH	5.9	5.88	5.86	5.83
Viscosity cps	5887	5860	5825	5791
Spreadability g.cm/sec	6.00	7.00	8.00	9.00
Drug content %	87	86.8	86.7	86.7

Table no. 12 Stability data of 3 months

DISCUSSION

The present study successfully developed a ketoconazole-loaded niosomal gel using the thin-film hydration method and Carbopol 934 as the gel base. The optimized formulation showed a vesicle size of 81.71 nm, 83% entrapment efficiency, and 94% drug

content, which indicated successful drug incorporation and formation of nanosized vesicles.

The prepared gel was smooth and homogeneous, with a pH of 5.9, viscosity of 5887 cps, and spreadability of 6 g·cm/s, indicating suitable properties for topical application. The formulation showed

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gradual drug release, with 75.69% release at 240 min. Among all the tested kinetic models, the Korsmeyer–Peppas model showed the highest R^2 (0.9931).

The niosomal gel also showed better antifungal activity against *Candida albicans*, by producing a 16 mm zone of inhibition compared with 13 mm for conventional ketoconazole gel, while the blank gel showed no inhibition.

Overall, the developed niosomal gel showed good formulation properties, sustained drug release, and improved antifungal activity, indicating its potential as a topical delivery system for ketoconazole.

CONCLUSION

The study concludes that ketoconazole-loaded niosomal gel is a promising approach for topical antifungal drug delivery. The niosomal system helps to overcome some limitations of conventional topical formulations by improving drug incorporation and providing controlled delivery at the skin surface. The addition of niosomal dispersion into Carbopol 934 further provides a convenient dosage form which also gives cooling sensation due to the water present in it. Overall, the developed system has potential for the management of superficial fungal infections, although further stability, ex vivo, in vivo, and clinical studies are needed before practical application.

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