Design, Prepare and in Vitro Evaluation of Ketoconazole Ethosomal Drug Delivery System

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ABSTRACT

The present study was aimed at the design and evaluation of Ketoconazole-loaded ethosomes for enhanced dermal delivery. Ketoconazole, a broad-spectrum antifungal agent, exhibits limited skin penetration when administered through conventional topical formulations. To overcome this limitation, ethosomal vesicles were prepared using phospholipids and ethanol by using cold method. Four formulations (F1–F4) were developed and evaluated for particle size, zeta potential, surface morphology, and in vitro drug release characteristics. The particle size of the formulated ethosomes ranged from 289 nm to 369 nm, while zeta potential values ranged from −22 mV to −27 mV, indicating satisfactory vesicle stability. Scanning Electron Microscopy (SEM) studies revealed spherical and uniformly distributed vesicles with smooth surfaces. In vitro drug release studies demonstrated sustained release of Ketoconazole over a period of 8 hours. Among all formulations, F3 exhibited the highest cumulative drug release of 98.48%, along with the smallest particle size of 289 nm, suggesting enhanced drug diffusion and skin permeation potential. The results indicated that ethosomal vesicles effectively encapsulated Ketoconazole and provided controlled drug release with favorable physicochemical properties. The optimized formulation (F3) showed superior performance compared to other formulations and may serve as a promising carrier system for improved dermal delivery of Ketoconazole. Thus, ethosomal drug delivery systems offer a potential strategy for enhancing the therapeutic efficacy of topical antifungal treatment while improving patient compliance.

Keywords: Ketoconazole, Lipid, Cold method, Ethosomes, Dermal Delivery, Vesicular Drug Delivery System, In Vitro Drug Release.

INTRODUCTION

Ethosomes

Ethosomes are advanced vesicular carriers specifically designed for enhanced transdermal and topical drug delivery. They are structurally similar to liposomes but are distinguished by a high concentration of ethanol (20–45%) in their composition, which imparts unique properties to the vesicles.

The typical ethosomal system consists of:

Phospholipids: Form the vesicular bilayer, similar to liposomes.

Ethanol: Acts as a penetration enhancer, increasing vesicle fluidity and skin permeability.

Water: The aqueous medium in which the vesicles are dispersed.

First developed by Touitou et al. in the late 1990s, ethosomes were designed to overcome the limitations of conventional liposomes and niosomes in delivering drugs across the stratum corneum, the main barrier of the skin.

Advantages of Ethosomes

- Enhanced Skin Penetration:
- High ethanol content fluidizes both vesicle and stratum corneum lipids, allowing drugs to reach deeper skin layers.
- Versatile Drug Encapsulation:
- Can carry hydrophilic, lipophilic, and amphiphilic drugs, making them suitable for a wide range of therapeutics.
- Non-Invasive Delivery:
- Provides a patient-friendly alternative to injections or oral formulations for systemic or local effects.
- Controlled and Sustained Release:
- Vesicular structure allows gradual drug release, improving therapeutic outcomes.
- Improved Bioavailability:

Disadvantages of Ethosomes

- Stability Issues: High ethanol content may destabilize vesicles over time, requiring proper storage conditions.
- Skin Irritation: Ethanol can cause dryness, irritation, or burning sensations in sensitive individuals.
- Limited Drug Loading: Very high drug doses may be difficult to incorporate without affecting vesicle integrity.
- Manufacturing Complexity: Requires careful formulation techniques to maintain vesicle size, stability, and drug encapsulation efficiency.
- Short Shelf Life: Depending on the composition, ethosomes formulations may require refrigeration and protection from light to prevent degradation.

MATERIALS

Ketoconazole was procured from Hetero Labs, Hyd, Cholesterol, Phosphotidyl choline, and Ethanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY

Formulation development

Table-1: Composition of Ketoconazole Ethosomes (F1 to F4)

S.No.	Ingredients (mg)	F1	F2	F3	F4
1	Ketoconazole	20	20	20	20
2	Phosphotidyl choline	100	200	300	400
3	Ethanol	5	5	5	5
4	Water	10	10	10	10

Cold Method Procedure

Ethosomal formulations were prepared by using the cold method. This is the most common and widely used method for the ethosomal preparation. Phospholipid and drug and other pharmaceutical ingredient listed in table were dissolved in ethanol in a covered vessel at room temperature with vigorous stirring. This mixture was heated to 30^0C ± 10C and a fine stream of distilled water was added slowly, with constant mixing at 1000 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 30^0 C ± 10C. The preparation was left to cool at room temperature for 30 min and then it was sonicated at 4^0 C for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator.

CHARACTERIZATION

Particle size:

All the prepared batches of ethosomal gel were viewed under microscope to study their size. Size of Ethosomes from each batch was measured at different location on slide by taking a small drop of ethosomes on it and average size of ethosomes were determined.

SEM analysis

The morphology of Ethosomes was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the Ethosomes was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.

Zeta Potential:

The zeta potential means the charges which are present on the surface of Ethosomal. The many time the charge is present on the surface of Ethosomal. This charge is come due to the component or ingredient which was used during the manufacturing. Some charge is must be required on surface of all transferosome present in formulation, due to some charge all Ethosomal particle repeal to each other and coagulation of particle are avoided. The zeta potential of Ethosomal was taken in zeta sizer instrument having Malvern software. The analysis of sample was carried out at 25°c with the angle of detection 90°. The ideal zeta potential value must be required in range between +30 to -30mV. These ranges prevent the aggregation of Ethosomal particle.

Drug entrapment efficiency

Prepare ethosomal dispersion (known total drug concentration). Record volume and total drug added Transfer aliquots into centrifuge tubes. Include blank/control tubes. Centrifuge at 15,000 rpm (~20,000 × g) for 30–45 min at 4°C. (Adjust if pellet not forming; nanosized ethosomes may need ultracentrifugation. Carefully collect the supernatant (avoid disturbing pellet). This supernatant contains free (unentrapped) drug**.** If desired, wash pellet once with small volume of buffer, recentrifuge and combine washes with supernatant (to quantify any loosely bound drug). Determine drug

concentration in supernatant using a validated analytical method (UV at λmax). Multiply by supernatant volume to get mass of free drug.

$$\text{EE (\%)} = \frac{\text{Total drug added} - \text{Free (unencapsulated) drug}}{\text{Total drug added}} \times 100$$

In-Vitro Drug release studies:

The in-vitro study of drug permeation through the Dialysis membrane was performed using a modified Franz type glass diffusion cell. The modified cell having higher capacity is (10 ml) is used to maintain sink condition. The samples were analyzed for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal.

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{\text{Da}}{\text{Dt}} \times 100$$

Where, Dt = Total amount of the drug in the patch

Da = The amount of drug released

Conditions:

Medium: Phosphate buffer pH 7.4
RPM: 200
Temperature: 37 ± 0.5^{0}C
Time intervals: 1, 2, 3, 4, 5, 6, 7, 8 hours

Stability studies:

Optimized medicated niosomes were subjected to short term stability testing. The niosomes were sealed in aluminium foils and kept in a humidity chamber maintained at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 month as per ICH guidelines. Changes in the appearance and drug content of the stored patches were investigated after storage at the end of every month.

RESULTS AND DISCUSSION

Determination of Size

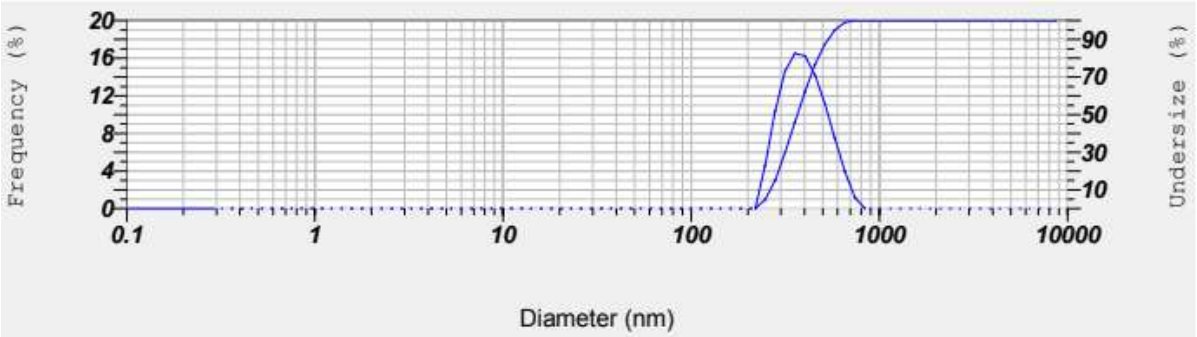


Fig-1: Particle size analysis of Optimized formulation of Ethosomes

Zeta potential

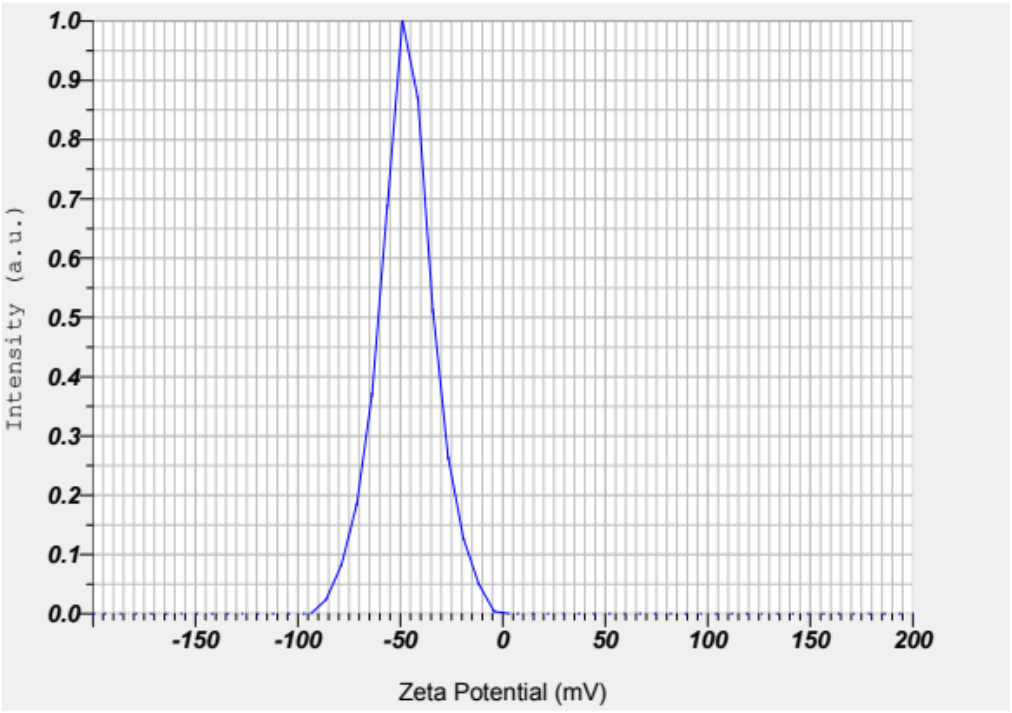


Fig-2: Zeta potential of Ethosomes

Table-2: Evaluation Studies of particle size and Zeta potential Ethosomes

F. No	Particle size (nm)	Zeta potential (mV)
F1	369	-26
F2	343	-27
F3	289	-22
F4	293	-26

SEM Analysis

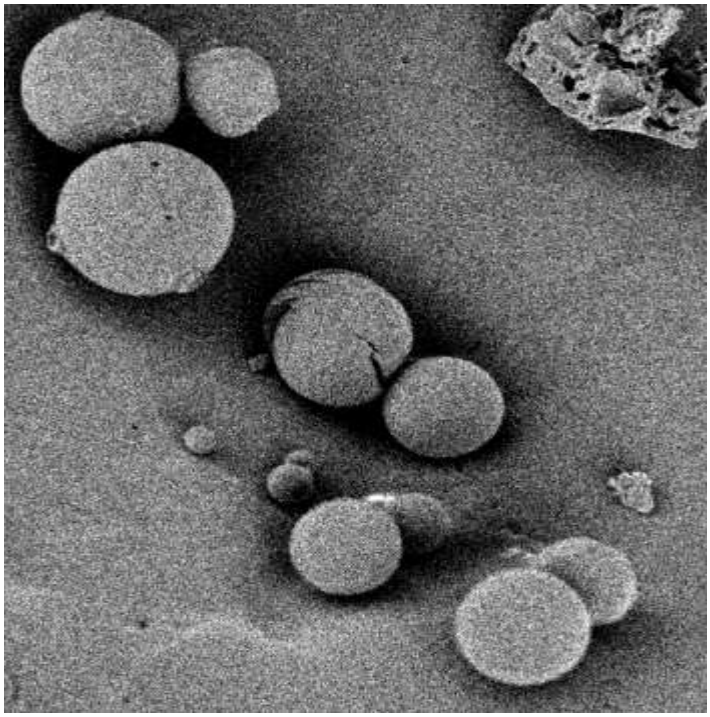


Fig-3: SEM Analysis of Ethosomes

The particle size, zeta potential, and SEM studies demonstrated successful preparation of ketoconazole-loaded ethosomes. The optimized formulation (F3) exhibited the smallest particle size (289 nm) with satisfactory zeta potential (−22 mV), indicating good stability and enhanced potential for skin penetration. SEM analysis confirmed the presence of uniformly distributed, spherical vesicles without aggregation. These findings suggest that the developed ethosomal system is a promising carrier for enhancing the dermal delivery of ketoconazole.

In vitro release study:

Table-3: In vitro drug release profiles of Ketoconazole Ethosomes (F1-F4)

Time (hr)	F1	F2	F3	F4
0	0	0	0	0
1	24.69	25.96	27.49	28.93
2	37.51	38.47	39.86	39.90
3	42.25	43.10	44.53	41.82
4	53.16	54.98	57.82	56.63
5	65.82	67.12	69.83	66.35
6	72.49	73.59	78.98	77.25
7	83.10	85.20	89.63	88.20
8	95.35	96.15	98.48	97.19

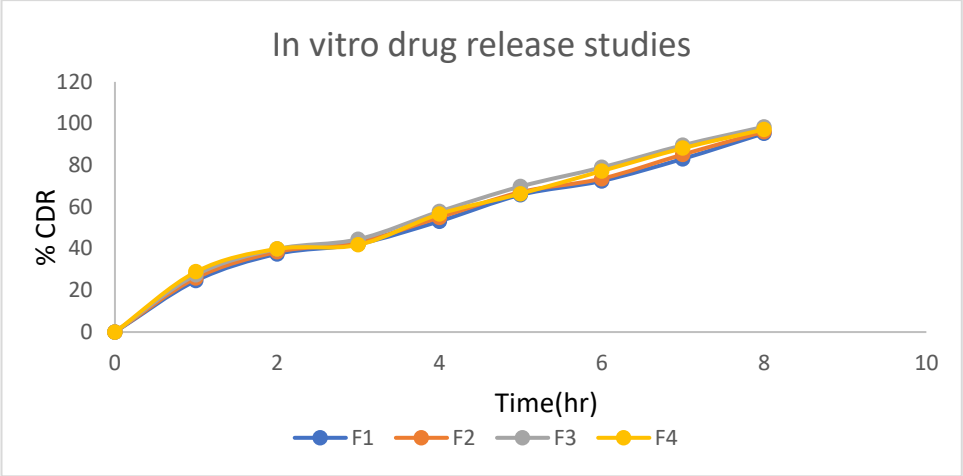


Fig-4: In vitro drug release studies of F1-F4 formulations

Overall, the results indicate that the optimized formulation F3 provided the most desirable release profile, achieving nearly complete drug release (98.48%) within 8 hours. Therefore, F3 can be considered the optimized ethosomal formulation for enhanced dermal delivery of Ketoconazole due to its superior release characteristics and potential therapeutic effectiveness.

Stability studies

There was no significant change in physical and chemical properties of the Ethosomes optimized formulation after 90 days. Parameters quantified at various time intervals were shown.

Table-4: Results of stability studies of optimized formulation

F. Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-3	25^{0}C/60%RH % Release	98.48	97.68	96.30	95.64	Not less than 85 %
F-3	30^{0}C/75% RH % Release	98.48	97.43	96.20	95.32	Not less than 85 %
F-3	40^{0}C/75% RH % Release	98.48	97.12	96.13	95.21	Not less than 85 %

SUMMARY AND CONCLUSION

The present study focused on the design and evaluation of Ketoconazole-loaded ethosomes for enhanced dermal delivery. Ethosomal formulations (F1–F4) were successfully prepared and evaluated for various physicochemical parameters, including particle size, zeta potential, surface morphology, and in vitro drug release. The particle size analysis revealed that all formulations were within the nanometric range, with sizes varying from 289 nm to 369 nm. Among the formulations, F3 exhibited the smallest particle size (289 nm), indicating its potential for enhanced skin penetration. Zeta potential values ranged from −22 mV to −27 mV, demonstrating adequate electrostatic stability of the ethosomal vesicles. SEM analysis confirmed the formation of spherical, smooth, and uniformly distributed vesicles without significant aggregation. The in vitro drug release study showed a sustained and controlled release pattern for all formulations over an 8-hour period. The cumulative drug release ranged from 95.35% to 98.48%, with formulation F3 exhibiting the highest drug release (98.48%). The superior performance of F3 may be attributed to its optimized composition, smaller vesicle size, and efficient drug encapsulation, which facilitated enhanced drug diffusion.The overall evaluation results demonstrated that ethosomal vesicles effectively encapsulated Ketoconazole and provided sustained drug release, indicating their suitability as carriers for topical antifungal therapy. Therefore, the developed Ketoconazole-loaded ethosomal system represents a promising approach for enhancing dermal delivery and antifungal efficacy. The study concludes that ethosomes can serve as an effective vesicular carrier for topical administration of

Ketoconazole, potentially improving treatment outcomes in fungal skin infections while reducing dosing frequency and improving patient compli**ance.**

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