

“VESICULAR INNOVATION IN TOPICAL ANTIFUNGAL DELIVERY: DEVELOPMENT OF KETOCONAZOLE-LOADED UFASOMAL GEL”

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Abstract:

Ufasomes are unsaturated fatty acid-based vesicular carriers that have potential for topical drug delivery by improving drug incorporation and providing controlled drug release. Ketoconazole is a broad-spectrum antifungal drug widely used for the treatment of superficial fungal infections; however, its poor aqueous solubility may limit its effective topical delivery. The present study aimed to formulate and characterize ketoconazole-loaded ufasomes and incorporate the optimized vesicular formulation into a topical gel for improved antifungal delivery. Ketoconazole-loaded ufasomes were prepared using oleic acid, Tween 80, cholesterol, and vitamin E oil by thin-film hydration (TFH) and alcohol addition methods. A Central Composite Design was employed to optimize the formulation by varying oleic acid and Tween 80 concentrations, with particle size and entrapment efficiency as the responses. The optimized formulation prepared by thin film hydration contained 50 mg ketoconazole, 24.41 mg oleic acid, 80 mg Tween 80, 10 mg cholesterol, and one drop of vitamin E oil, and exhibited a particle size of 202.5 nm and an entrapment efficiency of 84.76%. The gel prepared using Carbopol 934 using the optimized ufasomal formulation showed a pH of 5.5, viscosity of 5842 cP, and drug content of 96.52%. In-vitro drug release studies demonstrated sustained release over 24 h, with 85.00% release from the TFH ufasomal gel. The formulation also exhibited antifungal activity against *Aspergillus niger*, producing a zone of inhibition of 3.4 ± 0.2 cm. Overall, the findings indicate that ketoconazole-loaded ufasomes can be successfully incorporated into a topical gel and may provide sustained drug release with satisfactory antifungal activity, suggesting their potential as a vesicular approach for topical ketoconazole delivery.

Keywords: Ketoconazole; ufasomes; oleic acid; Tween 80; topical gel; central composite design; antifungal activity; controlled release.

1. Introduction:

Fungal infections are common infectious disorders that primarily affect the skin, hair, and nails. Superficial fungal infections are particularly prevalent under warm and humid conditions and may cause significant discomfort and impairment of quality of life.¹ Topical therapy is commonly preferred for localized fungal infections because it provides direct drug delivery to the affected site while minimizing systemic exposure. However, effective topical drug delivery remains challenging because the skin acts as a highly efficient barrier to drug permeation.^{2,3} The stratum corneum, consisting of corneocytes embedded within a lipid-rich matrix, represents the principal barrier to the penetration of many therapeutic agents.⁴ The physicochemical properties of the drug and the characteristics of the formulation strongly influence its penetration and retention within the skin. Conventional topical dosage forms such as creams and gels may therefore have limitations in achieving adequate drug penetration and prolonged drug availability at the target site.^{5,6} Various advanced drug delivery systems, including nanocarriers and vesicular systems, have been investigated to overcome these limitations and improve dermal and transdermal drug delivery.⁷ Ketoconazole is a broad-spectrum imidazole antifungal agent widely used for the treatment of superficial fungal infections. However, its limited aqueous solubility can affect drug dissolution and availability from conventional formulations. Therefore, the development of an appropriate carrier system capable of

improving drug localization, penetration, and release at the site of infection may enhance the effectiveness of topical ketoconazole therapy.

Among the different vesicular systems, ufasomes are vesicular carriers composed primarily of unsaturated fatty acids and have demonstrated their potential as drug delivery carriers.⁸ Ufasomes commonly utilize unsaturated fatty acids such as oleic acid and possess amphiphilic characteristics that facilitate the formation of vesicular structures under appropriate physicochemical conditions.^{9,10}

The presence of unsaturated fatty acids makes ufasomes particularly attractive for topical and transdermal drug delivery. Fatty acid-based vesicles can interact with the lipid components of the stratum corneum and may enhance drug penetration through the skin.^{11,12} Their vesicular structure also provides the potential for drug encapsulation and controlled release. Several studies have demonstrated the application of ufasomes for dermal delivery of drugs, including dexamethasone, methotrexate, itraconazole, albendazole, terbinafine, clotrimazole, and other therapeutic agents.¹³⁻¹⁸ In particular, itraconazole-loaded ufasomes and oleic acid vesicles have demonstrated potential for topical antifungal delivery, supporting the application of fatty-acid-based vesicular systems in antifungal therapy.¹⁴

The characteristics of ufasomes are influenced by several formulation and processing variables, including the type and concentration of fatty acid, surfactant concentration, cholesterol content, pH, and preparation method. These variables can affect vesicle size, surface charge, entrapment efficiency, stability, and drug-release characteristics.^{9,10} Therefore, systematic optimization of formulation variables is important for obtaining ufasomes with desirable physicochemical and drug-delivery properties.

In the present study, ketoconazole-loaded ufasomes were developed for topical antifungal delivery and subsequently incorporated into a gel base. Different formulation variables and preparation approaches were investigated, and the prepared ufasomes were characterized for particle size, zeta potential, entrapment efficiency, and in-vitro drug release. A systematic formulation optimization approach was employed to identify suitable formulation conditions based on the selected critical responses. The optimized ketoconazole-loaded ufasomal formulation was subsequently incorporated into a topical gel and evaluated for its physicochemical properties and in-vitro antifungal activity. The developed formulation was compared with conventional ketoconazole formulations to assess its potential as an improved topical delivery system.

2. Materials and Methodology

2.1 Materials

Ketoconazole, used as the active pharmaceutical ingredient, was obtained from Cipla Ltd., Bengaluru. Oleic acid, used as an unsaturated fatty acid and vesicle-forming component, was procured from S.D. Fine Chemicals Ltd., Mumbai. Tween 80, used as a non-ionic surfactant, was obtained from Ultra Pure-Lab Chem Industries LLP, India. Cholesterol, used as a stabilizing agent, was procured from Avra Synthesis Pvt. Ltd., Hyderabad. Vitamin E oil, used as an antioxidant, was obtained from DeveHerbes, New Delhi. Chloroform, used as an organic solvent, was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, while methanol, used as a cosolvent, was obtained from Nice Chemicals Pvt. Ltd. Ethanol, used as a cosolvent, was obtained from Trault Bioenergy Ltd. Carbopol 934, used as a gelling agent, was procured from Central Drug House (P) Ltd., New Delhi. Triethanolamine, used as a neutralizing agent, was obtained from S.D. Fine Chemicals Ltd., Mumbai. All other chemicals and reagents used in the study were of analytical grade.

2.1 Preparation of Calibration Curve

A primary stock solution of ketoconazole (100 µg/mL) was prepared by accurately weighing 10 mg of the drug and dissolving it in a suitable solvent, followed by making up the volume to 100 mL. The maximum absorption wavelength (λ_{max}) of ketoconazole was determined to be 203 nm using a UV–Visible spectrophotometer. A secondary stock solution was prepared by transferring 1 mL of the primary stock solution into a 10 mL volumetric flask and diluting with phosphate buffer (pH 5.5) to obtain a concentration of 10 µg/mL. Further dilutions were prepared to obtain working concentrations in the range of 1–5 µg/mL. The absorbance of each solution was measured at 203 nm, and a calibration curve was constructed by plotting absorbance against the corresponding concentration of ketoconazole.

2.3 Preparation of ketoconazole-loaded ufasomes^{15,19–25}

- For TFH (thin film hydration), ketoconazole, oleic acid, Tween 80, cholesterol and vitamin E oil were dissolved in chloroform:methanol (2:1; 10mL:5mL). The organic phase was transferred to a 500 mL round-bottom flask and evaporated under reduced pressure at 60°C until a thin dry film formed. The film was hydrated with 20 mL pH 5.5 phosphate buffer and the resulting vesicular suspension was sonicated for 30 min.
- For alcohol addition, ketoconazole, oleic acid, cholesterol and Tween 80 were dissolved in 10mL ethanol. Phosphate buffer pH 5.5 was kept under continuous stirring on the magnetic stirrer at 60–70°C. The organic phase was added dropwise using a syringe, followed by stirring for 20–30 min to facilitate solvent removal. The resulting suspension was sonicated for 30mins to reduce particle size.

2.4 Design of experiments and optimization

Design-Expert @Stat-Ease 360 software was used. A central composite design was employed with oleic acid (20, 40 and 60 mg) and Tween 80 (80, 100 and 120 mg) as quantitative factors. Preparation method was a categorical factor with two levels: TFH and alcohol addition. Particle size was selected as a response to minimize, and entrapment efficiency as a response to maximize.

Table 1: Factors and levels selected for the optimization of ketoconazole ufasomes

Factor	Variable	Low (-1)	Middle (0)	High (+1)	Unit/Type
A	Oleic acid	20	40	60	mg
B	Tween 80	80	100	120	mg
C	Preparation method	TFH	—	Alcohol addition	categorical

Table 2: Central composite design (3²) of ketoconazole loaded ufasomes using thin film hydration method (T1- T9)

Formulation Code	Ketoconazole (mg)	Oleic acid (mg)	Tween 80 (mg)	Cholesterol (mg)	VitE Oil (drops)	Chloroform (ml)	Methanol (ml)
T1	50	20	80	10	1	10	5
T2	50	20	100	10	1	10	5
T3	50	20	120	10	1	10	5
T4	50	40	80	10	1	10	5
T5	50	40	100	10	1	10	5
T6	50	40	120	10	1	10	5
T7	50	60	80	10	1	10	5
T8	50	60	100	10	1	10	5
T9	50	60	120	10	1	10	5

Table3:Central composite design(3²) of ketoconazole loaded ufasomes using alcohol addition method (A1- A9)

Formulation Code	Ketoconazole (mg)	Oleic acid (mg)	Tween80 (mg)	Cholesterol (mg)	Ethanol (ml)
A1	50	20	80	10	10
A2	50	20	100	10	10
A3	50	20	120	10	10
A4	50	40	80	10	10
A5	50	40	100	10	10
A6	50	40	120	10	10
A7	50	60	80	10	10
A8	50	60	100	10	10
A9	50	60	120	10	10

Based on the formulation chart generated by the Design of Experiments (DoE), a total of 18 formulations were prepared, comprising 9 formulations using the thin-film hydration (TFH) method and 9 formulations using the alcohol addition method.

The influence of independent variables on dependent variables is studied for each of the preparation method , in addition, the preparation method was considered a categorical factor to study the impact of process on the dependent variables.

2.5 Evaluation of Ufasomes

2.5.1 Particle Size and Polydispersity Index (PDI)^{26,27}

The particle size and PDI of the prepared ufasomal formulations were determined using dynamic light scattering (DLS), Horiba Scientific Nanoparticle SZ- 100 at a fixed angle of 90° and a temperature of

25°C. 0.1mL of the suspension was diluted to 10mL with buffer and analyzed to determine the mean vesicle size and size distribution.

2.5.2 Zeta Potential²⁸

The zeta potential of the prepared ufasomal formulations was determined using a zeta potential analyzer. 0.1mL suspension was diluted with 10mL buffer before analysis, and the surface charge of the vesicles was recorded to assess their physical stability.

2.5.3 Entrapment Efficiency(by indirect method)¹⁴

The entrapment efficiency of ketoconazole-loaded ufasomes was determined by centrifugation. 2ml of the ufasomal suspension was centrifuged at a high speed (10000-15000 rpm) for half an hour to separate the untrapped drug from the vesicles. The amount of free drug present in the supernatant was determined spectrophotometrically at 203 nm. The entrapment efficiency was calculated using the following equation:

$$\%EE = \frac{\text{Total drug} - \text{unentrapped drug}}{\text{Total drug}} \times 100$$

2.5.4 Drug Content

The drug content of the optimized ufasomal formulation was determined by diluting 0.5ml in 10ml methanol. The content was filtered through whatmann filter paper No 41. 1ml of above solution was diluted to 10ml with phosphate buffer (pH 5.5). The absorbance was measured at 203 nm using a UV–Visible spectrophotometer, and the drug content was calculated from the previously prepared calibration curve.

2.5.5 Microscopic Examination of Optimized Ufasomes²⁹

The optimized ufasomal preparation was examined using a bright-field microscope equipped with an image analyzer to observe the morphology and vesicular characteristics. A small quantity of the optimized ufasomal dispersion was placed on a clean glass slide and covered with a coverslip. The preparation was examined under 40X magnification, and the vesicular structures were observed and recorded using the image analyzer.

2.6 Preparation of ufasomal gel³⁰

The optimized ufasomal suspension was incorporated into Carbopol 934 gel. Preliminary trials compared 1.5% and 2% Carbopol 934, and 2% was selected in the source study based on the resulting consistency/viscosity. Triethanolamine was added dropwise for pH adjustment.

2.7 Evaluation of Ufasomal Gel

2.7.1 Physical Appearance

The prepared ufasomal gel was visually examined for its color, homogeneity, clarity, consistency, and presence of any lumps or grittiness. The formulation was also checked for phase separation and other visible physical changes.

2.7.2 Determination of pH

The pH of the prepared ufasomal gel was determined using a calibrated digital pH meter.

2.7.3 Viscosity³¹

The viscosity of the prepared ufasomal gel was determined using a Brookfield viscometer at a suitable spindle and rotational speed. The viscosity was recorded in centipoise (cP) to evaluate the consistency and flow characteristics of the gel.

2.7.4 Spreadability³²

The spreadability of the ufasomal gel was determined using the parallel-plate method. One gram of gel was placed on a transparent Petri plate and the initial area occupied by the gel was marked. Another Petri plate was placed over the gel, and incremental weights of 100 g were applied until maximum spreading was achieved. The diameter of the spread gel was measured and the area was calculated using the formula:

$$A = \pi r^2$$

$$Si = (d^2 \times \frac{\pi}{4})$$

Where,

Si = Spreading area (mm²) depending on mass,

d = Spreading area diameter (mm)

2.7.5 Drug Content

The drug content of the prepared ufasomal gel was determined by accurately weighing a known quantity of gel and extracting the drug using a suitable solvent. The resulting solution was suitably diluted and analyzed spectrophotometrically at 203 nm. The drug content was calculated using the calibration curve of ketoconazole and expressed as a percentage of the theoretical drug content.

2.7.6 In-vitro drug release¹⁵

Drug release was assessed using a dialysis membrane bag diffusion technique. The membrane had a molecular weight cut-off of 12,000–14,000 Da and was soaked overnight in pH 5.5 phosphate buffer. An amount equivalent to 5 mg ketoconazole was placed in the dialysis bag. The receptor medium consisted of 200 mL phosphate buffer pH 5.5:ethanol (70:30), maintained at 37 ± 0.5 °C and agitated at 100 rpm. 5mL samples were withdrawn periodically and replaced with fresh medium. Samples were analyzed at 203 nm.

Release data were fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models. The release exponent n from the Korsmeyer–Peppas model was used to describe the release mechanism.

2.8 Antifungal activity:^{33–35}

Antifungal activity was evaluated against *Aspergillus niger* by the agar well diffusion method using Potato Dextrose Agar. Potato extract, dextrose and agar were prepared and sterilized by autoclaving at 121 °C and 15 psi for 15 min. The fungal culture was spread on the solidified medium using a sterile cotton swab and wells were prepared with a sterile cork borer. Pure drug solution, ketoconazole-loaded ufasomal suspension prepared by thin film hydration method, ufasomal gel prepared by thin film hydration method and marketed ketoconazole gel were tested at an equivalent ketoconazole concentration of 2 mg/mL. Dimethyl sulfoxide was used as the negative control. Zones of inhibition were measured after 48 h, with experiments performed in triplicate.

3. Results and discussion

3.1 Preparation of Standard Curve

The standard curve of ketoconazole was prepared in phosphate buffer pH 5.5 over the concentration range of 1–5 µg/mL. The absorbance increased progressively with an increase in drug concentration, indicating good linearity. The regression equation was $y = 0.2014x$, with an R² value of **0.9975** (Table 4). The standard curve is shown in Figure 1.

Table 4: Standard curve of ketoconazole

Conc (µg/ml)	Average Absorbance (n=3)
1	0.232±0.016
2	0.402±0.023
3	0.618±0.022
4	0.815±0.038
5	0.985±0.048

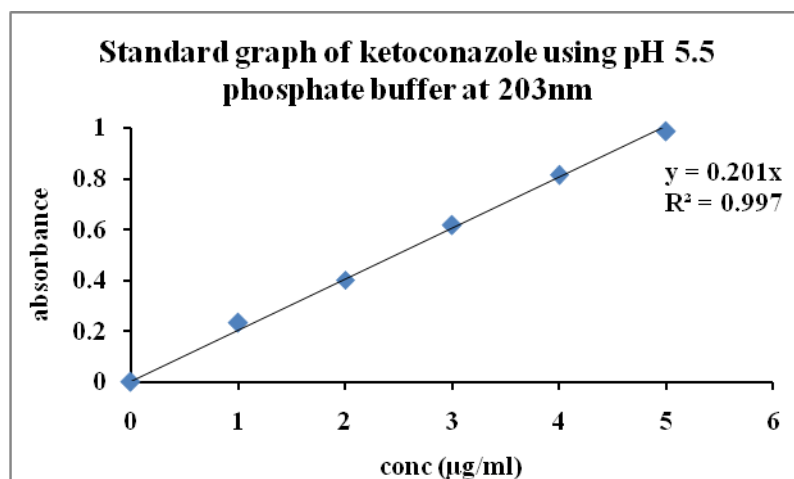


Figure 1: Standard graph of ketoconazole

3.2 Preparation of Ketoconazole-Loaded Ufasomes

Ketoconazole-loaded ufasomes were prepared by thin film hydration (TFH) and alcohol addition methods using ketoconazole, oleic acid, Tween 80, cholesterol and vitamin E oil. The effect of oleic acid and Tween 80 concentrations on particle size and entrapment efficiency was investigated using a Central Composite Design.

3.2.1 Evaluation of Ufasomes Prepared by Thin Film Hydration

The formulations prepared by the thin film hydration method showed particle sizes ranging from **179.5 to 476.4 nm**, while entrapment efficiency ranged from **68.42 to 92.63%**. The results demonstrated that changes in the concentrations of oleic acid and Tween 80 influenced the characteristics of the prepared ufasomes (Table 5).

Table 5: Evaluation of ketoconazole-loaded ufasomes prepared by thin film hydration as per CCD design

Formulation code	Oleic acid (mg)	Tween 80 (mg)	Particle size (nm)	Entrapment efficiency (%)	Zeta potential (mV)	Drug content (%)
T1	20	80	275.8	78.16	-20.5	91.8 ± 0.8
T2	20	100	263.9	73.84	-19.6	93.5 ± 0.7
T3	20	120	179.5	68.42	-23.9	92.4 ± 0.9
T4	40	80	410.6	83.17	-26.3	95.1 ± 0.6
T5	40	100	357.9	79.95	-19.3	97.6 ± 0.5
T6	40	120	339.5	74.63	-22.8	96.2 ± 0.7
T7	60	80	476.4	92.63	-23.2	96.0 ± 0.8
T8	60	100	442.8	86.85	-22.6	98.4 ± 0.4
T9	60	120	296.8	81.14	-25.7	97.1 ± 0.6

3.2.1.1 Evaluation of ufasomes by DOE by thin film hydration method:

a. Particle Size:

The particle size of obtained ufasome prepared by thin film hydration was ranged from 179.5nm to 476.4nm as shown in table 5.

The ANOVA (Analysis of Variance) for linear model was used to analyse the particle size of the prepared nanoparticles. The p-value and R^2 value was found to be <0.0001 and 0.9616 respectively, showing the model is significant.

The **Model F-value** of 75.12 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant.

Table 6: Fit statistics of particle size by thin film hydration method:

Std. Dev.	21.87	R²	0.9616
Mean	351.37	Adjusted R²	0.9488
C.V. %	6.22	Predicted R²	0.9031
		Adeq Precision	22.2733

The **Predicted R²** of 0.5122 is in reasonable agreement with the **Adjusted R²** of 0.6864; i.e. the difference is less than 0.2.

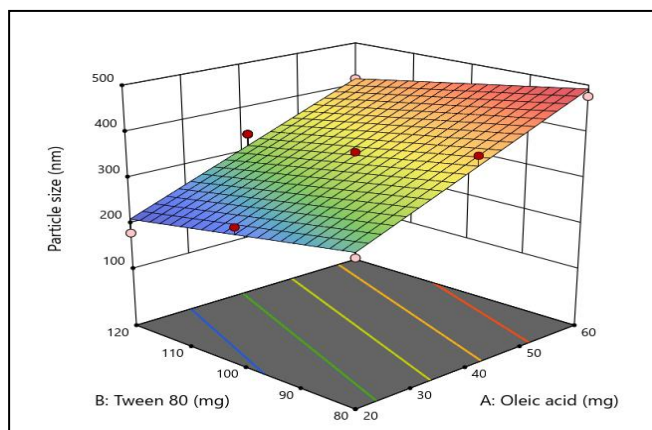


Figure 2: 3-D graph of particle size by thin film hydration method

Final Equation in Terms Of Coded Factors

$$\text{Particle Size} = +351.37 + 102.65 \cdot A - 37.98 \cdot B$$

where A represents oleic acid and B represents Tween 80.

The positive coefficient of oleic acid indicates an increase in particle size with increasing concentration, while the negative coefficient of Tween 80 suggests a reduction in particle size due to improved interfacial stabilization and formation of smaller vesicles. The response surface plot (**Figure 2**) illustrates the combined effect of oleic acid and Tween 80 on particle size.

b. Entrapment efficiency:

The entrapment efficiency of obtained ufasome prepared by thin film hydration was ranged from 68.42% to 92.63% as shown in table 5.

The ANOVA (Analysis of Variance) for linear model was used to analyse the entrapment efficiency showed the p-value and R² value 0.0082 and 0.7985 respectively, showing the model is significant.

The **Model F-value** of 11.89 implies the model is significant. There is only a 0.82% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant.

Table 7: Fit statistics of particle size by thin film hydration method:

Std. Dev.	3.76	R²	0.7985
Mean	79.87	Adjusted R²	0.7313
C.V. %	4.71	Predicted R²	0.5909
		Adeq Precision	9.2533

The **Predicted R²** of 0.9707 is in reasonable agreement with the **Adjusted R²** of 0.9930; i.e. the difference is less than 0.2.

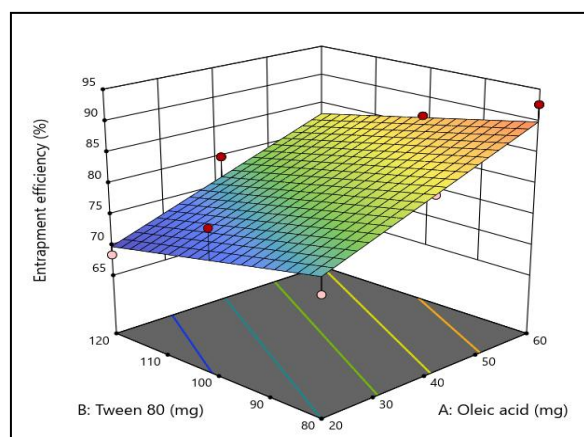


Figure 3: 3-D graph of entrapment efficiency by thin film hydration method

Final Equation in Terms Of Coded Factors

$$\text{Entrapment efficiency} = +79.87 + 6.70 \cdot A - 3.35 \cdot B$$

Where A represents oleic acid and B represents Tween 80.

The positive coefficient of oleic acid indicates that increasing its concentration improved drug entrapment, likely by providing a larger hydrophobic domain for ketoconazole incorporation. In contrast, the negative coefficient of Tween 80 suggests that higher concentrations may reduce entrapment efficiency, possibly due to increased drug partitioning into the external phase. The combined effect of oleic acid and Tween 80 on entrapment efficiency is illustrated in **Figure 3**.

3.2.2 Evaluation of Ufasomes Prepared by alcohol addition method

The formulations prepared by the thin film hydration method showed particle sizes ranging from **263.9nm to 529.1nm**, while entrapment efficiency ranged from **67.78% to 96.94%**. The results demonstrated that changes in the concentrations of oleic acid and Tween 80 influenced the characteristics of the prepared ufasomes (**Table 8**).

Table 8: Evaluation of ketoconazole-loaded ufasomes prepared by alcohol addition method as per CCD design

Formulation code	Oleic acid (mg)	Tween 80 (mg)	Particle size (nm)	Entrapment efficiency (%)	Zeta potential (mV)	Drug content (%)
A1	20	80	292.6	96.94	-23.9	99.12 ± 0.42
A2	20	100	275.8	94.63	-18.5	97.24 ± 0.45
A3	20	120	263.9	90.84	-19.3	98.35 ± 0.38
A4	40	80	341.3	79.54	-22.8	94.68 ± 0.51
A5	40	100	326.5	76.89	-17.4	93.95 ± 0.47
A6	40	120	314.2	77.72	-24.6	94.23 ± 0.49
A7	60	80	529.1	75.68	-20.8	93.48 ± 0.54
A8	60	100	482.5	70.34	-24.9	91.86 ± 0.56
A9	60	120	476.4	67.78	-23.5	90.94 ± 0.59

3.2.2.1 Evaluation of ufasomes by DOE by alcohol addition method:

a. Particle Size:

The particle size of obtained ufasome prepared by alcohol addition method was ranged from 263.9nm to 529.1nm as shown in table 8.

The ANOVA (Analysis of Variance) for linear model was used to analyse the particle size of the prepared nanoparticles. The p-value and R^2 value was found to be 0.0004 and 0.9391 respectively, showing the model is significant.

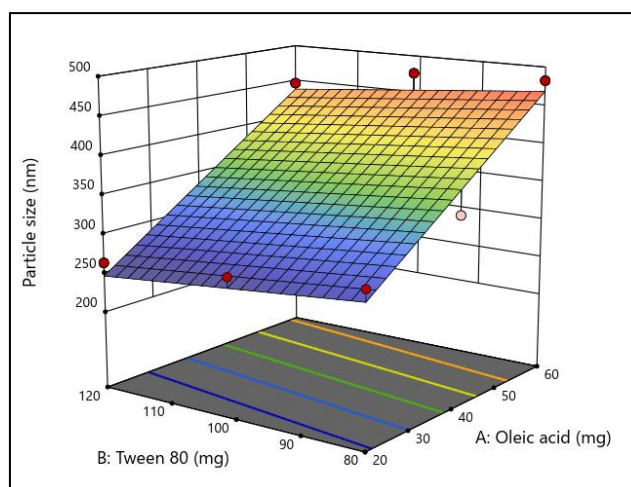
The **Model F-value** of 39.31 implies the model is significant. There is only a 0.04% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant.

Table 9: Fit statistics of particle size by alcohol addition method:

Std. Dev.	26.74	R²	0.9391
Mean	357.78	Adjusted R²	0.9055
C.V. %	7.47	Predicted R²	0.8574
		Adeq Precision	14.3582

The predicted R² of 0.8574 is in reasonable agreement with the Adjusted R² of 0.9055; i.e. the difference is less than 0.2.

**Figure 4: 3-D graph of particle size by alcohol addition method****Final Equation in Terms Of Coded Factors**

$$\text{Particle size} = +357.78 + 95.57 \cdot A - 15.25 \cdot B$$

Where A represents oleic acid and B represents Tween 80.

The positive coefficient of oleic acid indicates an increase in particle size with increasing concentration, whereas the negative coefficient of Tween 80 suggests a reduction in particle size. The response surface plot (**Figure 4**) illustrates these effects, indicating that oleic acid was the major variable influencing particle size in the alcohol addition method.

b. Entrapment efficiency:

The entrapment efficiency of obtained ufasome prepared by alcohol addition method was ranged from 67.78% to 96.94% as shown in table 8.

The ANOVA (Analysis of Variance) for linear model was used to analyse the entrapment efficiency showed the p-value and R² value 0.0002 and 0.9409 respectively, showing the model is significant.

The **Model F-value** of 47.74 implies the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant.

Table 10: Fit statistics of particle size by alcohol addition method:

Std. Dev.	2.95	R²	0.9409
Mean	81.15	Adjusted R²	0.9212
C.V. %	3.64	Predicted R²	0.8737
		Adeq Precision	16.6872

The Predicted R² of 0.8737 is in reasonable agreement with the adjusted R² of 0.9212; i.e. the difference is less than 0.2

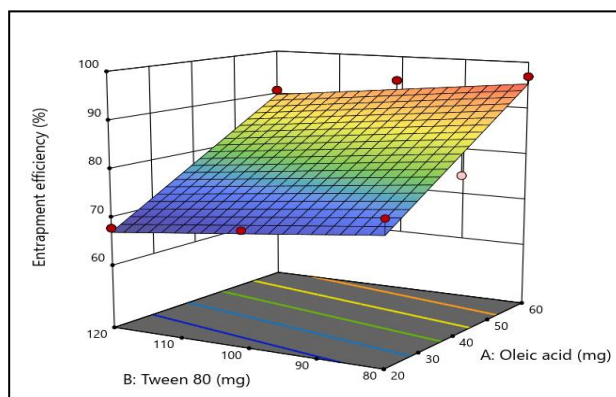


Figure 5: 3-D graph of entrapment efficiency by alcohol addition method

Final equation in terms of coded factors:

$$\text{Entrapment efficiency} = +81.15 + 11.44 \cdot A - 2.77 \cdot B$$

Where A represents oleic acid and B represents Tween 80.

Oleic acid showed a positive effect on entrapment efficiency, indicating improved drug entrapment with increasing concentration, whereas Tween 80 showed a negative effect. This may be attributed to the role of oleic acid in vesicle formation and increased drug solubilization in the external phase at higher surfactant levels. The combined effects are illustrated in Figure 5.

3.2.3 Comparative evaluation of thin film hydration and alcohol addition method as a type of preparation method in ufasomes:

The two preparation methods were compared based on particle size and entrapment efficiency. The thin film hydration method produced particle sizes in the range of **179.5–476.4 nm**, whereas the alcohol addition method produced particle sizes in the range of **263.9–529.1 nm**. Similarly, the entrapment efficiency ranged from **68.42–92.63%** for the thin film hydration method and **67.78–96.94%** for the alcohol addition method.

Based on the combined optimization of the responses, the **thin film hydration method** was selected for further development. Numerical optimization suggested a formulation containing **24.41 mg oleic acid and 80 mg Tween 80**. The predicted particle size was 300 nm and predicted entrapment efficiency was 77.5083%, with a desirability value of 0.883. On experimental evaluation, the optimized formulation showed a particle size of **202.5 nm** and entrapment efficiency of **84.76%** (Table 11).

Table 11. Comparison of DOE-predicted and experimental values of the optimized formulation

	Oleic acid (mg)	Tween 80 (mg)	Particle size (nm)	Entrapment efficiency (%)	Desirability
DOE Prediction	24.41	80	300	77.5083	0.883
Actual results	24.41	80	202.5	84.76%	

3.3 Zeta potential:

The zeta potential of the ketoconazole-loaded ufasomes ranged from **−19.3 to −26.3 mV** for the thin film hydration formulations and **−17.4 to −24.9 mV** for the alcohol addition formulations. The negative charge was attributed to oleic acid, which promotes electrostatic repulsion between vesicles and helps reduce aggregation. The optimized formulation showed a zeta potential of **−20.5 mV**, indicating satisfactory surface-charge characteristics and physical stability of the vesicular system.

3.4 Microscopic Examination of Optimized Ufasomes

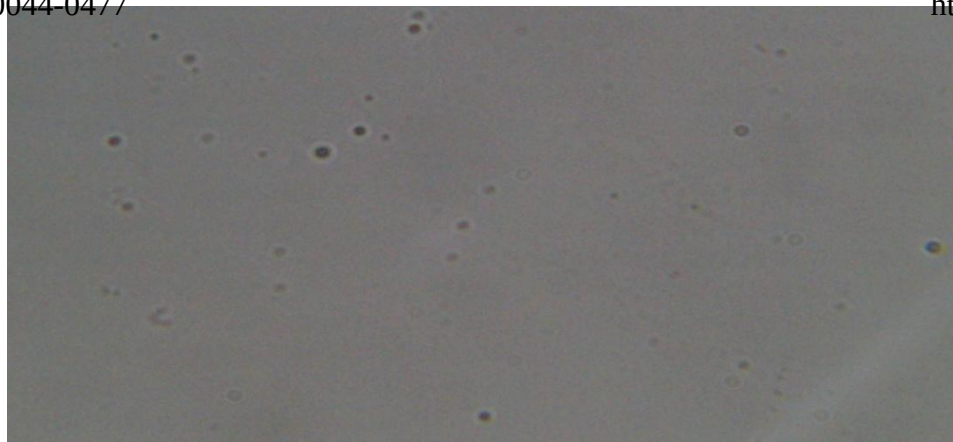


Figure 6: Bright-field microscopic image of the optimized ufasomal preparation.

Microscopic examination of the optimized ufasomal formulation revealed the presence of distinct, spherical to nearly spherical vesicular structures with relatively uniform distribution, as shown in **Figure 6**. The absence of major aggregation indicated good physical stability of the vesicles. The observed vesicular morphology supported the successful formation of ufasomes and was consistent with the particle-size results obtained by instrumental analysis.

3.5 Preparation and Evaluation of Optimized Ufasomal Gel

The optimized ketoconazole-loaded ufasomal suspension was incorporated into a Carbopol 934 gel to obtain a suitable topical dosage form. The optimized gel showed a **pH of 5.5**, viscosity of **5842 cP at 10 rpm**, and drug content of **96.52%** (**Table 12**). The pH was considered suitable for topical application, while the observed viscosity indicated adequate consistency of the gel. The drug content demonstrated satisfactory incorporation of ketoconazole into the gel.

Table 12. Evaluation parameters of optimized ketoconazole-loaded ufasomal gel

Formulation code	pH	Viscosity (cps)	% drug content	Spreadability
FG	5.5	5842	96.52%	13.84±0.0163mm ²

3.6 In-vitro Drug Release Study

The in-vitro drug release study was performed using dialysis membrane diffusion with phosphate buffer pH 5.5 and ethanol in the ratio of 70:30 as the receptor medium at $37 \pm 0.5^\circ\text{C}$. The release profiles of the optimized ufasomal formulations, corresponding suspensions and marketed gel are presented in **Table 13** and **Figure 7**.

Table 13: In-vitro drug release profile of ketoconazole formulations

Time (hrs)	Formulation suspension by thin film hydration method	Formulation suspension by alcohol addition method	Marketed gel	Ufasomal gel by thin film hydration method	Ufasomal gel by alcohol addition method
0	0	0	0	0	0
0.5	7.249255	3.67428	7.527309	5.620655	4.508441
1	9.572989	4.210526	8.877855	7.765641	6.236346
2	11.45978	4.726912	9.970209	8.341609	8.262165
4	12.61172	6.097319	11.73784	9.652433	9.692155
6	16.32572	10.18868	14.2006	10.32771	10.00993
8	19.82125	12.47269	18.03376	12.86991	11.7577
10	28.79841	18.80834	28.20258	18.62959	15.23337
12	47.26912	37.53724	44.8858	32.572	33.36643
24	88.57994	82.22443	87.98411	85.00497	73.08838
26	82.62165	79.44389	85.00497	81.82721	68.52036
28	71.10228	67.3287	73.68421	72.29394	51.83714

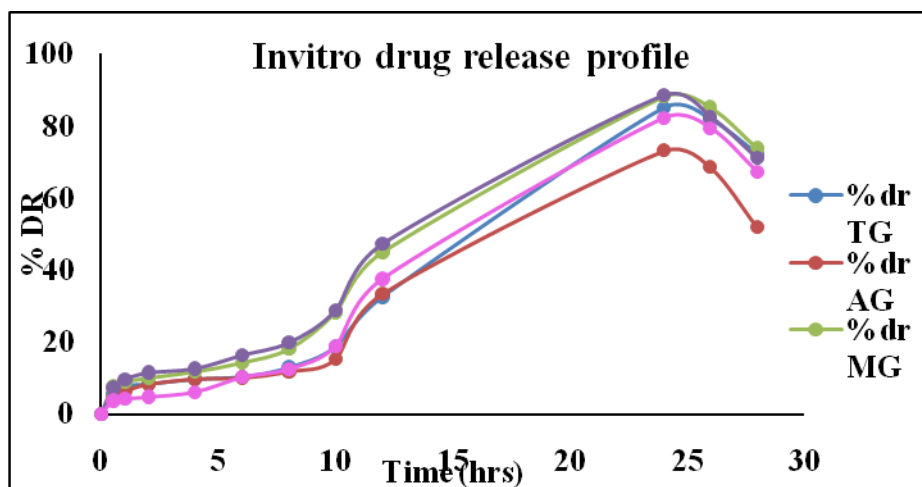


Figure 7: Comparison plot Of In-vitro drug release release of formulation, ufasomal gel and Marketed gel

The thin film hydration suspension showed the highest drug release at 1, 2, 12 and 24 h (9.57%, 11.46%, 47.27% and 88.58%, respectively). This may be related to its relatively favorable vesicle characteristics and greater surface area available for drug diffusion. The alcohol addition suspension showed lower release (4.21%, 4.73%, 37.54% and 82.22%), which may be attributed to differences in vesicle size, drug entrapment and drug distribution resulting from the preparation method.

The ufasomal gels showed slower drug release than their corresponding suspensions. The thin film hydration ufasomal gel released 7.77%, 8.34%, 32.57% and 85.00%, whereas the alcohol addition ufasomal gel showed 6.24%, 8.26%, 33.37% and 73.09% at 1, 2, 12 and 24 h, respectively. The slower release from the gels may be due to the Carbopol gel matrix providing an additional diffusion barrier.

The lower 24-h release from the alcohol addition ufasomal gel (73.09%) compared with the thin film hydration ufasomal gel (85.00%) may be associated with its vesicular characteristics, particularly larger particle size and/or higher drug entrapment, which can retard drug diffusion. Overall, the results indicate that particle size, drug entrapment, preparation method and incorporation into the gel matrix collectively influenced the release of ketoconazole.

3.7 Antifungal Activity

The antifungal activity of the formulations was evaluated against *Aspergillus niger* using the agar well diffusion method. All formulations were tested at an equivalent ketoconazole concentration of **2 mg/mL**. The pure drug solution showed the highest zone of inhibition (**4.3 ± 0.2 cm**), followed by the ufasomal suspension (**3.9 ± 0.2 cm**). The ufasomal gel and marketed gel showed zones of inhibition of **3.4 ± 0.2 cm** each (**Table 14**).

Table 14: Antifungal activity of ketoconazole formulations against *Aspergillus niger*

Formulation	Zone of inhibition ± SD
PD	4.3±0.2 cm
FS	3.9±0.2cm
FG	3.4±0.2cm
MG	3.4±0.2cm

Where,

PD = Pure drug solution

FS = Formulation suspension

FG = Formulation gel

MG = Marketed gel

The slightly lower zone of inhibition observed with the gel formulations may be associated with slower diffusion of ketoconazole from the vesicular and gel matrices. Nevertheless, the developed ufasomal gel

showed appreciable antifungal activity against *A. niger*. The antifungal activity assessment is presented in **Figure 8**.

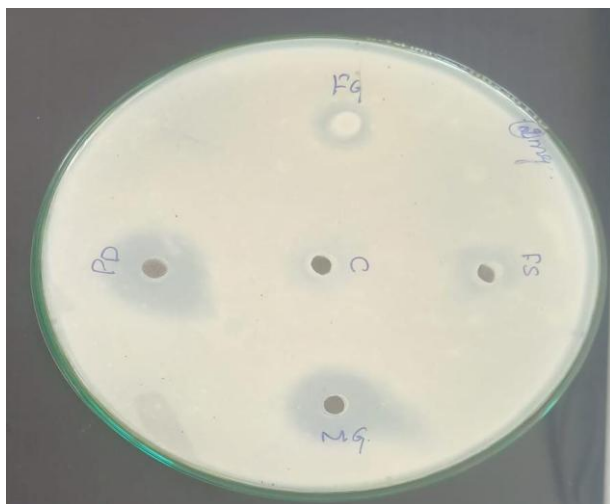


Figure 8: Antifungal activity of ketoconazole formulations against *Aspergillus niger*.

CONCLUSION:

Ketoconazole-loaded ufasomes were successfully formulated and optimized using the Design of Experiments (DoE) approach. The optimized formulation showed a particle size of 202.5 nm and an entrapment efficiency of 84.76%. The prepared Carbopol 934-based gel exhibited satisfactory physicochemical properties, including a pH of 5.5, viscosity of 5842 cP, and drug content of 96.52%. In-vitro drug release studies demonstrated sustained release over 24 hours. Antifungal studies against *Aspergillus niger* showed a zone of inhibition of 3.4 ± 0.2 cm for the ufasomal gel, comparable to the marketed gel. Overall, the findings suggest that ketoconazole-loaded ufasomal gel may be a promising approach for topical antifungal delivery.

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