Journal or Innovative Development in Pharmaceutical and Technical Science (JIDPTS)
Volume:9, Issue:11, Nov:2026

(J I D P T S)
ISSN(O):2581-6934

FORMULATION AND EVALUATION OF KETOCONAZOLE LOADED TRANSFEROSOMES FOR IMPROVED ANTI FUNGAL THERAPY

Medipalli Viswaja[1], Gouraram Akhila[2], Boddupally Anitha[3], Sunkaraboina Amulya[4], Avinesh Dwivedi[5], Maskuri Balakrishna[6]

[1]Associate Professor, [2,3,4,5,6] Students

[1,2,3,4,5,6] Department of pharmaceutics, Teegala Ram Reddy college of pharmacy, Meerpet, Hyderabad, Ranga Reddy Telangana - 500097, India.

Corresponding: Medipalli Viswaja, Associate professor

Department of pharmaceutics, Teegala Ram Reddy College of Pharmacy, Hyderabad - 500097, Telangana, India.

Abstract: The present study was aimed at the formulation and evaluation of Ketoconazole-loaded transferosomes for improved antifungal therapy. Ketoconazole is a broad-spectrum antifungal agent widely used in the treatment of superficial fungal infections; however, its therapeutic efficacy is limited due to poor skin penetration and low aqueous solubility. The objective of this study was to develop transferosomal formulations capable of enhancing drug permeation and improving antifungal effectiveness. FTIR studies confirmed the compatibility between the drug and selected excipients, indicating the absence of significant drug-excipient interactions. Ketoconazole-loaded transferosomes were prepared by the thin-film hydration method and evaluated for particle size, entrapment efficiency, surface morphology, in-vitro drug release, and stability studies. The particle size of the formulations ranged from 122 to 135 nm, indicating the formation of nanosized vesicles suitable for enhanced skin permeation. SEM analysis revealed spherical and smooth vesicles without aggregation. Entrapment efficiency ranged from 79.63% to 84.20%, with formulation F4 exhibiting the highest drug entrapment. In-vitro diffusion studies demonstrated sustained drug release from all formulations over 8 hours, with F4 showing the highest cumulative drug release of 97.53%.Stability studies conducted for three months under different storage conditions indicated that the optimized formulation remained stable with no significant changes in drug release characteristics.

Keywords: Ketoconazole, Transferosomes, Thin-Film Hydration Method, Entrapment Efficiency, In-vitro Drug Release.

INTRODUCTION

Fungal infections are among the most common dermatological disorders affecting millions of people worldwide. These infections are primarily caused by dermatophytes, yeasts, and molds, leading to conditions such as tinea corporis, tinea pedis, candidiasis, and seborrheic dermatitis. The increasing prevalence of fungal infections, coupled with the emergence of drug-resistant strains, has created a growing need for more effective and targeted drug delivery systems. Conventional topical antifungal formulations, including creams, ointments, and gels, often exhibit limited penetration through the stratum corneum, resulting in inadequate drug concentration at the site of infection and prolonged treatment duration. Ketoconazole is a broad-spectrum imidazole antifungal agent widely used for the treatment of superficial fungal infections. It exerts its antifungal activity by inhibiting the synthesis of ergosterol, an essential component of the fungal cell membrane, thereby increasing membrane permeability and causing fungal cell death. Although ketoconazole is highly effective against a wide range of fungi, its therapeutic efficacy following topical administration is often limited by poor skin penetration, short residence time, and reduced drug availability at the infected site. These limitations may lead to incomplete eradication of fungal pathogens, frequent application, and poor patient compliance. Novel vesicular drug delivery systems have attracted considerable attention for improving topical drug

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delivery. Among these, transferosomes are highly deformable and elastic lipid vesicles composed of phospholipids and edge activators such as Tween 80, Span 80, or sodium cholate. The presence of edge activators imparts remarkable flexibility to the vesicles, enabling them to penetrate through the narrow intercellular spaces of the stratum corneum more efficiently than conventional liposomes. This unique property allows transferosomes to deliver drugs into deeper layers of the skin, resulting in enhanced bioavailability, improved therapeutic efficacy, and sustained drug release. Transferosomal drug delivery offers several advantages, including enhanced skin permeation, higher drug entrapment efficiency, controlled drug release, reduced dosing frequency, and minimized systemic side effects. Owing to their excellent deformability and biocompatibility, transferosomes have emerged as a promising carrier for topical and transdermal delivery of antifungal, anti-inflammatory, anticancer, and analgesic drugs. Incorporation of transferosomes into topical gel formulations further improves formulation stability, ease of application, prolonged skin retention, and patient acceptability.

MATERIALS

Ketoconazole was procured from Synpharma Research Labs, Cholesterol, Carbopol 934, Span 80, Lecithin and Methanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY

Formulation development

Table-1: Formulation table of Ketoconazole

Ingredients (mg)	F1	F2	F3	F4
etoconazole	100	100	100	100
Soya lecithin	100	125	150	175
Cholesterol	50	50	50	50
Span 80	0.1	0.2	0.3	0.4
Chloroform	10	10	10	10
Methanol	5	5	5	5

Preparation method Transfersomes

A thin-film hydration technique was employed to form transferosomes. Specific amounts of lipids were dissolved in a mixture of organic solvents consisting of methanol and chloroform (1:2) in a dry round-bottom flask. The evaporation of organic solvents was performed under vacuum using a rotary evaporator at 100 rpm, at 48-50 °C. The thin lipid film thus obtained was then hydrated using water containing surfactant and drug by rotation for 1 hour at 50 °C. Each formulation was placed in an ultra sonicator at 150 W for 20 seconds. A dialysis bag was used to remove the free drug, and the final formulation was stored at 4 °C for further use. Blank transfersomes were prepared using the same method for each formulation.

Characterization

Vesicle size

Transfersomes vesicles can be visualized by SEM and optical microscope. The Morphological characterization of transfersome vesicle such as shape and surface feature were projected by using optical microscope and SEM.

SEM Analysis

The shape, surface characteristics, and size of the Transferosomes were observed by scanning electron microscopy. Once again, 0.2 ml of the Transferosomes in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The Transferosomes were mounted on an aluminum stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage.

Determination of entrapment efficiency percentage:

The amount of Ketoconazole entrapped in transfersomes was estimated by centrifugation method. 1gm of Transfersomes was taken and diluted with 10ml phosphate buffer (pH 7.4). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution was placed in centrifugation tube and centrifuged at 14000 rpm for 30 minutes. 0.5ml of supernatant was withdrawn and diluted before going for absorbance measurement using UV spectrophotometer (UV-2960 Lab India). This gives us the total amount of unentrapped drug. Entrapment efficiency is expressed as the percent of drug trapped.

$$\% \text{ Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

In-vitro drug release studies

Modified Franz diffusion cell with a receiver compartment volume of 10ml and effective diffusion area of 2cm2 was used for this study. In-vitro drug study was performed by using dialysis membrane in phosphate buffer solution (pH 7.4). To perform in-vitro drug release study, dialysis membrane was mounted horizontally on the receptor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2cm2 and capacity of receptor compartment was 10ml. The receptor compartment was filled with 10ml of phosphate buffer (pH 7.4) maintained at 37± 0.50C and stirred by a magnetic bar at 100rpm. Transfersomes formulation equivalent to 80mg drug was placed on the dialysis membrane and the top of the diffusion cell was covered. At appropriate time intervals 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffer (pH 7.4) to maintain sink conditions. The samples were analyzed spectrophotometrically at λ max 225 nm.

Stability Studies of Transfersomes

After measuring the initial percentage entrapment of the drug in the optimized formulation, the three batches of the same formulation were stored in sealed glass ampoules (one each) at refrigeration temperature (4±2oC), room temperature (25±2oC) and body temperature (37±2oC) for a period of at least 3 months. The percentage entrapment of the drug and % drug content was determined in the formulations after 90 days to know the amount of drug leaked out. The percent drug lost was calculated taking the initial entrapment of drug as 100%.

RESULTS & DISCUSSION

Particle size of Transferosomes

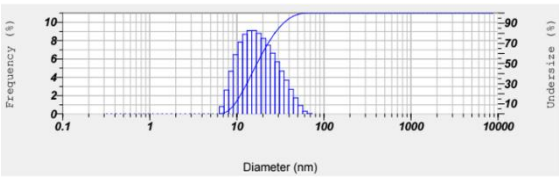


Fig-1: Particle size of Optimized formulation

The mean particle size of optimized transferosomes was found to be 122 nm

Table-2: Particle size

F. No	Particle size
F1	131
F2	135
F3	122
F4	125

SEM Analysis

Scanning electron microscopy (SEM) revealed that the Ketoconazole transferosome's were smooth and spherical without any aggregation.

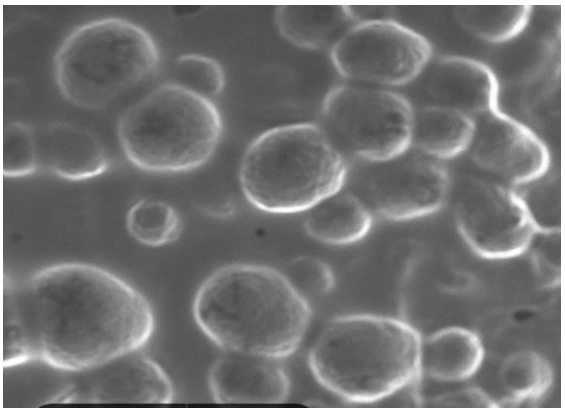


Fig-2: SEM Analysis of Transfersomes

Entrapment efficiency

Table-3: Percentage entrapment efficiency

F. No	% EE
F1	79.63
F2	81.23
F3	82.39
F4	84.20

The optimized formulation (F4) was selected based on its highest entrapment efficiency (84.20%), indicating superior drug loading capacity.

In vitro drug release studies

The diffusion medium used was phosphate buffer solution pH 7.4. In the Franz diffusion cell, the Dialysis membrane was mounted between donor and receptor compartment of diffusion cell. Area of membrane separating two compartments was 1.7662 cm2. Transfersomes vesicle (2 ml) volume was accurately pipetted into donor compartment which was then covered with aluminum foil to avoid any evaporation. Diffusion medium (10 ml) was maintained at 37 ±1 o C, so that

the membrane just touches the receptor medium surface. A magnetic bar continuously stirred in the diffusion medium at 100 rpm. Each of 1 ml volume was withdrawn at required time intervals and replaced by 1 ml volume of receptor medium (phosphate buffer pH 7.4) to maintain the sink condition. These samples were analyzed by UV spectrophotometer at maximum wavelength 225 nm.

Table-4: Diffusion studies of Transfersomes

Time	F1	F2	F3	F4
0	0	0	0	0
1	27.10	24.60	26.39	30.25
2	35.13	32.99	38.56	42.16
3	45.32	55.37	56.83	49.80
4	56.35	65.10	67.89	69.86
6	78.10	77.52	78.15	79.54
8	94.50	95.69	92.38	97.53

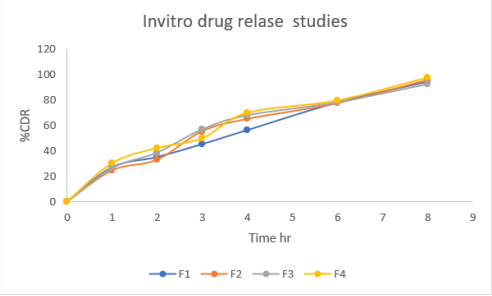


Fig-3: Diffusion studies of Transfersomes (F1-F4)

Stability studies:

There was no significant change in physical and chemical properties of the transferosomes formulation F4 after 3 months. Parameters quantified at various time intervals were shown.

Table-5: Results of stability studies of optimized formulation F-4

F. Code	Parameters	Initial	1st Month	2ndMonth	3rd Month	Limits as per Specifications
F-4	250C/60%RH % Release	97.53	96.60	95.55	94.46	Not less than 85 %
F-4	300C/75% RH % Release	97.53	96.57	95.50	94.46	Not less than 85 %
F-4	400C/75% RH % Release	97.53	96.16	95.12	94.46	Not less than 85 %

SUMMARY AND CONCLUSION

The present study was undertaken to formulate and evaluate Ketoconazole-loaded transferosomes for improved antifungal therapy. Preformulating studies were performed to assess the suitability of Ketoconazole for transferosomal formulation development. FTIR compatibility studies indicated the absence of significant interactions between Ketoconazole and the selected excipients, confirming the compatibility and stability of the drug within the formulation. Ketoconazole-loaded transferosomes were successfully prepared and evaluated for particle size, entrapment efficiency, surface morphology, in-vitro drug release, and stability. The particle size of the prepared formulations ranged from 122 to 135 nm, indicating the formation of nanosized vesicles suitable for enhanced skin permeation. SEM analysis revealed that the transferosomes were spherical, smooth, and free from aggregation. Entrapment efficiency studies demonstrated efficient drug incorporation within the vesicular system, with formulation F4 exhibiting the highest entrapment efficiency of 84.20%. In-vitro diffusion studies showed sustained drug release from all formulations over a period of 8 hours. Among the formulations tested, F4 exhibited the highest cumulative drug release of 97.53%, indicating superior release characteristics. Stability studies conducted for three months under various storage conditions demonstrated that the optimized formulation (F4) remained stable, with drug release values remaining within acceptable limits, indicating good physical and chemical stability.

Conclusion

The present study successfully formulated and evaluated Ketoconazole-loaded transferosomes for improved antifungal therapy. The transferosomal formulations exhibited nanosized vesicles with satisfactory entrapment efficiency, spherical morphology, and sustained drug release characteristics. Among all the formulations, F4 was identified as the optimized formulation based on its highest entrapment efficiency (84.20%) and maximum cumulative drug release (97.53%). The enhanced drug release profile and nanoscale particle size suggest the potential of transferosomes to improve skin permeation and therapeutic effectiveness of Ketoconazole. Furthermore, stability studies confirmed that the optimized formulation remained stable under different storage conditions for a period of three months. Therefore, Ketoconazole-loaded transferosomes can be considered a promising vesicular drug delivery system for enhancing antifungal therapy by improving drug delivery, increasing therapeutic efficacy, and potentially reducing the frequency of administration.

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