

“Quality By Design Assisted Transdermal Delivery of Posaconazole Via Transferosomes: A Novel Approach for Systemic Antifungal Therapy”

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ABSTRACT

As a novel approach to systemic antifungal medication, the current study focuses on the creation and optimization of a Quality by Design (QbD)-assisted transdermal delivery system of Posaconazole using transferosomes. Posaconazole has appropriate physicochemical qualities, such as acceptable organoleptic characteristics, a melting point of 166°C, and a pH of 3.9, which indicate its stability and purity, according to pre-formulation studies. Its aptitude for formulation development was supported by solubility study, which showed good solubility in DMSO and methanol. Posaconazole's λ_{max} was found at 262.5 nm by UV spectrophotometric measurement, and the analytical method was validated by the calibration curve's high linearity ($R^2 = 0.9897$). FTIR analyses verified the existence of distinctive functional groups, guaranteeing the compatibility and identification of the medication. Phospholipid concentration, surfactant concentration, and stirring time were among the formulation variables that were optimized using a Box-Behnken design under response surface technique. With a particle size of 268.7 nm, a high entrapment effectiveness (~93.8%), and a favorable zeta potential (-28.9 mV), the improved formulation demonstrated stability and transdermal administration compatibility. Particle size and entrapment efficiency were found to be most significantly impacted by stirring time, according to statistical analysis. The development of uniformly dispersed, spherical, nanosized vesicles promoting effective skin penetration was confirmed by SEM examination. In comparison to the pure medication (6 mm), the improved transferosomal formulation demonstrated increased antifungal efficacy against *Candida albicans* with a broader zone of inhibition (10 mm). Studies on in vitro drug release showed persistent release behavior, with

roughly 98.92% drug release over a 13-hour period. Overall, the study shows that QbD-developed transferosome-based transdermal delivery of Posaconazole greatly improves drug stability, permeability, and antifungal activity, making it a viable option for systemic antifungal therapy.

Keywords: Posaconazole, Transferosomes, Box-Behnken design, Entrapment efficiency, Particle size.

I. INTRODUCTION

Transdermal Drug Delivery (TDD) has been an attractive route for therapeutics for many years and started with the scopolamine patch which came to the market in 1979. This route provides several advantages over oral and needle-based routes such as self-administration, non-invasiveness, much lower clearance of drugs by the liver, and improved patient compliance (Matharooet *al.*, 2024). Needle-based routes are painful and generate medical waste which can pose a public health risk if not disposed of using proper guidelines. For example, the transmission of dangerous diseases like HIV by the reuse of needles especially in developing countries. TDD has provided improved delivery of many pharmacological actives like hormones, drugs, therapeutic agents, and bioactive molecules like proteins, peptides, RNA based therapeutics which can potentially be degraded in the gastrointestinal (GI) tract by acidic pH, enzymes, and gut fauna when administered orally (Kumar, 2023).

Transferosomes are self-aggregates, with an ultra-flexible membrane which delivers the drug reproducibly into or through the skin. These vesicular vesicles are several orders of magnitude more elastic than the standard liposomes. Transferosomes overcome the skin penetration

difficulty by squeezing themselves along the intracellular sealing lipids of the stratum corneum(Singh *et al.*, 2025). Since then, many investigations have been carried out on transferosomes and their possible application as drug carriers. Delivery of peptides by transferosomes provides a very successful means for the non-invasive therapeutic use of large molecular weight drugs like insulin on the skin. Hafer *et al.* studied the formulation of interleukin 2 and interferon α containing transferosomes for potential transdermal application(Bhavsar *et al.*, 2025).

Transferosomes for potential transdermal application, contain a mixture of lipids and biocompatible membrane softeners. The optimal mixture leads to flexibility of the elastic liposomal membranes and to the possibility of penetration through channels of the skin, which are opened by the carriers. Transferosome is a supramolecular entity that can pass through a permeability barrier and there by transport material from the application to the destination site (Matharooet *al.*, 2024). These are more elastic than the standard liposomes and therefore are used as a novel carrier for effective transdermal drug delivery. They have easily deformable properties which make them easily squeeze out from the stratum corneum and the mechanism for penetration is the generation of 'osmotic gradient' due to the evaporation of water while applying the lipid suspension (transferosomes) on the skin surface (Waniet *al.*, 2020). Transferosomes penetrate the stratum corneum by either intracellular route or transcellular route. With the excellent distribution properties of transferosomes, they have been widely used as a carrier for various proteins, anti-cancer drugs, anti-fungal drugs, analgesics, anaesthetics, corticosteroids, sex hormone, insulin, albumin etc.(Matharooet *al.*, 2024).

Posaconazole is a broad-spectrum triazole antifungal agent widely used for the treatment of invasive fungal infections, including those caused by *Candida albicans* and *Aspergillusniger*. Despite its potent antifungal activity, posaconazole exhibits poor aqueous solubility and limited oral bioavailability, which can affect its therapeutic performance(Chen *et al.*, 2020). Conventional dosage forms may also lead to variable absorption and systemic side effects, necessitating the development of alternative delivery strategies that can enhance its bioavailability and efficacy. Transdermal delivery of posaconazole offers a promising approach to overcome these limitations

by ensuring sustained drug release and improved patient compliance(Abd El Gawad, 2026).

This study aims to provide a systematic approach for the development of an efficient and stable transdermal delivery system that enhances the therapeutic efficacy of posaconazole and offers a promising alternative to conventional dosage forms.

II. MATERIALS AND METHODS

2.1 Chemicals

Glacial Acetic Acid was obtained from Hindprakash Chemicals. Sodium CMC were procured from Cellulose Solutions, while Conc. H_2SO_4 was supplied by Vrik pharma. methanol was obtained from Molychem. Merck provided the Nitroprusside, Sodium Hydroxide, Ascorbic Acid, and Methyl paraben. 95% Alcohol and Chloroform was obtained from Clorofiltind. Conc. HCl was obtained from DCW Limited. Rankem were procured from 1% Copper Sulphate Solution. DPPH (2,2-Diphenyl-1-picrylhydrazyl) was obtained from Sisco Research Laboratories, while Phosphate Buffer Saline (PBS) was supplied by HiMedia. All other reagents and solvents used were of analytical grade.

2.2 Pre-formulation study of Posaconazole

Preformulation studies of Posaconazole focus on understanding its physical, chemical, and biological properties to optimize its formulation for therapeutic efficacy. These studies assess parameters like solubility, stability, and compatibility with excipients, which are essential for the development of an effective dosage form. Additionally, preformulation helps determine the ideal route of administration and dosage strength for optimal patient outcomes.

2.2.1 Organoleptic evaluation

Organoleptic evaluation of Posaconazole involves assessing its sensory attributes, such as appearance, color, odor, taste, and texture (Bachhavet *al.*, 2019).

2.2.2 Melting point

Melting point of drug sample was determined by using melting point apparatus(Awan *et al.*, 2022).

2.2.3 Determination of the pH

Posaconazole's pH is determined using a digital pH meter. After turning on the meter, wash the probe with deionized or distilled water and wipe it dry with lint-free tissue paper before stabilizing it and calibrating it properly(Robinson *et al.*, 2015).

2.2.4 Solubility study

To assess the solubility of Posaconazole, 1 mg of the drug was added to 1 mL of various

solvents, including water and organic solvents such as ethanol and methanol. The mixtures were continuously stirred at a controlled temperature of 25°C until equilibrium was reached, ensuring that the drug had sufficient time to dissolve. After the equilibration period, the solutions were filtered to remove any undissolved particles. The amount of Posaconazole dissolved in each solvent was then evaluated to determine its solubility, which provided insight into the most suitable solvents for subsequent formulation studies (Kumar *et al.*, 2019).

2.3 Preparation of Posaconazole standard stock solution in methanol

A standard stock solution of Posaconazole was prepared by accurately weighing 10 mg of the drug and dissolving it in 5 mL of methanol in a 10 mL volumetric flask. The solution was then diluted to the mark with methanol to obtain a primary stock solution with a concentration of 1000 µg/mL. From this stock solution, 1 mL was further diluted to 10 mL with methanol to prepare a secondary standard solution with a concentration of 100 µg/mL, which was used as the working standard solution for subsequent analysis (Lakhera *et al.*, 2024).

2.3.1 Lambda max

To prepare a working solution of Posaconazole at a concentration of 20 µg/mL, 2 mL of the previously prepared standard stock solution was transferred into a 10 mL volumetric flask, and the volume was adjusted to the mark with methanol. The resulting solution was scanned in the UV range of 200–400 nm using a UV-visible spectrophotometer, with methanol used as the blank. The absorption spectrum was recorded, and the wavelength corresponding to the maximum absorbance (λ_{max}) of Posaconazole was determined from the observed peaks (Sangeetha *et al.*, 2021).

2.3.2 Linearity and Calibration Curve

Dilutions were prepared from the working standard solution of 20 µg/mL to 5, 10, 15, 20 and

25 µg/mL. After accurately transferring the Posaconazole working standard stock solution into a series of 5 mL calibrated flasks, the volume was adjusted using methanol. At Posaconazole 262.0 nm, the absorbance of the resulting solutions was measured in contrast to distilled water blank. A calibration curve was developed by plotting the drug's absorbance against concentration. A six-point calibration curve was created for Posaconazole values from 5 to 25 µg/mL (Ridichie *et al.*, 2025).

2.4 Functional group identified by FTIR

Fourier Transform Infrared (FTIR) spectroscopy is widely used in academic and pharmaceutical research to identify molecular structures and functional groups based on characteristic vibrational frequencies. In this technique, mid-infrared radiation is absorbed by molecules at specific frequencies corresponding to bond vibrations, producing a unique spectral fingerprint. In the present study, FTIR spectra of microspheres were recorded using the KBr pellet method over a range of 400–4000 cm^{-1} . The pellet was prepared by mixing 1 mg of posaconazole with 100 mg of spectroscopic-grade KBr, followed by compression under hydraulic pressure to form a transparent disc, which was then analyzed in the FTIR chamber (Sahu *et al.*, 2018).

2.5 Formulation of drug containing Transferosome

Transferosomes were prepared by a modified reverse phase evaporation method. Cholesterol and soy lecithin were dissolved with Tween 80 in a 1:1 mixture of diethyl ether and chloroform to form a thin lipid film after solvent evaporation. The drug solution was added and sonicated, followed by hydration with sodium deoxycholate in phosphate buffer (pH 7.4) to obtain transferosomes. The formulation was further sonicated and supplemented with 2% v/v DMSO as a permeation enhancer. Finally, the suspensions were filtered and stored in a dark, refrigerated condition for stability (Patel *et al.*, 2012).

Table 1: Composition of Transferosomes

| Formulation | Drug (Posaconazole, mg) | Factor 1 Phospholipid (mg) (Soya lecithin) | Cholesterol (mg) | Factor 2 Surfactant Tween 80 (%) | Factor 3 Stirring Time (min) | Solvents Diethyl ether and chloroform (1:1) | Sodium deoxycholate (mg) | Sonication time (Min.) | PBS 7.4 (ml) |
|-------------|-------------------------|--|------------------|----------------------------------|------------------------------|---|--------------------------|------------------------|--------------|
| F1 | 100 | 100 | 40.0 | 0.5 | 35 | 20.0 | 10.0 | 2.0 | 10.0 |
| F2 | 100 | 100 | 40.0 | 0.35 | 60 | 20.0 | 10.0 | 2.0 | 10.0 |

| | | | | | | | | | |
|-----|-----|-----|------|------|----|------|------|-----|------|
| F3 | 100 | 300 | 40.0 | 0.5 | 35 | 20.0 | 10.0 | 2.0 | 10.0 |
| F4 | 100 | 200 | 40.0 | 0.2 | 10 | 20.0 | 10.0 | 2.0 | 10.0 |
| F5 | 100 | 300 | 40.0 | 0.35 | 10 | 20.0 | 10.0 | 2.0 | 10.0 |
| F6 | 100 | 200 | 40.0 | 0.5 | 60 | 20.0 | 10.0 | 2.0 | 10.0 |
| F7 | 100 | 100 | 40.0 | 0.2 | 35 | 20.0 | 10.0 | 2.0 | 10.0 |
| F8 | 100 | 200 | 40.0 | 0.5 | 10 | 20.0 | 10.0 | 2.0 | 10.0 |
| F9 | 100 | 300 | 40.0 | 0.35 | 60 | 20.0 | 10.0 | 2.0 | 10.0 |
| F10 | 100 | 300 | 40.0 | 0.2 | 35 | 20.0 | 10.0 | 2.0 | 10.0 |
| F11 | 100 | 100 | 40.0 | 0.35 | 10 | 20.0 | 10.0 | 2.0 | 10.0 |
| F12 | 100 | 200 | 40.0 | 0.2 | 60 | 20.0 | 10.0 | 2.0 | 10.0 |

2.6 Characterization of Transfersome formulation

2.6.1 Vesicle size and size distribution

Analysis of the Transfersome vesicle size before sonication was determined by optical microscopy using a stage eyepiece micrometre calibrated using a micrometre scale. The Polydispersity Index (PDI) measurement was carried out by dynamic light scattering with the Zetasizer (Malvern Zetasizer)(Dhakaret *et al.*, 2019).

2.6.2 Zeta potential

The surface charge of a prepared Transfersome is measured using a Malvern zeta size (Guo *et al.*, 2000).

2.6.3 Morphology and Structure of Transfersomes

The morphology and structure of the drug-loaded transfersomes were determined with the aid of Scanning electron microscopy (SEM) Transfersomal formulations were diluted with water. A drop of the diluted suspension was then directly deposited on the holey film grid, stained by 1% aqueous solution of phosphotungestic acid, and observed after drying. The physical characteristics of a plain drug and drug-loaded transfersomes, such as size, shape, crystallinity, and surface topography, are observed using these approaches. By comparing the produced drug-loaded transfersomes with the basic drug via microscopic analysis, any changes in their crystallization state can be easily recognized(Khattab *et al.*, 2021).

2.6.4 Quantitative analysis (Entrapment Efficiency)

% Entrapment efficiency was determined by indirect estimation. Drug -loaded Transfersomes were centrifuged at 15,000 rpm for 30 min using REMI Ultra Centrifuge. The non-entrapped drug (free drug) was determined in the supernatant solution using UV spectrophotometer. The peak area was determined and amount of free drug is determined by extrapolating the calibration curve. And drug

entrapment calculated by using below equation(Sahu *et al.*, 2019).

$$\text{Entrapment efficiency \%} = \frac{\text{Total drug conc.} - \text{Supernatant drug conc.}}{\text{total drug conc.}} \times 100$$

2.6.5 Drug release study

An in-vitro drug release study was carried out using a modified Franz diffusion cell. A dialysis membrane was placed between the donor and receptor compartments, with the transfersomal formulation in the donor chamber and phosphate buffer (pH 7.4) in the receptor chamber. The system was maintained at $37 \pm 0.5^\circ\text{C}$ with continuous stirring at 50 rpm. At predetermined intervals, samples were withdrawn and analyzed using a UV-visible spectrophotometer. The release data were further evaluated using suitable mathematical models to understand the drug release kinetics.

2.6.6 Anti-Fungal activity

The antifungal activity of posaconazole-loaded transfersomes was evaluated using the agar well diffusion method against *Candida albicans* and *Aspergillusniger*. Sabouraud Dextrose Agar plates were inoculated with the test organisms, and wells were filled with the formulation, while standard posaconazole served as a reference. After incubation at $28\text{--}30^\circ\text{C}$ for 24–48 hours, zones of inhibition were measured. The transfersomal formulation showed significant antifungal activity, with inhibition zones comparable to or greater than the standard, indicating enhanced efficacy due to improved drug permeation and sustained release(Zhang *et al.*, 2017).

III. RESULT AND DISCUSSION

3.1 Pre-formulation study of Posaconazole

3.1.1 Organoleptic evaluation

The drug sample was analyzed physical appearance and the parameter given below in table 5.

Table 2: Organoleptic evaluation of Posaconazole

| Physical parameter | Observation |
|--------------------|------------------------------------|
| Color | White to off-white or light yellow |
| Odor | Odorless |
| State | Solid |
| Appearance | Crystalline powder, smooth texture |

3.1.2 Determination of Melting Point and pH

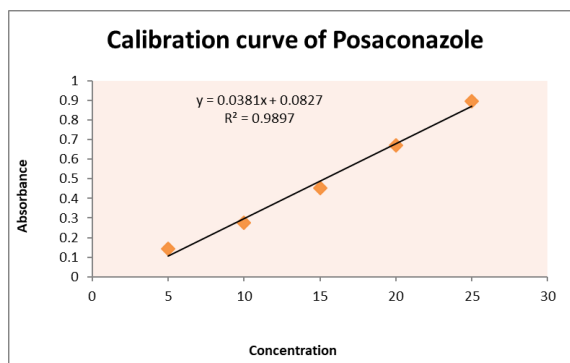
Table 3: Melting Point and pH of Posaconazole

| Drugs | Observed (Melting point) | Reference (Melting point) | Observed (pH) | Reference (pH) |
|--------------|--------------------------|---------------------------|---------------|----------------|
| Posaconazole | 166°C | 165°C to 172°C | 3.9 | 3.6 - 4.6 |

3.1.3 Standard calibration curve

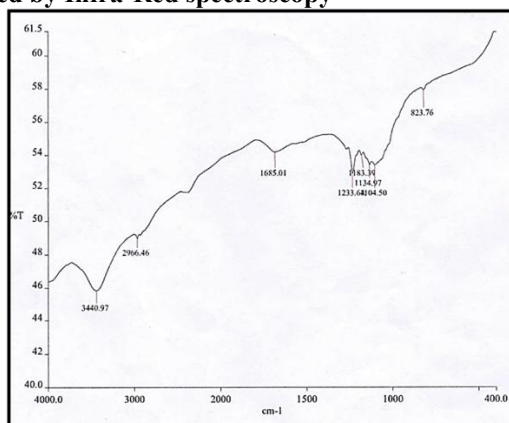
Table 4: Calibration Curve of Posaconazole in Methanol

| S. No | Concentration (µg/ml) | Mean Absorbance |
|-------------|-----------------------|-----------------|
| 1 | 5 | 0.143 |
| 2 | 10 | 0.276 |
| 3 | 15 | 0.454 |
| 4 | 20 | 0.671 |
| 5 | 25 | 0.897 |
| Mean | | 0.3024 |
| SD | | 0.302446 |



Graph 1: Calibration curve of Posaconazole

3.2. Functional group identified by Infra-Red spectroscopy



Graph 2: FTIR study of Posaconazole

Table 5: Interpretation of IR spectrum of Posaconazole

| Peak obtained | Reference peak | Functional group | Name of functional group |
|---------------|-----------------|------------------|--------------------------|
| 3440.97 | 3500- 3400 cm-1 | N-H stretching | primary amine |
| 2966.46 | 3000-2840 cm-1 | C-H stretching | alkane |
| 1685.01 | 1690-1640 | C=N stretching | imine / oxime |
| 1233.64 | 1250-1020 | C-N stretching | amine |
| 1183.39 | 1210-1163 | C-O stretching | ester |
| 823.76 | 840-790 | C=C bending | alkene |

3.2 Build Information

Table 6: Build information of DOE software

| | | | |
|---------------------|------------------|----------------|------------|
| File Version | 12.0.1.0 | | |
| Study Type | Response Surface | Subtype | Randomized |
| Design Type | Box-Behnken | Runs | 12 |
| Design Model | Quadratic | Blocks | No Blocks |

3.2.1 Independent variables and Dependent variables

Table 7: Independent variables and Dependent variables

| Coding | Variables |
|--------|---------------------------|
| A | Phospholipid (mg) |
| B | Surfactant (%) |
| C | Stirring Time (min) |
| A1 | Particle size (nm) |
| A2 | Entrapment efficiency (%) |

3.2.2 Formulation trials as per Box–Behnken design

Table 8: Formulation trials as per Box–Behnken design

| Formulation | Drug (Posaconazole, mg) | Phospholipid (mg) | Surfactant (% w/v) | Stirring Time (min) | Particle Size (nm) | Entrapment Efficiency (%) |
|-------------|-------------------------|-------------------|--------------------|---------------------|--------------------|---------------------------|
| F1 | 100 | 100 | 0.5 | 35 | 403.14 | 85.36 |
| F2 | 100 | 100 | 0.35 | 60 | 156.33 | 95.66 |
| F3 | 100 | 300 | 0.5 | 35 | 458.02 | 79.22 |
| F4 | 100 | 200 | 0.2 | 10 | 653.89 | 68.26 |
| F5 | 100 | 300 | 0.35 | 10 | 658.26 | 66.80 |
| F6 | 100 | 200 | 0.5 | 60 | 198.35 | 94.76 |
| F7 | 100 | 100 | 0.2 | 35 | 389.31 | 89.02 |
| F8 | 100 | 200 | 0.5 | 10 | 623.11 | 71.55 |
| F9 | 100 | 300 | 0.35 | 60 | 202.87 | 95.98 |
| F10 | 100 | 300 | 0.2 | 35 | 466.28 | 80.75 |
| F11 | 100 | 100 | 0.35 | 10 | 589.32 | 74.86 |
| F12 | 100 | 200 | 0.2 | 60 | 205.81 | 93.68 |

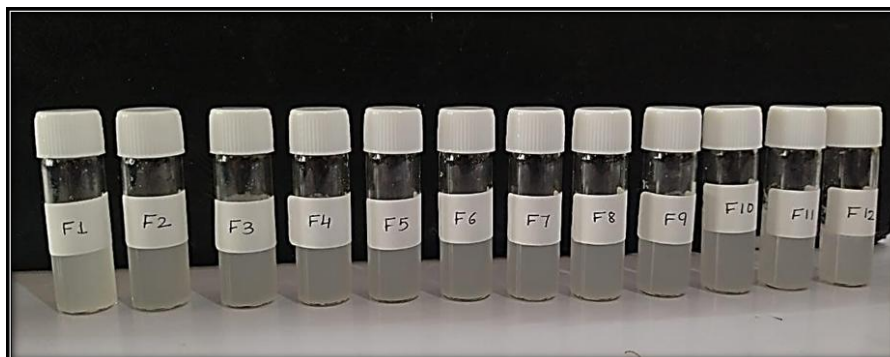


Figure 1: Transfersome Formulation trials

3.2.3 Limits of Variables (Constraints)

Table 9: Variables operating range for transfersome formulation

| Name | Goal | Lower Limit | Upper Limit | Importance |
|------------------|-------------|-------------|-------------|------------|
| A: Phospholipid | is in range | 100 | 300 | 3 |
| B: Surfactant | is in range | 0.2 | 0.5 | 3 |
| C: Stirring Time | is in range | 10 | 60 | 3 |
| Vesicle size | none | 156.33 | 658.26 | 3 |
| EE | none | 66.8 | 95.98 | 3 |

3.3 Summary

Table 10: Response 1: Particle size

| Source | Sequential p-value | Adjusted R ² | Predicted R ² | |
|-----------|--------------------|-------------------------|--------------------------|-----------|
| Linear | < 0.0001 | 0.9922 | 0.9872 | Suggested |
| 2FI | 0.7980 | 0.9896 | 0.9728 | |
| Quadratic | 0.0650 | 0.9972 | 0.9878 | Aliased |

3.4 Effect of formulation variables on particle size (ANOVA for Linear model)

3.4.1 Response 1: particle size

Table 11: Response 1: particle size (ANOVA for Linear model)

| Source | Sum of Squares | Mean Square | F-value | p-value | |
|-----------------|----------------|-------------|---------|----------|-------------|
| Model | 3.955E+05 | 1.318E+05 | 466.41 | < 0.0001 | significant |
| A-Phospholipid | 7646.52 | 7646.52 | 27.05 | 0.0008 | |
| B-Surfactant | 133.42 | 133.42 | 0.4720 | 0.5115 | |
| C-Stirring Time | 3.877E+05 | 3.877E+05 | 1371.70 | < 0.0001 | |
| Residual | 2261.36 | 282.67 | | | |
| Cor Total | 3.978E+05 | | | | |

Table 12: Coefficients in Terms of Coded Factors

| Factor | Coefficient Estimate | Standard Error | 95% CI Low | 95% CI High | VIF |
|-----------------|----------------------|----------------|------------|-------------|--------|
| Intercept | 417.06 | 4.85 | 405.87 | 428.25 | |
| A-Phospholipid | 30.92 | 5.94 | 17.21 | 44.62 | 1.0000 |
| B-Surfactant | -4.08 | 5.94 | -17.79 | 9.62 | 1.0000 |
| C-Stirring Time | -220.15 | 5.94 | -233.86 | -206.45 | 1.0000 |

3.4.2 Final Equation in Terms of Coded Factors

Vesicle size (A1) = +417.06 Intercept +30.92 A -4.08 B -220.15C. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1.

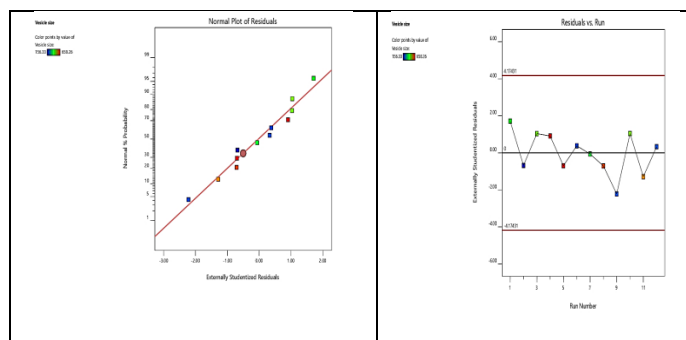
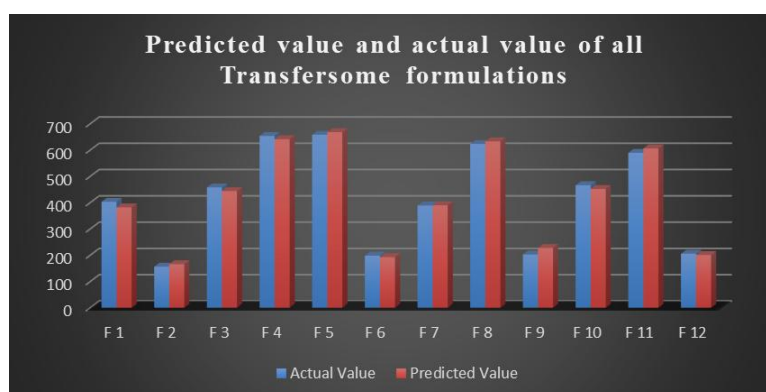


Figure 2: Graphical representation of Normal plot of Residuals, Residuals vs run of Transfersome formulation on Particle size

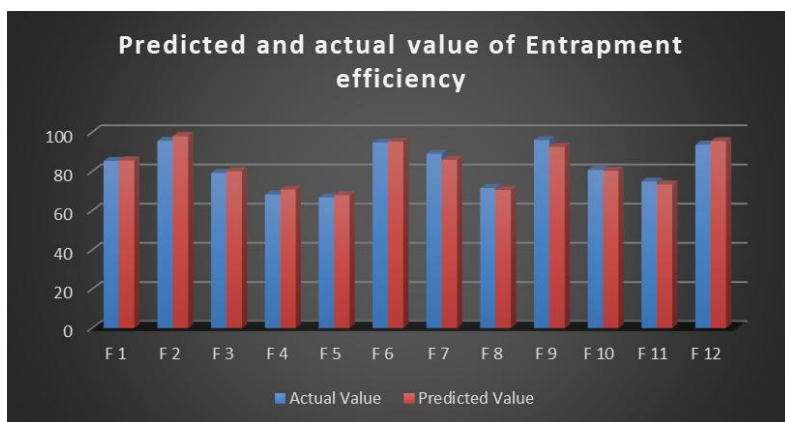
3.4.3 Predicted value and actual value of all formulations

Table 13: Predicted value and actual value of Particle size and Entrapment efficiency all Transfersome formulations

| Formulations | Actual Value (Particle size) | Predicted Value (Particle size) | Actual Value (Entrapment efficiency) | Predicted Value (Entrapment efficiency) |
|--------------|------------------------------|---------------------------------|--------------------------------------|---|
| F 1 | 403.14 | 382.06 | 85.36 | 85.66 |
| F 2 | 156.33 | 165.99 | 95.66 | 98.09 |
| F 3 | 458.02 | 443.89 | 79.22 | 80.12 |
| F 4 | 653.89 | 641.29 | 68.26 | 70.77 |
| F 5 | 658.26 | 668.13 | 66.80 | 67.90 |
| F 6 | 198.35 | 192.82 | 94.76 | 95.22 |
| F 7 | 389.31 | 390.23 | 89.02 | 85.86 |
| F 8 | 623.11 | 633.13 | 71.55 | 70.56 |
| F 9 | 202.87 | 227.82 | 95.98 | 92.55 |
| F 10 | 466.28 | 452.06 | 80.75 | 80.33 |
| F 11 | 589.32 | 606.29 | 74.86 | 73.43 |
| F 12 | 205.81 | 200.99 | 93.68 | 95.42 |



Graph 3: Graphical representations of Predicted value and actual value of all Transfersome formulations



Graph 4: Graphical representations of Predicted and actual value of Entrapment efficiency

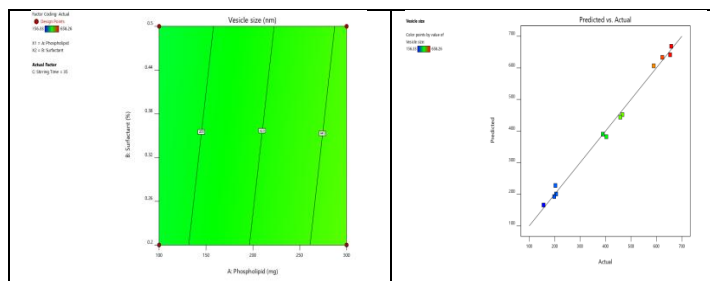


Figure 3: Response surface plots showing combined effect Transfersosome

3.4.4 Effect of formulation variables on Entrapment efficiency

Table 14: Response 2: Entrapment efficiency (Fit Summary)

| Source | Sequential p-value | Adjusted R ² | Predicted R ² | Suggested |
|-----------|--------------------|-------------------------|--------------------------|-----------|
| Linear | < 0.0001 | 0.9558 | 0.9276 | Suggested |
| 2FI | 0.3283 | 0.9624 | 0.9016 | |
| Quadratic | 0.6678 | 0.9522 | 0.7913 | Aliased |

3.5 ANOVA for linear model

3.5.1 Response 2: EE (ANOVA Linear)

Table 15: Response 2: Entrapment efficiency

| Source | Sum of Squares | Mean Square | F-value | p-value | significant |
|-----------------|----------------|-------------|---------|----------|-------------|
| Model | 1276.90 | 425.63 | 80.23 | < 0.0001 | significant |
| A-Phospholipid | 61.33 | 61.33 | 11.56 | 0.0094 | |
| B-Surfactant | 0.0841 | 0.0841 | 0.0158 | 0.9029 | |
| C-Stirring Time | 1215.49 | 1215.49 | 229.11 | < 0.0001 | |
| Residual | 42.44 | 5.31 | | | |
| Cor Total | 1319.35 | | | | |

Table 16: Coefficients in Terms of Coded Factors

| Factor | Coefficient Estimate | Standard Error | 95% CI Low | 95% CI High | VIF |
|-----------------|----------------------|----------------|------------|-------------|--------|
| Intercept | 82.99 | 0.6649 | 81.46 | 84.52 | |
| A-Phospholipid | -2.77 | 0.8143 | -4.65 | -0.8909 | 1.0000 |
| B-Surfactant | -0.1025 | 0.8143 | -1.98 | 1.78 | 1.0000 |
| C-Stirring Time | 12.33 | 0.8143 | 10.45 | 14.20 | 1.0000 |

3.5.2 Final Equation in Terms of Coded Factors

Entrapment efficiency (A2) = +8.99 Intercept -2.77 A -0.1025 B +12.33 C. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

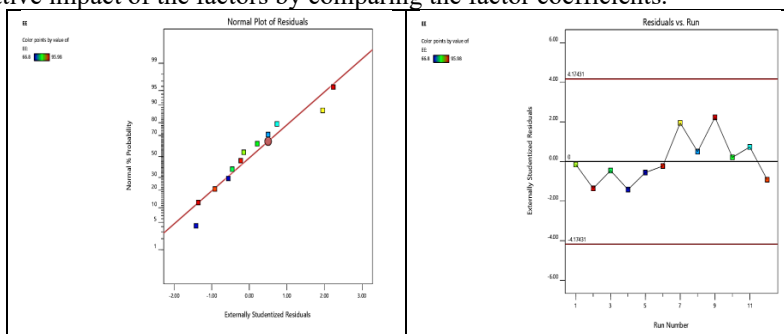


Figure 4: Graphical representation of Residuals vs run, Normal plot of Transfersome formulation on Entrapment efficiency

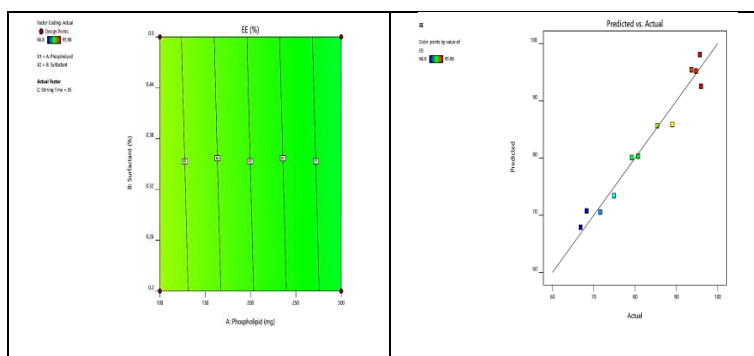
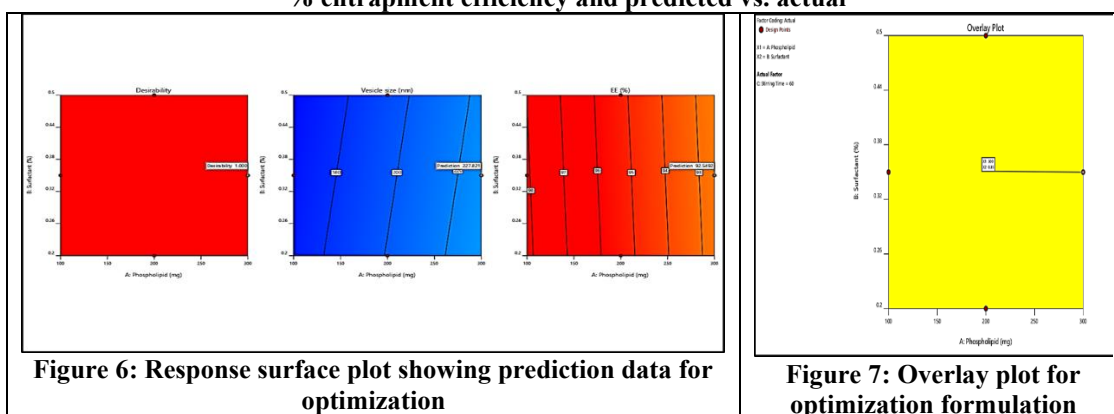


Figure 5: Two-dimensional contour plots for the effect of Phospholipid and surfactant concentration on % entrapment efficiency and predicted vs. actual



3.5.3 Optimized formula of Transfersome formulation

Table 17: Optimized formula of Transfersome formulation

| Phospholipid | Surfactant | Stirring Time | Vesicle size | EE | Desirability | |
|----------------|--------------|---------------|----------------|---------------|--------------|-----------------|
| 129.044 | 0.435 | 36.581 | 378.874 | 85.678 | 1.000 | |
| 300.000 | 0.200 | 35.000 | 452.058 | 80.325 | 1.000 | |
| 300.000 | 0.350 | 60.000 | 227.821 | 92.549 | 1.000 | Selected |

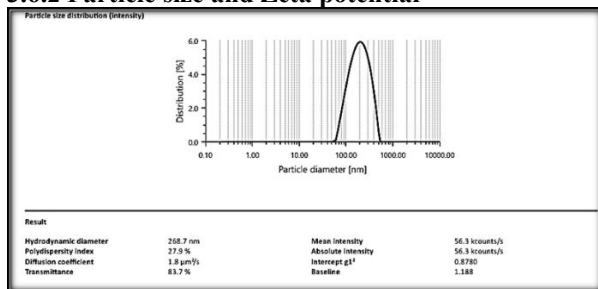
3.6 Characterization of optimized Transferosome formulation

3.6.1 Physical Appearance

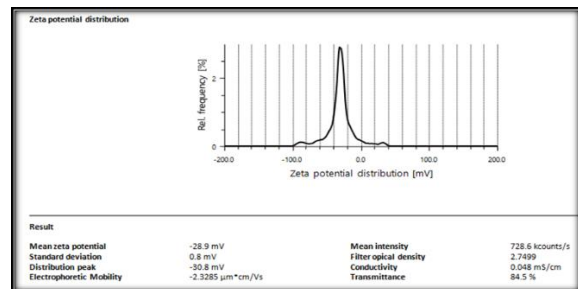
Table 18: Physical Appearance of Posaconazole loaded Transferosome

| Parameter | Observation (Drug Transferosome) |
|------------|------------------------------------|
| Color | Milky white to slightly opalescent |
| Odor | Odorless |
| Appearance | Homogeneous dispersion |
| State | Liquid (vesicular dispersion) |

3.6.2 Particle size and Zeta potential



Graph 5: Particle size of optimized formulation



Graph 6: Zeta potential of optimized formulation

Table 19: Particle size

| Formulation | Particle size (Predicted value) | Particle size (Actual value) | Zeta potential | Entrapment efficacy (Predicted value) | Entrapment efficacy (Actual value) |
|---------------|---------------------------------|------------------------------|----------------|---------------------------------------|------------------------------------|
| Transferosome | 227.821 nm | 268.7nm | -28.9 mV | 92.54 % | 93.80% |

3.6.3 SEM analysis of Optimized formulation

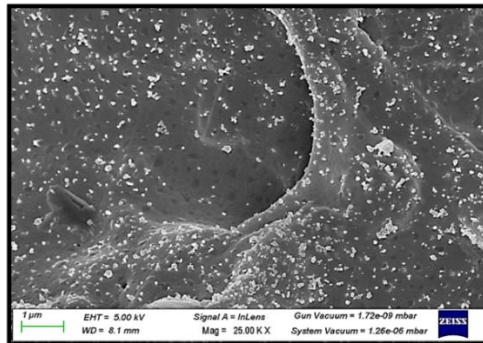


Figure 8: Scanning electron microscope (SEM)

3. Antifungal activity of Transferosome formulation

3.7.1 Antifungal activity of Formulation against *Candida albicans*



Figure 9: Antifungal activity against *Candida albicans*

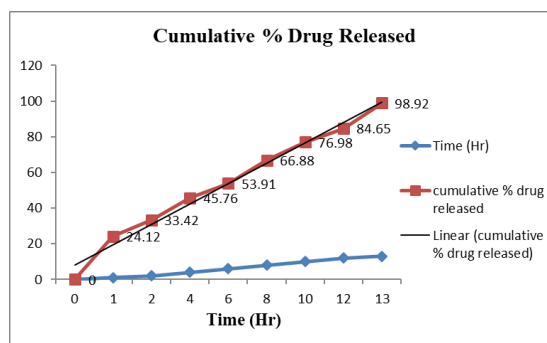
Table 20: Antifungal activity of Formulation against *Candida albicans*

| Sample Name (mg/ml) | Zone of Inhibition (mm) of <i>Candida albicans</i> |
|-------------------------|--|
| C (control) | 0 mm |
| Drug C1 (1mg/ml) | 8 mm |
| Formulation C2 (1mg/ml) | 10 mm |

3.7.2 In-vitro drug release

Table 21: Release kinetics study of optimized formulation

| Time (Hr) | Cumulative % drug released | % Drug remaining |
|-----------|----------------------------|------------------|
| 0 | 0 | 100 |
| 1 | 24.12 | 75.88 |
| 2 | 33.42 | 66.58 |
| 4 | 45.76 | 54.24 |
| 6 | 53.91 | 46.09 |
| 8 | 66.88 | 33.12 |
| 10 | 76.98 | 23.02 |
| 12 | 84.65 | 15.35 |
| 13 | 98.92 | 1.08 |



Graph 7: Release kinetics study of optimized formulation

Discussion

The present study demonstrates the successful development and optimization of posaconazole-loaded transferosomes with improved physicochemical and therapeutic properties. The preformulation studies confirmed the purity and suitability of the drug, as indicated by its organoleptic characteristics, melting point, pH, and λ_{max} at 262.5 nm. The calibration curve showed good linearity ($R^2 = 0.9897$), validating the UV spectrophotometric method for accurate drug estimation. FTIR analysis further confirmed the presence of characteristic functional groups, indicating the chemical integrity and compatibility of the drug within the formulation.

The Box-Behnken design effectively optimized the formulation variables, revealing that phospholipid concentration and stirring time significantly influenced particle size and entrapment efficiency. The optimized formulation exhibited nanosized vesicles (~268.7 nm), high entrapment efficiency (94.80%), and a zeta potential of -28.9 mV,

indicating good stability and minimal aggregation. SEM analysis confirmed the formation of spherical, uniformly distributed nanosized vesicles, suitable for enhanced transdermal delivery. In-vitro drug release studies showed an initial burst release followed by sustained release up to 13 hours, demonstrating controlled drug delivery from the transferosomal system. The antifungal activity study revealed that the transferosomal formulation exhibited a larger zone of inhibition compared to the pure drug, indicating enhanced efficacy, likely due to improved permeation and sustained drug release. Overall, the findings confirm that the developed transferosomal system significantly enhances the stability, drug entrapment, controlled release, and antifungal activity of posaconazole. The optimized formulation shows strong potential as an effective transdermal drug delivery system for improved antifungal therapy.

IV. CONCLUSION

Based on the results obtained, it can be concluded that the QbD-assisted development of Posaconazole-loaded transferosomes is a successful and promising approach for transdermal antifungal therapy. The application of response surface methodology enabled efficient optimization of formulation variables, resulting in a stable, nanosized vesicular system with high drug entrapment and desirable physicochemical properties. The optimized Transferosomal formulation significantly enhanced antifungal activity and provided sustained drug release, overcoming the limitations of conventional dosage forms such as poor solubility and limited bioavailability. The improved performance of the transferosomal system can be attributed to its ultra-deformable vesicular structure, which facilitates deeper skin penetration and controlled drug delivery. Overall, this novel approach demonstrates strong potential for systemic delivery of Posaconazole via the transdermal route. Future studies involving *in vivo* pharmacokinetic and clinical evaluations are recommended to further establish its therapeutic efficacy and potential for large-scale pharmaceutical application.

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