

Available online on 15.08.2026 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



Open Access Full Text Article



Research Article

Development of Ketoconazole-Loaded Transferosomal Hydrogel: Formulation Optimization and Evaluation for Enhanced Topical Antifungal Delivery

Anushka A. Shah ¹, Rachana B. Patil ¹, Preeti G. Karade ^{*1}¹Department of Pharmaceutics, Appasaheb Bimale College of Pharmacy, Sangli. 416416, Maharashtra, India

Article Info:



Article History:

Received 12 May 2026
Reviewed 28 June 2026
Accepted 22 July 2026
Published 15 August 2026

Cite this article as:

Shah AA, Patil RB, Karade PG, Development of Ketoconazole-Loaded Transferosomal Hydrogel: Formulation Optimization and Evaluation for Enhanced Topical Antifungal Delivery, Journal of Drug Delivery and Therapeutics. 2026; 16(8):73-82
DOI: <https://doi.org/10.22270/jddt.v16i8.7912>

For Correspondence:

Preeti G. Karade, Department of Pharmaceutics, Appasaheb Bimale College of Pharmacy, Sangli. 416416, Maharashtra, India

Abstract

The present study focuses on the development and evaluation of a novel ketoconazole-loaded transferosomal hydrogel designed to enhance transdermal delivery for effective treatment of fungal infections. Transferosomes, flexible vesicular carriers composed of phospholipids and edge activators, were employed to improve drug encapsulation and skin penetration. A 3² full factorial design was used to optimize critical formulation parameters. The optimized vesicles were thoroughly characterized for particle size, zeta potential, entrapment efficiency, and other physicochemical properties. To enable topical application, the transferosomes were incorporated into a hydrogel matrix, which was subsequently for drug content estimation, viscosity, *in vitro* diffusion, and antifungal activity. Drug release followed the Higuchi model, characterized as a diffusion-controlled process where the drug moves from a polymeric matrix into the surrounding medium. An excellent match is indicated by the high R² value (R² value of 0.9941), which confirms that Fickian diffusion through the hydrogel matrix. The results demonstrated that the transferosomal hydrogel exhibited zone of inhibition of 46mm and conventional formulation exhibited zone of inhibition of 30mm, transferosomal hydrogel exhibited superior antifungal efficacy against *Candida albicans* and significantly enhanced epidermal penetration and retention in contrast to traditional formulations. 85.86±5.68% of Transferosomal hydrogel drug was diffused through the goat skin while the marketed cream was only 45.78±2.56 diffused through the goat skin within 8 hours. These findings underscore the potential of this system as a promising and effective topical antifungal therapy.

Keywords: Ketoconazole, Phospholipid, Vitamin E, Transferosomes, Hydrogel, *Ex Vivo* Skin Permeation.

INTRODUCTION

Fungal infections commonly act on the external layers of the body, including the skin, nails, and mucous membranes. Candidiasis is one of the most common fungal infections and, in certain cases, can extend into deeper tissues, particularly among individuals with weakened immune systems. It typically develops in moist, warm, and creased regions of the body, such as under the arms and between the buttocks. Ketoconazole, a compound derived from the imidazole class, is recognized for its potent and wide-ranging antifungal properties. It is commonly employed in managing both superficial and systemic fungal infections. The drug's antifungal mechanism works by disrupting the synthesis of ergosterol, a vital constituent of fungal cell membrane. When administered orally, Ketoconazole (KTZ) is subject to substantial first-pass metabolism in the liver and gastrointestinal system, requires frequent dosage, and carries a risk of severe liver damage. For localized infections, topical formulations are generally preferred over oral administration, as they minimize systemic exposure

while providing targeted therapeutic effects directly at the site of infection¹.

One of the best medication delivery methods for topical administration is transferosomes, which are phospholipid vesicles containing edge activators. Due to their high deformability, transferosomes are capable of passing through the skin's microscopic pores as intact vesicles. This unique flexibility arising from the incorporation of edge activators, which impart the vesicles with their characteristic elasticity. The trans-epidermal osmotic gradient between the external and internal surfaces of the epidermis facilitates the elastic transport. They may pass through pores that are smaller than themselves because of their elasticity².

The purpose of this research was to formulate and assess a (KTZ) -loaded transferosomal gel with the goal of improving skin penetration and enhancing antifungal efficacy. Candidiasis served as the model infection to compare the antifungal performance of the developed (KTZ) -loaded transferosomal gel formulation against that of a commercially available product³.

MATERIALS AND METHODS

Materials:

Phospholipon 90 G was procured from Chemet India, while ketoconazole was obtained from Aarti Drugs Ltd. in Tarapur, India. TPGS was procured from Matrix Life science Pvt Ltd, Aurangabad, Maharashtra, India. We acquired Carbopol 934 from Charon Pharma Chem Industries in Mehsana, Gujarat, India. Other chemicals and solvents used were of analytical quality.

Methods of Formulation of Ketoconazole Transferosomes:

The ethanol injection technique was utilized for the preparation of the Ketoconazole (KTZ) Transferosomes (TFS). An aqueous phase was generated by dissolving 100 mg of KTZ and a fixed amount of surfactant (TPGS) in 10 mL of phosphate buffer (pH 7.4). Using a hot plate magnetic stirrer phospholipid was dissolved in 4 mL of anhydrous ethanol until a clear solution that serves as an organic phase was obtained.⁴ Both solutions were then heated to 50°C while being magnetically agitated at 600 rpm. An ethanol-based phospholipid solution was then added dropwise while the aqueous solution was constantly agitated for 20 minutes. Ethanol was removed by transferring the obtained dispersion to a vacuum evaporator, followed by sonication to reduce particle size. A 3² full factorial design was applied using Design-Expert® v13 to evaluate the combined influence of phospholipid and surfactant concentrations on particle size (PS) and entrapment efficiency (%EE).

Characterization of transferosomes

Particle Size and Polydispersity index: Particle size and surface charge of the prepared KTZ-loaded transferosomal dispersion was determined using the Horiba Zetasizer⁴.

Entrapment Efficiency: The entrapment efficiency of ketoconazole transferosomes was evaluated using centrifugation method. The transferosomal dispersion was placed into centrifuge and run at 3000 rpm for 30 min. The top layer was withdrawn and diluted with phosphate buffer (pH 7.4) and the amount of untrapped KTZ was measured using UV Spectrophotometer set at 225nm⁵.

$$\%EE = \frac{\text{Total Drug Content} - \text{Free Drug content}}{\text{Total Drug Content}} \times 100 \quad \text{Eq. 1}$$

Determination of Zeta Potential: The transferosome sample was suspended in Milli-Q water and screened for zeta potential at 25°C by Zetasizer (Horiba SZ-100)⁶.

Transmission Electron Microscopy: Morphological characterization of the formulation was performed using a JEOL TEM 2100 Plus transmission electron microscope operated at 200 kV. For sample preparation, a diluted vesicular dispersion was carefully applied onto a copper grid with a carbon support film. Excess material was removed by gently blotting with filter paper. Subsequently, a drop of 1% phosphotungstic acid was added as a negative stain. The samples were then air-dried prior to imaging⁷.

Differential Scanning Calorimetry: Differential scanning Calorimetry (Perkin Elmer DSC 4000) analysis was conducted for KTZ and optimized KTZ-TFS. Samples were heated from 20 – 300°C at rate of 10°C/min under nitrogen purge. Thermal transitions were observed to assess drug carrier interactions⁸.

In Vitro Drug Diffusion: The quantity of KTZ diffused from TFS nanovesicles was measured using the dialyzing technique (using Franz diffusion cells with cellulose membrane) for eight hours at 37°C and 100 rpm. The dialyzing medium was 100 mL of Phosphate Buffer pH 7.4, and a weighed amount of prepared transferosomal solution (equivalent to 2 mg of the KTZ) was added to the donor cell. The drug content of the aliquots was measured at 255 nm at intervals of 0.5, 1, 2, 3, 4, 5, 6, and 8 hours⁹.

Stability Study: For 30 days, TFSs were kept at temperature (25.0 ± 1.0 °C) and at 4.0 ± 1.0 °C.⁽⁴⁾ Stability of the formulation was evaluated by measuring the size of the particles at defined intervals the developed formulation was then visually inspecting for sedimentation¹⁰.

Incorporation of Optimized Ketoconazole loaded Transferosomal formulation into Hydrogel: -

Preparation of Transferosomes loaded Hydrogel:

The transferosome-loaded hydrogel was prepared by first dissolving Carbopol 934P in a measured volume of water under constant stirring at 600 rpm, followed by the addition of methyl paraben sodium (0.02% w/v) and propyl paraben sodium (0.1% w/v), with continued agitation for 30 minutes. The resulting gel base was allowed to stabilize for 24 hours. Subsequently, KTZ TFS was dispersed in propylene glycol and 1% ethanol, and this dispersion was gradually incorporated into the preformed Carbopol gel under stirring at 1000 rpm, with further mixing for 30 minutes to obtain the hydrogel. Finally, triethanolamine (TEA) was added to adjust the pH to 5.5–6.5, yielding a clear and homogenous gel formulation¹¹.

Evaluation of transferosomal hydrogel:

Homogeneity: Semisolid dosage forms spread uniformly over the skin surface need to be consistent for patient compliance. This was accomplished by pressing a little amount of the generated gels between the thumb and index finger. The consistency was evaluated to see if it was homogenous or not¹².

pH: The pH of the formulated transferosomal gels was determined using a calibrated pH meter. For measurement, each gel sample was diluted with 10 mL of distilled water to obtain a uniform solution, after which readings were recorded. Three independent measurements were taken, and the mean value along with the standard deviation (mean ± SD) was calculated to ensure accuracy and reproducibility of the results¹³.

Spreadability: By placing 0.5 g of the formulation placed between a pair of circular glass plates, the spreadability of the transferosomal gel was assessed. To maximize spreading, the system was left undisturbed for five

minutes while a steady weight was applied. The spreadability was determined by measuring the diameter of the resultant circular film¹⁴.

$$S = \frac{\text{Weight} \times \text{Length}}{\text{Time}} \quad \text{Eq. 2}$$

Viscosity: The Brookfield DV III Ultra programmable Rheometer was used to determine the viscosity of the gel. A spindle was used to test the viscosity of ten grams of gel in a beaker at 100 rpm¹⁵.

Drug content: The dissolution study was executed by dissolving one gram of gel in 100 mL of phosphate buffer at pH 7.4. Appropriate dilutions were then prepared using the same phosphate buffer (pH 7.4) to ensure consistency throughout the experiment. A UV spectrophotometer was used to detect absorbance at 225 nm¹⁶.

$$\text{Drug Content} = \frac{\text{Amount of drug found in Hydrogel}}{\text{Theoretical amount of drug added}} \times 100 \quad \text{Eq. 3}$$

Extrudability: The formulated gel was filled into standard capped collapsible aluminum tubes, which were sealed by crimping, and the net weight of each tube was recorded. For the extrudability test, each tube was placed between two glass slides and secured with clamps, after which a 500 g weight was applied to ensure uniform pressure. Upon removing the cap, the expelled gel was carefully collected and weighed, and extrudability was expressed as the percentage of gel obtained after extrusion relative to the total weight of the formulation in the tube. Based on these percentages, grades were assigned to evaluate the extrudability performance¹⁷.

In vitro Drug Diffusion Study: Franz diffusion cell was used to conduct an *in vitro* drug diffusion study. The donor and receptor compartments of the diffusion cell were separated by a dialysis membrane. The donor compartments were filled with one gram of transferosomal gel loaded with KTZ TFS. Then the recipient chambers were filled with 25 mL of 7.4 pH PBS. A magnetic stirrer was used to secure the entire unit. The solution in the receptor compartment was continuously swirled at 50 rpm with a magnetic bead while the temperature was maintained at $32 \pm 0.5^\circ\text{C}$. The drug concentration was measured using a UV-Vis spectrophotometer set to 225 nm after the samples were removed at different times. The receptor phase was substituted with an equal volume of the same medium. The *in vitro* drug release data from transferosomal gel were kinetically analysed using a range of mathematical models, including zero-order, first-order, Higuchi, and Kores Meyer-Peppas model equations¹⁸.

In Vitro Antifungal Activity: The agar well diffusion technique was used to measure the antifungal activity of KTZ TFS. The agar medium was made by combining 20 grams of sabouraud dextrose agar powder with 200 millilitres of distilled water. It was then autoclaved for 20 minutes at 121° degrees Celsius. Under aseptic circumstances, the produced agar medium was transferred into sterile glass petri plates. After the agar medium had solidified, the surface of each plate was

punctured with small wells using a sterile cork borer. The agar surface was then streaked with *Candida albicans* to achieve inoculation, and uniform wells of 6 mm diameter were created in each of the three petri dishes. Following the placement of the respective formulation samples into the wells, the plates were allowed to stand for at least 30 minutes before incubation at $25 \pm 1^\circ\text{C}$ for 24 hours. After incubation, the inhibitory zones were measured by recording the most consistent external diameter, which included the diameter of the wells. All experiments were performed in triplicate to ensure reliability of the results¹⁹⁻²⁰.

Ex Vivo Skin Permeation Study: Study was accomplished through the goat skin (hair removed from the dorsal side) using a Franz diffusion cell. For the permeation study, phosphate buffer at pH 5.5 was used as the receptor medium and maintained at $35 \pm 0.5^\circ\text{C}$. The transferosomal gel (T-gel) and the marketed gel (M-gel) were separately mounted onto excised goat skin, which was then positioned between the donor and receptor compartments to facilitate the experiment. At regular intervals, 1 mL of the sample was withdrawn and replaced with a fresh medium to maintain the sink for up to 7 h. Samples were analyzed using a UV-visible spectrophotometer at 260 nm. After completion of the test, the goat skin was cautiously detached, and the formulation attached to the skin was scraped off²¹.

Skin Retention Study: The skin was cut into small pieces and immersed in a 10 mL mixture of methanol and phosphate buffer solution with pH 5.5 (Kumar, 2015). The mixture was stirred for 30 min, and the extract was analyzed using a UV-visible spectrophotometer at 260 nm to determine the drug retained in the skin²².

Stability Studies: The optimized KTZ loaded hydrogel was stored in sealed containers at three different temperatures ($4 \pm 1^\circ\text{C}$, $25 \pm 1^\circ\text{C}$, and $40 \pm 1^\circ\text{C}$) with a relative humidity of $75 \pm 5\%$ for three months. Periodically, the pH and KTZ content of the gel formulation was measured. The gel was checked for its physicochemical properties and physical appearance²².

RESULT:

Characterization of transferosomes:

Particle size and polydispersity index: Using different drug-to-polymer ratios, KTZ-loaded TFS were effectively created. Particle size of the formulations ranged from 51.7 nm to 75.9 nm while their PDI values varied between 0.354 and 0.517. uniform dispersion and stability of Transferosomes was highlighted by their nanoscale particle size and good PDI value, which qualifies them for efficient drug delivery applications²³. Table No. 1 summarizes the particle size and PDI results for all formulations, and Fig no. 1 shows the particle size distribution. Formulation B3, the optimized batch, showed a PDI of 0.354 and a particle size of 73.9 nm. Polynomial equation proposed by the model for Particle size is as follows;

$$Y_1 = +58.71 + 9.42A + 0.6333B - 1.18AB + 7.48A^2 - 2.17B^2$$

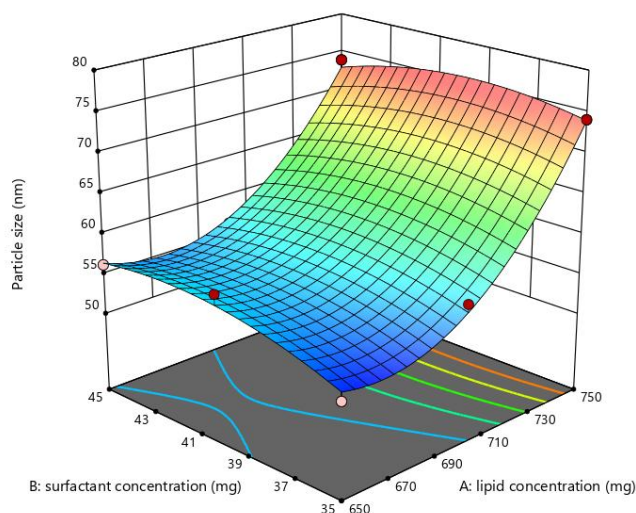


Figure 1: 3D surface plot showing the combined effect of Phospholipid concentration and TPGS concentration on Particle Size

Entrapment efficiency: The efficacy of these formulations in encapsulating the drug was demonstrated by their entrapment efficiency (%EE), which varied between 88.02 ± 0.025 and $97.95 \pm 0.02\%$. Table No.1 Displays the entrapment efficiency findings for each formulation and fig no.2 shows entrapment efficiency distribution. The capability of the TFS to load and maintain the medication is a crucial metric that determines entrapment efficiency, which plays a vital role for guaranteeing the intended therapeutic effects²⁴.

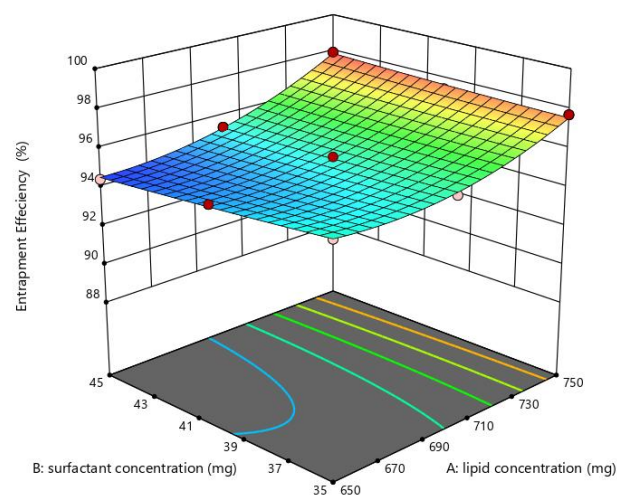


Figure 2: 3D surface plot showing the combined effect of Phospholipid concentration and TPGS concentration on Entrapment Efficiency

Polynomial equation proposed by the model for entrapment efficiency is as follows

$$Y_2 = +95.50 + 1.33A - 0.1800B + 0.3300AB + 0.8483A^2 + 0.0433B^2$$

The model recommended for the study design was a quadratic model based on the dependent and independent variables. Model F value 56.23 tells us that the model is significant. Model terms are significant. The small difference between Pred R^2 (0.8801) and Adj R^2 (0.9718) indicates good agreement. Adequate Precision of 19.145 (>4) confirms a strong signal, supporting reliable optimization and exploration of the design space.

Table 1: Formulation of Transfersomes by 3^2 factorial design

Batches	Independent variables		Dependent variables	
	Phospholipon 90 G (X_1 , mg)	TPGS (X_2 , mg)	Particle Size (Y_1 , nm)	Entrapment efficiency (Y_2 , %)
B1	650	45	56.2 ± 0.589	94.42 ± 0.026
B2	650	35	51.7 ± 0.789	95.57 ± 0.015
B3	700	45	56.4 ± 0.807	95.52 ± 0.02
B4	750	45	73.9 ± 0.963	97.95 ± 0.025
B5	700	40	58.5 ± 0.872	95.61 ± 0.021
B6	650	40	58.1 ± 1.178	95.16 ± 0.020
B7	750	35	74.1 ± 0.829	97.74 ± 0.020
B8	700	35	56.9 ± 0.822	95.54 ± 0.030
B9	750	40	74.5 ± 0.915	97.42 ± 0.020

Determination of Zeta potential: A zeta potential of -25 mV for KTZ-TFS fig no. 3(b) suggests that the vesicles will stay stable throughout time, lowering the possibility of particle aggregation and sedimentation. Maintaining

stability is crucial to ensuring consistent drug delivery, as aggregation can influence particle size distribution, disrupt release mechanisms, and reduce the formulation's therapeutic effectiveness²⁵.

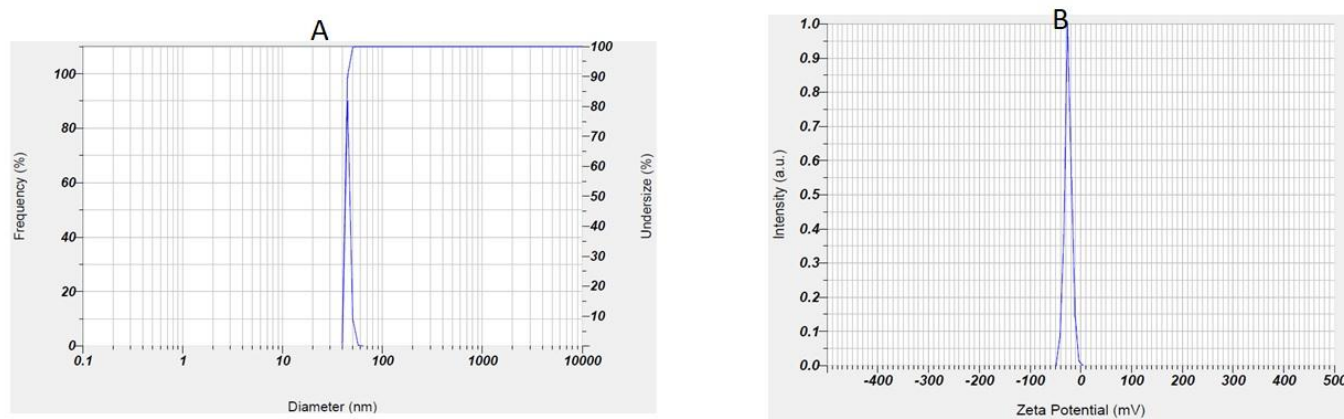


Figure 3:(a) Particle Size and PDI (b) Zeta Potential of optimized formulation

Transmission Electron Microscopy (TEM): Analysis of KTZ-TFS revealed a spherical form. TEM was used to view morphology of formulation. Vesicles with a consistent spherical shape were visible in the TEM

micrographs. The smooth surface and narrow size distribution of the vesicles gave the impression that they were not aggregated²⁶.

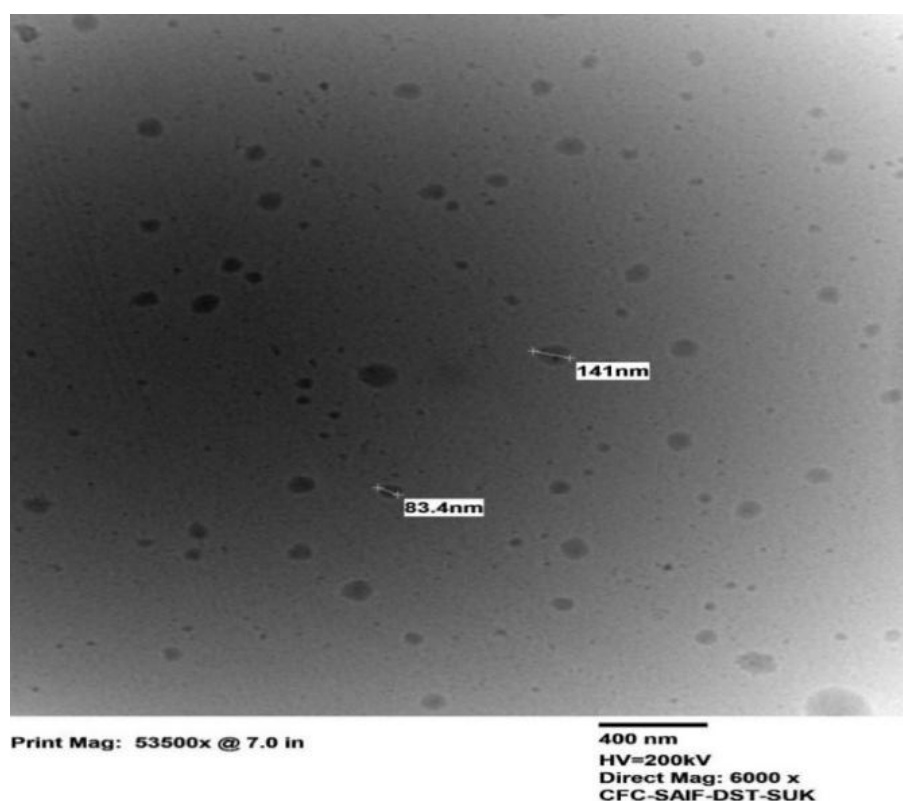


Figure 4: Transmission Electron Microscopy image of the optimized Ketoconazole loaded Transfersomes

Differential Scanning Calorimetry of KTZ-TFS: Differential Scanning Calorimetry thermogram of pure KTZ revealed a clear, impulsive endothermic peak at 150.47 °C, indicating its melting point in fig no.5.a). The sharp endothermic melting peak of pure KTZ (around 150–151 °C) is either significantly reduced in intensity, shifted, or completely disappears in the DSC thermogram of the TFS formulation showed in fig

no.5.b). This alteration signifies that ketoconazole is no longer in its crystalline state but is now molecularly diffused or present in an amorphous state within the lipid bilayer of the TFS. The disappearance or broadening of the melting peak confirms successful encapsulation and interaction of KTZ with the TFS components, reflecting improved drug solubilization and possible enhancement in bioavailability²⁷.

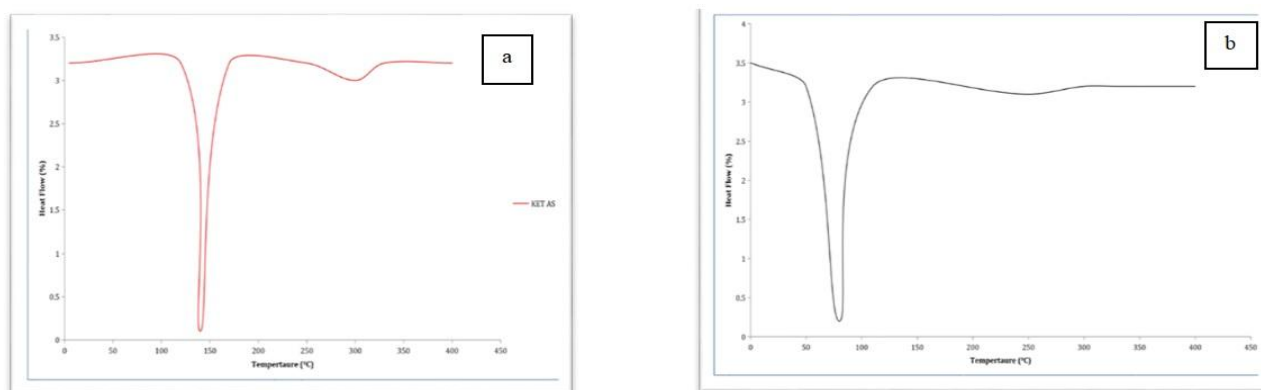


Figure 5:(a) DSC thermogram of pure KTZ and (b) KTZ TFS

In-vitro drug release study: Majority of KTZ stays encapsulated within the transferosomal vesicles throughout the early phase, as seen by the low percentage of drug released (10.22%) at one hour, which supports a limited burst effect. In addition to lowering the possibility of local discomfort, this controlled release makes sure that KTZ doesn't quickly run out of the formulation. Most of the ketoconazole stays encapsulated within the TFS vesicles throughout the early phase, as evidenced by the low proportion of medication released at one hour, which supports a modest burst effect. The ability of the transferosomes to transport drugs continuously is demonstrated by the noticeable rise in cumulative release over an 8-hour period. For topical antifungal therapy, this profile is beneficial because it can sustain therapeutic drug levels at the application site for an extended period of time, which may lower the frequency of doses and increase patient compliance²⁸.

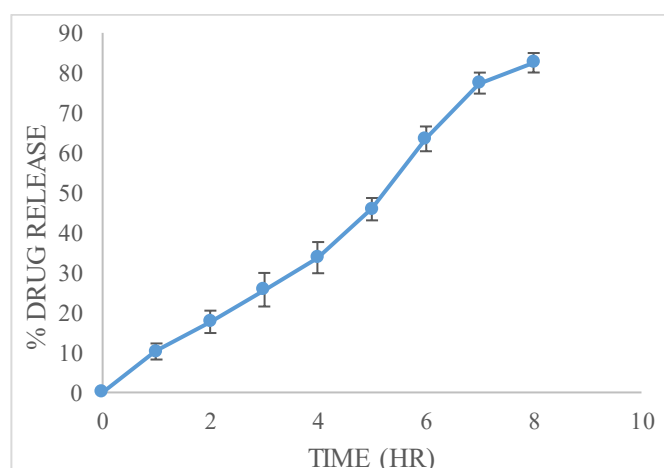


Figure 6: *In-vitro* drug release of TFS Carrier System.

Stability study: Formulations of TFSs that were refrigerated at 4.0°C revealed no sedimentation and were resistant to fusion and aggregation. They also displayed polydispersity and a non-significant decrease in size. On the other hand, those kept at 40°C displayed a small amount of sedimentation that was readily redispersed with a little shaking. They demonstrated a

shift in particle size and entrapment efficiency. Furthermore, the contents remained constant throughout the storage period, demonstrated improved stability. Augmentation of particle size may be potentially due to vesicle aggregation and swelling at this elevated temperature, which may also help improve their physical stability. The Particle size was noted to be 73.9 nm before study and after one month was found to be 75.7 nm while entrapment efficiency changes from 97.93 ± 0.003 to 95.81 ± 0.003 after 1 month²⁹.

Incorporation of KTZ-loaded transferosomes into hydrogel (HDG): Transferosomal suspension containing the drug was successfully incorporated into a Carbopol-based hydrogel. The dispersion was mixed thoroughly with the hydrated Carbopol gel base using gentle mechanical stirring to ensure uniform distribution of vesicles throughout the gel matrix³⁰.

Homogeneity: The homogeneity test of TFS hydrogel formulations confirms that the gel was smooth, uniform, and free from lumps or phase separation.

pH: The pH of the optimized KTZ-TFS hydrogel was evaluated to ensure its suitability for topical administration without causing irritation to the skin. The pH of the optimized batch was found to be 6.34 ± 0.24 . This result confirms that the prepared formulation falls within the acceptable physiological range for topical application, indicating its suitability and safety for topical administration.

Spreadability: Transferosomal hydrogels were shown to have a spreadability of 0.352 g·cm/sec, indicating a formulation that is ideal for topical drug delivery, easy to apply, and distributed uniformly.

Viscosity: Viscosity is crucial to ensure that the transferosomal hydrogel remains at the site of application on the skin. Low-viscosity formulations, such as simple vesicular suspensions, tend to flow away or fail to adhere, resulting in poor retention and reduced therapeutic efficacy. The rheology of the hydrogel affects its spreadability. A formulation with appropriate viscosity ensures easy application and uniform coverage, improving user experience and adherence to treatment. The viscosity of KTZ-TFS-based hydrogel was demonstrated to be 938.8 ± 12.50 Cp.

Drug content: A UV spectrophotometer was used to estimate the drug content. The KTZ TFS hydrogel formulation exhibited a drug content of $98.42 \pm 0.55\%$.

Extrudability: Extrudability of KTZ-TFS-based hydrogel was found to be 0.45 g/10 sec.

In Vitro Diffusion Study: The investigation was conducted to compare the release patterns of KTZ from the KTZ TFS hydrogel formulation and a marketed KTZ cream over an 8-hour period. At the end of 8 hours, the KTZ-TFS hydrogel exhibited a cumulative drug release of 85.86%, demonstrating a significantly higher and sustained release profile. In contrast, the marketed ketoconazole cream showed a cumulative drug release of only 58.78 % at the same time point. These findings indicated that the KTZ-TFS loaded hydrogel enables a more efficient and prolonged release of KTZ compared to the conventional marketed cream. The enhanced drug release from the transferosomal system can be attributed to its nanosized vesicular structure, which facilitates better drug permeation and diffusion across the membrane. This improved release profile suggests that the TFS hydrogel may provide superior therapeutic efficacy and sustained antifungal action compared to the marketed cream³¹.

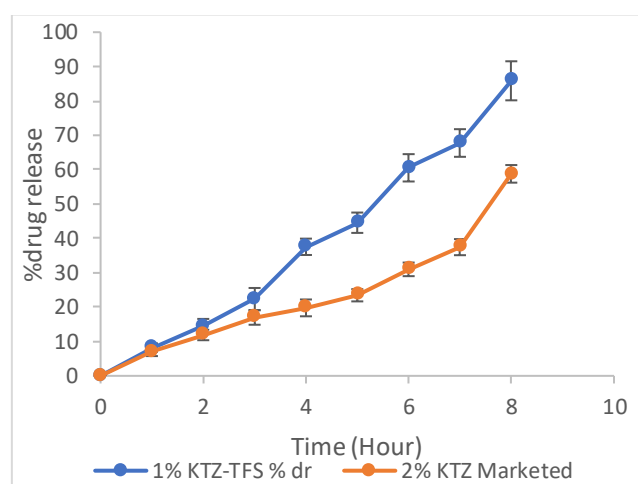


Figure 7: *In Vitro* diffusion study of 1 % KTZ TFS Hydrogel and 2% KTZ Marketed Cream.

In Vitro drug release Kinetics Studies: Drug release follows the Higuchi model, characterized as a diffusion-controlled process where the drug moves from a matrix system into the surrounding medium. An excellent match is indicated by the high R^2 value (R^2 value of 0.9941), which confirms that Fickian diffusion through the hydrogel matrix is the primary mechanism governing KTZ release. The diffusion-driven release mechanism indicates that KTZ is slowly transported from regions of higher concentration within the hydrogel toward the surrounding medium. This form of release is especially advantageous for topical drug delivery systems, as it facilitates a prolonged and regulated drug release profile. This, in turn, may enhance therapeutic outcomes and support patient adherence by minimizing the frequency of repeated application. The observed sustained release is generally credited to the synergistic action of the hydrogel's

structural matrix and the vesicle-based architecture of TFS, which together modulate the drug's outward diffusion³².

In Vitro Antifungal Activity: Using the agar well diffusion technique, the antifungal efficacy of the 1% KTZ-TFS hydrogel was evaluated and contrasted with that of a commercially marketed 2% ketoconazole cream. The TFS hydrogel exhibited a prominent zone of inhibition measuring 46 mm, indicative of potent antifungal activity. Under comparable test conditions, the marketed cream produced a smaller inhibition zone of 30 mm. Despite containing a lower concentration of KTZ, the TFS hydrogel demonstrated superior antifungal performance against *Candida albicans*. This enhanced effectiveness is attributable to the improved drug delivery and deeper skin penetration facilitated by the TFS carrier system³³.

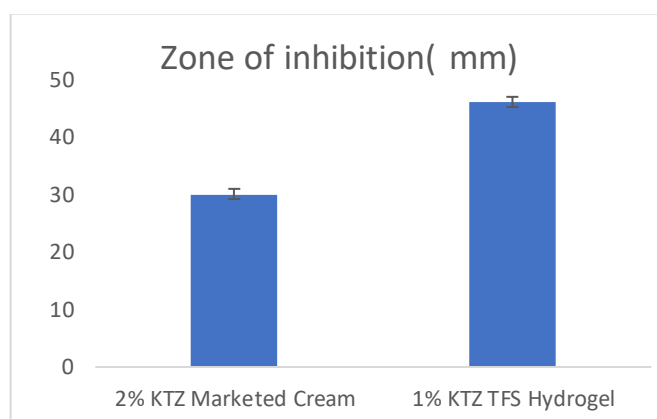


Figure 8: *In Vitro* Antifungal Activity.

Ex Vivo Skin Permeation Study: Transferosomal Hydrogel formulation significantly increased the diffusion of ketoconazole through the goat skin in comparison with marketed Cream. $79.86 \pm 5.68\%$ of TFS HDG drug was diffused through the goat skin while the marketed cream was only 45.78 ± 2.56 diffused through the goat skin within 8 hours. Thus, this test suggested that the diffusion of TFS HDG was more in contrast to commercial cream because of the ultra-deformability property of transferosomes³⁴.

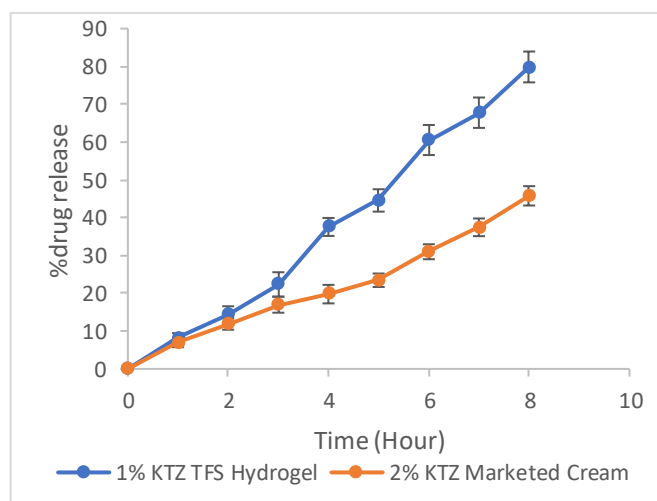


Figure 9: *Ex Vivo* % Skin Permeation

Skin Retention Study: The transferosomal hydrogel shows significantly higher skin retention, because transferosomes can change their membrane flexibility, contributes easy passage of drugs through the skin layer. Promotes skin adhesion and hydration, enhances the residence time.³⁴

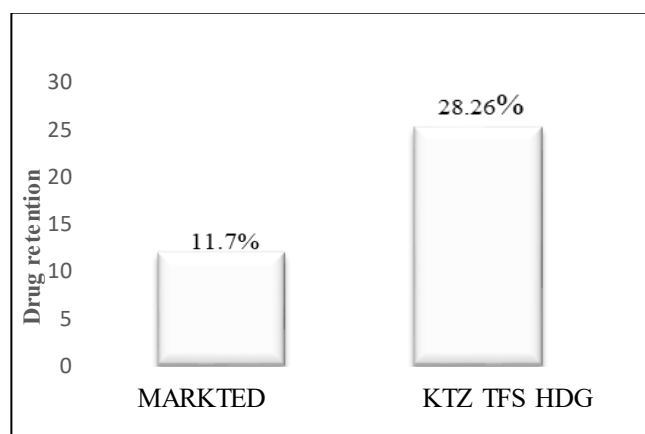


Figure 10: Skin Retention Study

Stability Studies: Transferosomal gels loaded with KTZ were analysed for physical appearance and pH. Physical appearance and pH characteristics of the transferosomal gels did not significantly alter, according to the stability study results. The drug content was slightly reduced at both the temperature. The Physical appearance initially at 4°C and at 25°C was observed to be white and clear and after 3 months appeared to be clear and white. The pH at 4°C and 25°C initially was found to be 5.26 and 5.57 and the pH after 3 months at 4°C and 25°C was found to be 5.26 and 5.58³⁵.

DISCUSSION

The present study successfully developed and optimized a ketoconazole-loaded transferosomal (KTZ-TFS) hydrogel for enhanced topical antifungal therapy. The transferosomes exhibited desirable physicochemical characteristics, with particle sizes ranging from 51.7 to 75.9 nm and PDI values of 0.354–0.517, indicating a narrow size distribution and homogeneous vesicle population. The optimized formulation (B3) demonstrated a particle size of 73.9 nm with a PDI of 0.354, confirming the suitability of the formulation for efficient topical delivery. High entrapment efficiencies (88.02–97.95%) reflected the excellent drug-loading capacity of the vesicular system. Statistical analysis further confirmed the adequacy and significance of the optimization model, indicating that the selected formulation variables effectively influenced the critical quality attributes.

The optimized transferosomes also exhibited a zeta potential of -25 mV, suggesting good colloidal stability by preventing vesicle aggregation through electrostatic repulsion. TEM images revealed uniformly distributed spherical vesicles, confirming successful transferosome formation. DSC analysis demonstrated the disappearance of the crystalline drug peak, indicating the conversion of ketoconazole into an amorphous state within the lipid bilayer. This transformation is expected to improve drug dissolution and facilitate sustained

drug release. Accordingly, the in vitro release study showed an initial controlled release followed by gradual drug diffusion over 8 hours, providing prolonged drug availability at the application site.

Incorporation of the optimized transferosomes into a Carbopol hydrogel produced a formulation with appropriate physicochemical properties for topical administration. The hydrogel exhibited good homogeneity, a skin-compatible pH (6.34 ± 0.24), satisfactory spreadability (0.352 g·cm/sec), suitable viscosity (938.8 ± 12.50 Cp), adequate extrudability, and high drug content ($98.42 \pm 0.55\%$). These characteristics indicate that the formulation is convenient to apply and capable of maintaining uniform drug distribution.

The transferosomal hydrogel showed significantly improved drug release compared with the marketed 2% ketoconazole cream, releasing 85.86% of the drug within 8 hours, whereas the marketed formulation released only 58.78%. The enhanced release can be attributed to the nanosized, highly deformable transferosomal vesicles, which facilitate improved drug diffusion through the hydrogel matrix. Drug release followed the Higuchi kinetic model ($R^2 = 0.9941$), indicating that diffusion was the predominant mechanism controlling drug release.

The enhanced performance of the transferosomal hydrogel was further supported by biological and permeation studies. The formulation produced a larger zone of inhibition against *Candida albicans* (46 mm) than the marketed 2% ketoconazole cream (30 mm), demonstrating superior antifungal efficacy despite containing only 1% ketoconazole. Ex vivo permeation studies also showed substantially greater drug permeation ($79.86 \pm 5.68\%$) compared with the marketed cream ($45.78 \pm 2.56\%$) after 8 hours, which can be attributed to the ultradeformable nature of transferosomes that enables efficient passage through the outermost skin layer. In addition, the transferosomal hydrogel exhibited higher skin retention, likely due to enhanced adhesion, hydration of the skin, and prolonged residence of vesicles within the skin layers, thereby increasing local drug availability.

Finally, stability studies demonstrated that the optimized hydrogel maintained its appearance, pH, and drug content with only minimal variations during 3 months of storage, indicating satisfactory formulation stability. Overall, these findings demonstrate that the transferosomal hydrogel is a promising topical drug delivery system capable of improving ketoconazole release, skin permeation, local retention, and antifungal efficacy, making it a potential alternative to conventional topical ketoconazole formulations.

CONCLUSION

The present study successfully developed and optimized a ketoconazole-loaded transferosomal hydrogel with desirable physicochemical properties, high drug encapsulation, and good stability. The optimized hydrogel exhibited sustained drug release, boosted skin permeation and retention, and superior antifungal

activity against *Candida albicans* compared with the marketed ketoconazole cream. These findings suggest that the transferosomal hydrogel is a promising Cutaneous drug delivery system for improving the therapeutic efficacy of ketoconazole for combating fungal skin infections.

Conflict of Interest: The authors have no conflicts of interest regarding this investigation.

Authors Contribution: Anushka A Shah: Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, and Final Approval of the Manuscript.

Preeti G Karde: Examination and interpretation of the data; final approval of the version to be published

Rachna B Patil: Analysis and interpretation of the data; final approval of the version to be published

Acknowledgments: The authors would like to sincerely thank Aarti Drugs Ltd., Tarapur, India for providing Ketoconazole.

REFERENCES:

- Bhosale, V. B., Koparde, A. A. & Thorat, V. M. Vulvovaginal candidiasis-an overview of current trends and the latest treatment strategies. *Microb. Pathog.* 107359 (2025). <https://doi.org/10.1016/j.micpath.2025.107359> PMID:39921042
- Opatha, S. A. T., Titapiwatanakun, V. & Chutoprapat, R. Transfersomes: A promising nanoencapsulation technique for transdermal drug delivery. *Pharmaceutics* 12, 1-23 (2020). <https://doi.org/10.3390/pharmaceutics12090855> PMID:32916782 PMCid:PMC7559928
- Öztürk, A. A. & Aygöl, A. Design of cefaclor monohydrate containing nanoparticles with extended antibacterial effect by nano-spray dryer: A nanoenglobing study. *J. Res. Pharm.* 24, 100-111 (2020). <https://doi.org/10.35333/jrp.2020.115>
- Fathalla, Z. M. A., Khaled, K. A., Hussein, A. K., Alany, R. G. & Vangala, A. Formulation and corneal permeation of ketorolac tromethamine-loaded chitosan nanoparticles. *Drug Dev. Ind. Pharm.* 42, 514-524 (2016). <https://doi.org/10.3109/03639045.2015.1081236> PMID:26407208
- J, R. K. Transfersomes: a wide perspective of novel vesicular drug delivery. *J. Emerg. Technol. Innov. Res.* 9, 299-306 (2022).
- Deng, S. et al. Dually cross-linked core-shell structure nanohydrogel with redox-responsive degradability for intracellular delivery. *Pharmaceutics* 13, 1-32 (2021). <https://doi.org/10.3390/pharmaceutics13122048> PMID:34959330 PMCid:PMC8708258
- Rauwel, P., Küünal, S., Ferdov, S. & Rauwel, E. A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM. *Adv. Mater. Sci. Eng.* 2015, (2015). <https://doi.org/10.1155/2015/682749>
- Raina, N., Rani, R., Thakur, V. K. & Gupta, M. New Insights in Topical Drug Delivery for Skin Disorders: From a Nanotechnological Perspective. *ACS Omega* 8, 19145-19167 (2023). <https://doi.org/10.1021/acsomega.2c08016> PMID:37305231 PMCid:PMC10249123
- Ng, S. F., Rouse, J., Sanderson, D. & Eccleston, G. A Comparative study of transmembrane diffusion and permeation of ibuprofen across synthetic membranes using franz diffusion cells. *Pharmaceutics* 2, 209-223 (2010). <https://doi.org/10.3390/pharmaceutics2020209> PMID:27721352 PMCid:PMC3986717
- Exert, L., Activity, B. A. & Virolisis, L. crossm. 94, 1-24 (2020).
- Shetty, K. & Sherje, A. P. Nano intervention in topical delivery of corticosteroid for psoriasis and atopic dermatitis-a systematic review. *J. Mater. Sci. Mater. Med.* 32, (2021). <https://doi.org/10.1007/s10856-021-06558-y> PMID:34331599 PMCid:PMC8325647
- Motawea, A., Maria, S. N., Maria, D. N., Jablonski, M. M. & Ibrahim, M. M. Genistein transfersome-embedded topical delivery system for skin melanoma treatment: in vitro and ex vivo evaluations. *Drug Deliv.* 31, (2024). <https://doi.org/10.1080/10717544.2024.2372277> PMID:38952058 PMCid:PMC11221477
- Abdellatif, A. A. H. & Tawfeek, H. M. Transfersomal Nanoparticles for Enhanced Transdermal Delivery of Clindamycin. *AAPS PharmSciTech* 17, 1067-1074 (2016). <https://doi.org/10.1208/s12249-015-0441-7> PMID:26511937
- Rahamathulla, M. et al. Simvastatin-Encapsulated Topical Liposomal Gel for Augmented Wound Healing: Optimization Using the Box-Behnken Model, Evaluations, and In Vivo Studies. *Pharmaceutics* 17, (2024). <https://doi.org/10.3390/ph17060697> PMID:38931364 PMCid:PMC11206487
- Raknam, P., Balekar, N., Teanpaisan, R. & Amnuaitkit, T. Thermoresponsive sol-gel containing probiotic's cell free supernatant for dental caries prophylaxis. *J. Oral Microbiol.* 14, (2022). <https://doi.org/10.1080/20002297.2021.2012390> PMID:34992736 PMCid:PMC8725760
- Makwana, S. B., Patel, V. A. & Parmar, S. J. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. *Results Pharma Sci.* 6, 1-6 (2016). <https://doi.org/10.1016/j.rinphs.2015.06.001> PMID:26949596 PMCid:PMC4760229
- Mu, R., Wang, B., Lv, W., Yu, J. & Li, G. Improvement of extrudability and self-support of emulsion-filled starch gel for 3D printing: Increasing oil content. *Carbohydr. Polym.* 301, 120293 (2023). <https://doi.org/10.1016/j.carbpol.2022.120293> PMID:36436851
- Fatima, A., Quraishi, N. & Babu, M. S. Formulation and In-Vitro Evaluation of Gabapentin Loaded Transfersosomal Gel. *J. Drug Deliv. Ther.* 13, 60-70 (2023). <https://doi.org/10.22270/jddt.v13i12.6081>
- Veeraswamy, S. D., Raju, I. & Mohan, S. An Approach to Antifungal Efficacy through Well Diffusion Analysis and Molecular Interaction Profile of Polyherbal Formulation. *Biomed. Pharmacol. J.* 15, 2069-2084 (2022). <https://doi.org/10.13005/bpj/2544>
- Hemanth, A. R., Kumar, G. B. K., Goudanavar, P. & S, D. S. Formulation and Evaluation of Hydrogels containing Liposomes Entrapped with Antifungal Agent. *Res. J. Pharm. Technol.* 14, 4947-4950 (2021). <https://doi.org/10.52711/0974-360X.2021.00860>
- Cheng Z, Kandekar U, Ma X, Bhabad V, Pandit A, Liu L, Luo J, Munot N, Chorage T, Patil A, Patil S. Optimizing fluconazole-embedded transfersosomal gel for enhanced antifungal activity and compatibility studies. *Frontiers in pharmacology.* 2024 Mar 28; 15:1353791. <https://doi.org/10.3389/fphar.2024.1353791> PMID:38606182 PMCid:PMC11007155
- Jacobs, G. A. et al. Topical delivery of acyclovir and ketoconazole. *Drug Deliv.* 23, 641-651 (2016). <https://doi.org/10.3109/10717544.2014.933283> PMID:25005585
- Mirza, R. et al. Brain targeting of cefepime loaded transfersomes based thermosensitive in situ gel via intranasal delivery: In vitro and in vivo studies. *J. Drug Deliv. Sci. Technol.* 95, 105585 (2024). <https://doi.org/10.1016/j.jddst.2024.105585>
- Fahmy, O. M., Eissa, R. A., Mohamed, H. H., Eissa, N. G. & Elsabahy, M. Machine learning algorithms for prediction of entrapment efficiency in nanomaterials. *Methods* 218, 133-140 (2023). <https://doi.org/10.1016/j.jymeth.2023.08.008> PMID:37595853
- Németh, Z. et al. Quality by Design-Driven Zeta Potential Optimisation Study of Liposomes with Charge Imparting Membrane Additives. *Pharmaceutics* 14, (2022). <https://doi.org/10.3390/pharmaceutics14091798> PMID:36145546 PMCid:PMC9503861
- Rana, I. et al. Solid lipid nanoparticles-mediated enhanced antidepressant activity of duloxetine in lipopolysaccharide-induced

- depressive model. *Colloids Surfaces B Biointerfaces* 194, 111209 (2020). <https://doi.org/10.1016/j.colsurfb.2020.111209> PMID:32599505
27. Leyva-porras, C., Cruz-alcantar, P., Espinosa-sol, V. & Saavedra-leos, M. Z. *Polymers | Free Full-Text | Application of Differential Scanning Calorimetry (DSC) and Modulated Differential Scanning Calorimetry (MDSC) in Food and Drug Industries | HTML*. 1-21.
28. Yasam, V. R., Jakki, S. L., Natarajan, J. & Kuppusamy, G. A review on novel vesicular drug delivery: Proniosomes. *Drug Deliv.* 21, 243-249 (2014). <https://doi.org/10.3109/10717544.2013.841783> PMID:24128089
29. Tawfeek, H., Abdellatif, A. A. H., Abd Elaleem Elnashar, J., Abdelaleem, Y. & Fathalla, D. Transfersomal gel nanocarriers for enhancement the permeation of lornoxicam. *J. Drug Deliv. Sci. Technol.* 56, (2020). <https://doi.org/10.1016/j.jddst.2020.101540>
30. Shareef, A. et al. Leflunomide loaded transfersomes based hydrogel against rheumatoid arthritis: In vitro and In vivo evaluation. *J. Drug Deliv. Sci. Technol.* 109, 106962 (2025). <https://doi.org/10.1016/j.jddst.2025.106962>
31. Singh, V. et al. Stimuli-sensitive hydrogels: A novel ophthalmic drug delivery system. *Indian J. Ophthalmol.* 58, 477-481 (2010). <https://doi.org/10.4103/0301-4738.71677> PMID:20952830 PMCID:PMC2993976
32. Lin, Y. S. & Tsay, R. Y. Drug release from a spherical matrix: Theoretical analysis for a finite dissolution rate affected by geometric shape of dispersed drugs. *Pharmaceutics* 12, 1-16 (2020). <https://doi.org/10.3390/pharmaceutics12060582> PMID:32585967 PMCID:PMC7357057
33. Amra, K. & Momin, M. Formulation evaluation of ketoconazole microemulsion-loaded hydrogel with nigella oil as a penetration enhancer. *J. Cosmet. Dermatol.* 18, 1742-1750 (2019). <https://doi.org/10.1111/jocd.12945> PMID:30980617
34. Kumar, N., and Shishu, H. (2015). D-Optimal experimental approach for designing topical microemulsion of itraconazole: characterization and evaluation of antifungal efficacy against a standardized tinea pedis infection model in wistar rats. *Eur. J. Pharm.Sci.* 67, 97-112. doi:10.1016/j.ejps.2014.10.014 <https://doi.org/10.1016/j.ejps.2014.10.014> PMID:25445834
35. Kaur, D. et al. Formulation and Evaluation of Transfersosomal Gel Loaded With Cefadroxil for Transdermal Delivery. *Ymer* 23, 121-47 (2024).