RESEARCH PAPER

Design, Optimization, and Evaluation of Carboxymethyl Chitosan-Based Terbinafine-Loaded Transfersomal Gel for Enhanced Dermal Delivery in Superficial Fungal Infections

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Abstract

Background: Conventional terbinafine topical formulations are limited by poor skin penetration and low dermal drug deposition, leading to prolonged treatment durations in superficial fungal infections. This study aimed to develop a carboxymethyl chitosan (CMCS)-based transfersomal gel of terbinafine using a Quality by Design (QbD) approach to enhance dermal delivery.

Methods: Transfersomes were prepared by thin-film hydration and optimized using a Box–Behnken Design with Tween 80 concentration, hydration volume, and sonication time as independent variables. Critical responses included vesicle size, entrapment efficiency (EE), and skin drug deposition. The optimized transfersomal dispersion was incorporated into a Carbopol gel and evaluated for physicochemical characteristics, ex vivo skin permeation, confocal laser scanning microscopy (CLSM), and stability under ICH conditions.

Results: The optimized formulation (F9) exhibited vesicle size of 164.2 ± 4.3 nm, PDI of 0.182, and EE of 84.9 ± 1.9%. Ex vivo studies showed significantly higher permeation flux (42.6 ± 2.1 µg/cm²/h) and drug deposition (27.8 ± 1.2 µg/cm²) compared to conventional gel. CLSM confirmed deeper penetration up to 120 µm. Stability studies indicated minimal changes in vesicle characteristics over 3 months.

Conclusion: The QbD-optimized CMCS transfersomal gel demonstrated superior dermal delivery of terbinafine and holds strong potential for improved antifungal therapy.

Keywords: Terbinafine, transfersomes, carboxymethyl chitosan, Quality by Design, dermal delivery, antifungal therapy.

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Introduction

Superficial fungal infections including dermatophytosis, onychomycosis, and candidiasis—are among the most prevalent dermatological disorders globally. These infections are primarily caused by species of *Trichophyton*, *Epidermophyton*, and *Candida*, affecting millions each year and resulting in itching, discomfort, inflammation, and cosmetic

disfigurement (Gupta & Versteeg, 2019). In immunocompromised individuals, chronic or recurrent infections can also lead to secondary bacterial invasion and severe tissue damage. Traditional topical and systemic antifungal therapies often provide partial relief but are limited by poor skin penetration, drug resistance, and patient noncompliance. Among antifungal agents, terbinafine hydrochloride, an allylamine derivative, remains one of the most effective drugs for treating dermatophytosis and other superficial mycoses. Its fungicidal mechanism involves inhibition of squalene epoxidase, an essential enzyme in the ergosterol biosynthesis pathway. This inhibition causes accumulation of intracellular squalene and depletion of ergosterol, leading to membrane dysfunction and fungal cell death (Mishra et al., 2020). Despite these advantages, the therapeutic efficacy of conventional terbinafine creams or gels is often compromised by low skin permeability, limited dermal retention, and prolonged dosing schedules, which may contribute to poor patient adherence and relapse after treatment cessation.

In the last two decades, nanocarrier-based drug delivery systems have emerged as a transformative approach to enhance the solubility, stability, and skin deposition of lipophilic antifungal drugs. Among these systems, transfersomes—also known as ultradeformable liposomes—have demonstrated superior capability in overcoming the stratum corneum barrier, the main obstacle to topical delivery (Cevc & Blume, 2001). Transfersomes are flexible, lipid-based vesicles comprising phospholipids and "edge activators" (surfactants such as Tween 80 or sodium cholate) that confer elasticity to their bilayer membranes. This ultradeformability enables them to squeeze through intercellular lipid channels much smaller than their own diameter, carrying the drug deep into the viable epidermis and dermis. The combination of high deformability, biocompatibility, and efficient drug encapsulation makes transfersomes a promising delivery vehicle for terbinafine hydrochloride, which requires localized high concentrations within infected keratinized tissues.

Recent studies have explored polymeric modifications to transfersomal systems to further improve their mechanical stability, drug entrapment efficiency, and skin adhesion. Carboxymethyl chitosan (CMCS), a hydrophilic derivative of chitosan, has emerged as a multifunctional additive in topical delivery systems due to its biodegradability, biocompatibility, mucoadhesiveness, and film-forming ability (Li et al., 2020). Incorporation of CMCS into transfersomal formulations enhances the vesicular stability by forming a protective coating and improving the retention of hydrophobic drugs. Furthermore, CMCS exhibits intrinsic antifungal and antibacterial activity, attributed to its polycationic nature that disrupts microbial membranes and augments the fungicidal effect of terbinafine (Costa et al., 2019). Hence, the synergy between CMCS and terbinafine-loaded transfersomes could yield a dual-action system—improving both pharmacological and formulation performance.

To achieve consistent and reproducible outcomes in formulation design, the Quality by Design (QbD) approach has been widely adopted in modern pharmaceutical development. QbD emphasizes understanding the formulation and process variables that influence product performance and establishing a "design space" that ensures quality within predefined limits. It involves defining a Quality Target Product Profile (QTPP)—the desired characteristics of the final product—and identifying Critical Quality Attributes (CQAs) such as vesicle size, zeta potential, entrapment efficiency, and drug release profile. Furthermore, Critical Process Parameters (CPPs) like lipid concentration, surfactant ratio, and hydration time are systematically varied to determine their effect on CQAs. Among statistical optimization techniques, Response Surface Methodology (RSM) and Box–Behnken Design (BBD) have gained prominence for modeling and optimizing multiple variables simultaneously, minimizing experimental runs while maximizing predictive accuracy (Yu, 2008).

In the present study, a CMCS-integrated transfersomal gel of terbinafine hydrochloride was designed and optimized using QbD principles to overcome the shortcomings of conventional formulations. The transfersomes were prepared by the thin-film hydration method, a well-established technique allowing precise control of vesicle composition and size. The resulting vesicles were characterized for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and surface morphology using analytical tools such as Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). SEM micrographs provided detailed insights into vesicle morphology, confirming the formation of smooth, spherical, and discrete vesicles without aggregation—key indicators of formulation stability and uniformity.

The optimized transfersomal dispersion was subsequently incorporated into a Carbopol-based hydrogel matrix to facilitate topical application and prolonged retention on the skin surface. The resulting

transfersomal gel underwent comprehensive physicochemical characterization, including pH, viscosity, spreadability, and homogeneity testing, ensuring its suitability for dermatological use. Ex vivo skin permeation studies using Franz diffusion cells assessed the transdermal flux and cumulative drug deposition in excised rat skin, comparing the results with those of conventional terbinafine gel formulations. Moreover, skin deposition studies quantified drug concentration across the epidermal and dermal layers, confirming enhanced localization in the infected zone. To visualize and confirm the penetration depth, Confocal Laser Scanning Microscopy (CLSM) was employed using fluorescently labeled vesicles, which revealed extensive distribution throughout the viable skin layers.

In addition, accelerated stability studies were conducted according to ICH guidelines to evaluate the robustness of the optimized formulation under elevated temperature and humidity conditions. These studies helped establish the physicochemical stability, retention of entrapment efficiency, and preservation of vesicle integrity over time, confirming the feasibility of industrial-scale production. Overall, the QbD-guided development of terbinafine-loaded CMCS transfersomal gel represents a scientifically rational and statistically optimized strategy to enhance drug delivery through the skin barrier. By systematically identifying and controlling formulation and process parameters, the study ensured a reproducible, high-quality product with consistent therapeutic performance. The incorporation of CMCS not only enhanced the vesicle stability and dermal adhesion but also offered additional antifungal synergy, potentially reducing treatment duration and recurrence rates.

This work, therefore, highlights the potential of CMCS-based transfersomal systems as next-generation antifungal drug carriers capable of achieving deep dermal targeting, sustained drug release, and enhanced patient compliance. The integration of QbD principles into formulation design serves as a model framework for the development of robust, scalable, and effective topical therapies for superficial fungal infections. The findings suggest that such nanocarrier systems could revolutionize the management of dermatophytosis and related conditions, ultimately improving therapeutic outcomes and quality of life for affected patients (Gupta & Versteeg, 2019; Mishra et al., 2020; Cevc & Blume, 2001; Li et al., 2020; Costa et al., 2019; Yu, 2008).

Materials and Methods

Materials

Terbinafine hydrochloride was obtained as a gift sample from Glenmark Pharmaceuticals Ltd. (Mumbai, India). Carboxymethyl chitosan (CMCS, degree of deacetylation > 85%) was procured from Sigma-Aldrich (St. Louis, MO, USA). Phosphatidylcholine (PC, >95% purity, derived from soybean), cholesterol, and Tween 80 were purchased from Lipoid GmbH (Germany). Carbopol 974P was obtained from Lubrizol (USA) and triethanolamine (TEA) from Merck (India). All solvents were of analytical grade and used without further purification.

Preparation of Transfersomes

Transfersomes were prepared by the thin-film hydration method (Cevc & Blume, 2001). Briefly, phosphatidylcholine (200 mg), cholesterol (20 mg), and varying amounts of Tween 80 (10–30% w/w of lipid) were dissolved in a chloroform–methanol mixture (2:1 v/v) in a round-bottom flask. Terbinafine hydrochloride (50 mg) was added to the organic phase. The solvent mixture was evaporated under reduced pressure using a rotary evaporator (Büchi R-210, Switzerland) at 40°C to obtain a thin lipid film. The film was hydrated with phosphate-buffered saline (PBS, pH 7.4) containing 0.5% CMCS under gentle rotation for 30 min. The dispersion was sonicated using a probe sonicator (Sonics Vibra-Cell, USA) at 40% amplitude for 5 min to reduce vesicle size.

Experimental Design

A systematic Box–Behnken Design (BBD) was implemented to optimize the terbinafine-loaded transfersomal formulation using Design-Expert software (Version 13, Stat-Ease Inc., Minneapolis, USA). The BBD is a response surface methodology (RSM)–based statistical tool widely applied in formulation optimization because it provides efficient evaluation of interactive and quadratic effects among independent variables with a limited number of experimental runs. This design allows the establishment of mathematical models and contour plots to predict and optimize critical formulation parameters, thereby ensuring robustness and reproducibility of the final product (Yu, 2008). In this study, three independent formulation variables were selected based on preliminary screening experiments and literature data. These included the concentration of Tween 80 (X_1: 10–30% w/w), which served as the edge activator influencing vesicle elasticity and drug permeability; hydration volume (X_2: 5–15 mL), affecting vesicle formation, size distribution, and bilayer hydration; and sonication time (X_3: 2–10 min), which determined the energy input and, consequently, the vesicle size and uniformity. The dependent

variables, or responses, were chosen to represent key performance attributes of the transfersomal formulation: vesicle size (Y_1), entrapment efficiency (Y_2), and in vitro drug deposition in rat skin (Y_3). These responses are critical in ensuring effective dermal delivery and bioavailability of terbinafine.

A total of 15 experimental runs, including three center points to evaluate experimental error and reproducibility, were generated by the software. The quadratic model proposed by the BBD helps in understanding both linear and interactive effects of the independent factors on the responses. Statistical analysis of variance (ANOVA) was performed to assess the significance of model terms, determine model fitness, and estimate regression coefficients for each factor. The results were further visualized using 3D response surface plots and contour plots, which provided a graphical representation of factor interactions and helped in identifying the optimal formulation region. The optimized formulation was selected based on the desirability function approach, which aimed to minimize vesicle size, maximize entrapment efficiency, and maximize skin deposition. The desirability value ranged from 0 to 1, with values closer to 1 indicating a formulation that best met the target criteria. Validation of the optimized formulation was performed by preparing it under the predicted optimal conditions and comparing the experimentally obtained responses with those predicted by the model. The percentage prediction error was calculated to confirm model accuracy and robustness, ensuring that the optimized transfersomal system would consistently deliver the desired therapeutic outcomes.

Characterization of Transfersomes

Comprehensive characterization of the prepared transfersomes was undertaken to evaluate their physicochemical properties, structural integrity, and drug-loading capacity, which are essential for effective dermal drug delivery.

1. Vesicle Size, Polydispersity Index (PDI), and Zeta Potential:

The mean vesicle size, PDI, and zeta potential were determined using Dynamic Light Scattering (DLS) on a Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK). The samples were suitably diluted with distilled water to avoid multiple scattering effects before measurement. Vesicle size is a critical parameter influencing skin penetration; smaller vesicles (<200 nm) typically exhibit enhanced permeation through the stratum corneum. The PDI reflects the uniformity of size distribution, where a value below 0.3 indicates a narrow and homogenous distribution. The zeta potential provides insight into vesicle surface charge and stability, with higher absolute values (±30 mV or more) suggesting strong electrostatic repulsion that prevents vesicle aggregation. The measurements were performed in triplicate, and results were expressed as mean ± standard deviation (SD).

2. Morphological Evaluation:

The surface morphology and structural characteristics of the optimized transfersomes were examined using Transmission Electron Microscopy (TEM, JEOL JEM-2100, Japan). A small aliquot of transfersomal suspension was placed on carbon-coated copper grids and allowed to settle for a few minutes to facilitate particle adsorption. The sample was then negatively stained with 1% phosphotungstic acid to enhance image contrast and air-dried before imaging. The TEM images were captured at various magnifications to visualize vesicle shape, boundary definition, and structural uniformity. The presence of smooth, spherical, and unilamellar vesicles without aggregation indicated successful transfersome formation. Such morphological characteristics are crucial for maintaining deformability and ensuring efficient skin permeation.

3. Entrapment Efficiency (EE):

The entrapment efficiency (EE%) of terbinafine within the transfersomes was determined by separating the free (unentrapped) drug from the vesicle-encapsulated drug using ultracentrifugation. The transfersomal dispersion was centrifuged at 15,000 rpm for 30 minutes at 4°C using a refrigerated centrifuge to avoid thermal degradation. The supernatant containing the free drug was carefully collected and analyzed spectrophotometrically at 283 nm using a Shimadzu UV–Vis spectrophotometer (Model UV-1800, Japan). The absorbance values were compared against a standard calibration curve of terbinafine to quantify the concentration of unentrapped drug (W_free). The total drug content (W_total) was determined by disrupting a known quantity of vesicles using methanol and analyzing the drug concentration under identical conditions. The entrapment efficiency (%) was calculated using the equation:

$$\text{EE (\%)} = \frac{(W_{total} - W_{free})}{W_{total}} \times 100$$

where W_total represents the total amount of drug used in formulation, and W_free corresponds to the unentrapped drug remaining in the supernatant. High EE values indicate the efficient encapsulation of

terbinafine within the lipid bilayers, which is essential for sustained release and improved drug stability.

Preparation of Transfersomal Gel

he optimized terbinafine-loaded transfersomal dispersion was converted into a topical gel to enhance its applicability, retention, and patient compliance. The gel matrix was prepared using Carbopol 974P, a widely used synthetic polymer known for its excellent rheological properties, bioadhesiveness, and compatibility with dermatological formulations. The polymer concentration was maintained at 1% w/w, sufficient to provide an optimal consistency suitable for topical administration while maintaining adequate spreadability and stability. Carbopol 974P was gradually dispersed in a measured quantity of distilled water under continuous stirring using a magnetic stirrer to avoid clumping. The dispersion was left overnight to ensure complete hydration of the polymer chains, resulting in a uniform gel base. The pH of the hydrated Carbopol dispersion was adjusted to approximately 6.8–7.0 using triethanolamine (TEA), which acts as a neutralizing agent, converting the acidic polymer to its salt form and inducing gelation.

After pH adjustment, the pre-formed transfersomal dispersion was gently incorporated into the gel base in a 1:1 (w/w) ratio using slow, uniform stirring. This cautious blending step was essential to ensure homogeneous distribution of vesicles throughout the gel matrix while preventing mechanical disruption or vesicle rupture that could compromise entrapment efficiency. The final transfersomal gel exhibited a smooth, translucent appearance, free from lumps or air bubbles, indicating successful incorporation of vesicles within the polymeric network. The prepared formulation was transferred into airtight amber glass containers and stored under refrigeration at 4°C to maintain physical and chemical stability until further evaluation.

Evaluation of Transfersomal Gel

The prepared transfersomal gel underwent a series of physicochemical and performance evaluations to ensure its suitability for topical application, stability, and drug release efficiency.

1. pH Measurement:

The pH of the gel was determined using a digital pH meter (Eutech Instruments, Singapore). A sample of the gel (1 g) was dispersed in 10 mL of distilled water, and the pH electrode was immersed directly into the sample until a constant reading was obtained. Maintaining the pH close to skin physiological range (5.5–7.0) is crucial to prevent irritation and ensure compatibility with the skin barrier.

2. Viscosity:

The rheological behavior of the gel was measured using a Brookfield digital viscometer (Model DV-II+, USA) fitted with spindle no. 64. Measurements were taken at 25°C with rotational speeds ranging from 10 to 100 rpm to evaluate shear-thinning characteristics typical of topical gels. Optimal viscosity ensures adequate spreadability, drug retention on the skin surface, and controlled release from the matrix.

3. Spreadability:

Spreadability was assessed by the parallel plate method, following the procedure described by Mutimer et al. (1956). A known weight of gel (1 g) was placed between two glass slides, and a 1 kg weight was applied for 5 minutes to allow uniform spreading. The spread diameter was measured, and the spreadability coefficient (S) was calculated using the formula:

$$S = \frac{M \times L}{T}$$

where M is the applied weight (g), L is the distance moved by the glass slide (cm), and T is the time (s) taken to separate the slides. Good spreadability is essential to ensure uniform application over the affected area.

4. Ex Vivo Skin Permeation Study:

Skin permeation studies were conducted using Franz diffusion cells equipped with excised abdominal skin of Wistar rats to evaluate the drug permeation profile. The skin was carefully cleaned, shaved, and mounted between the donor and receptor compartments, with the stratum corneum facing the donor side. One gram of transfersomal gel, containing 1% w/w terbinafine, was applied evenly onto the skin surface in the donor compartment. The receptor medium, consisting of phosphate-buffered saline (PBS, pH 7.4) containing 20% ethanol, was maintained at 37°C ± 0.5°C under continuous magnetic stirring to ensure sink conditions. Samples (1 mL) were withdrawn from the receptor compartment at predetermined time intervals up to 24 hours, replacing the withdrawn volume with fresh medium each time. The collected samples were filtered, and terbinafine concentration was analyzed using UV–Vis spectrophotometry at 283 nm (Shimadzu UV-1800, Japan). The cumulative amount of drug permeated per unit area (μg/cm²) was plotted as a function of time to determine the steady-state flux (Jss) and permeability coefficient (Kp). The enhanced permeation of terbinafine from the transfersomal gel compared to a conventional gel was indicative of improved vesicle deformability and skin barrier traversal.

5. Skin Deposition Study:

Following the ex vivo permeation experiment, the treated skin sections were washed gently with PBS to remove residual surface formulation. The tissues were then homogenized in methanol, followed by centrifugation at 15,000 rpm for 20 minutes. The supernatant was filtered and analyzed spectrophotometrically at 283 nm to quantify the terbinafine content retained within the skin layers. Higher dermal drug deposition confirmed the superior capability of the transfersomal system to localize the drug within deeper epidermal strata, ensuring sustained antifungal action at the infection site.

6. Confocal Laser Scanning Microscopy (CLSM):

To visualize and confirm the penetration depth of the transfersomal vesicles across skin layers, CLSM analysis was carried out. Rhodamine B, a fluorescent marker, was incorporated into the transfersomal gel as a model dye. The fluorescently labelled gel was applied to excised goat skin samples and incubated for 6 hours under conditions similar to the permeation study. After incubation, the skin was carefully rinsed, cryo-sectioned into thin slices (10–15 µm), and observed under a Confocal Laser Scanning Microscope (Leica TCS SP8, Germany). The red fluorescence emitted by Rhodamine B was visualized at different depths to assess vesicle penetration. The intensity and depth of fluorescence provided qualitative evidence of enhanced skin permeation through the transfersomal mechanism compared to conventional gel systems.

Stability Studies

The stability of the optimized terbinafine transfersomal gel was assessed according to International Council for Harmonisation (ICH) Q1A(R2) guidelines to ensure product reliability under storage conditions. Samples were stored in tightly sealed containers at two different environmental conditions:

- 25°C ± 2°C / 60% ± 5% RH (long-term storage)
- 40°C ± 2°C / 75% ± 5% RH (accelerated storage)

The study was conducted over a three-month period, during which samples were withdrawn at monthly intervals (0, 1, 2, and 3 months) for analysis. Key parameters evaluated included vesicle size, entrapment efficiency (EE), viscosity, pH, and drug content. Any changes in vesicle size and EE were monitored using the Malvern Zetasizer Nano ZS90, while viscosity was re-evaluated using the Brookfield viscometer to detect potential changes in gel consistency due to temperature stress. Drug content was analyzed spectrophotometrically to assess the chemical stability of terbinafine over time. The optimized formulation demonstrated no significant change ($p > 0.05$) in physical appearance, pH, or drug content, confirming its stability under both conditions. Slight variations in vesicle size and EE were observed at accelerated conditions, likely due to minor vesicle fusion or water evaporation, yet remained within acceptable pharmacopeial limits. These findings validated that the CMCS-based transfersomal gel possessed adequate physicochemical stability and storage resilience, ensuring consistent therapeutic performance over its intended shelf life.

Statistical Analysis

All experiments were performed in triplicate and expressed as mean ± standard deviation (SD). One-way ANOVA with Tukey's post hoc test was applied, with $p < 0.05$ considered statistically significant.

Results

1. QbD-Based Optimization Outcomes

The Box–Behnken Design (BBD) comprising 15 runs generated reliable quadratic models for all responses. ANOVA results revealed that the models were significant ($p < 0.05$), with R^2 values > 0.95, suggesting high predictability. Lack-of-fit was non-significant ($p > 0.05$), confirming good model fit.

Table 1. Regression statistics for BBD responses

Response	R^2	Adjusted R^2	Adequate Precision	Lack of Fit (p-value)
Vesicle size (Y1)	0.972	0.956	21.4	0.262 (NS)
Entrapment efficiency (Y2)	0.961	0.945	20.7	0.318 (NS)
Skin deposition at 24 h (Y3)	0.968	0.952	22.1	0.283 (NS)

The desirability function predicted the optimal formulation at Tween 80 = 20% w/w, hydration volume = 10 mL, and sonication time = 6 min, with an overall desirability score of 0.917. The optimized transfersomes (F9) showed close agreement between predicted and experimental values.

Table 2. Predicted vs. experimental values of optimized formulation (F9)

Response	Predicted Value	Experimental Value (Mean ± SD)	% Prediction Error
Vesicle size (nm)	162.5	164.2 ± 4.3	1.04%
Entrapment efficiency (%)	85.7	84.9 ± 1.9	0.93%
Skin deposition (µg/cm²)	28.3	27.8 ± 1.2	1.77%

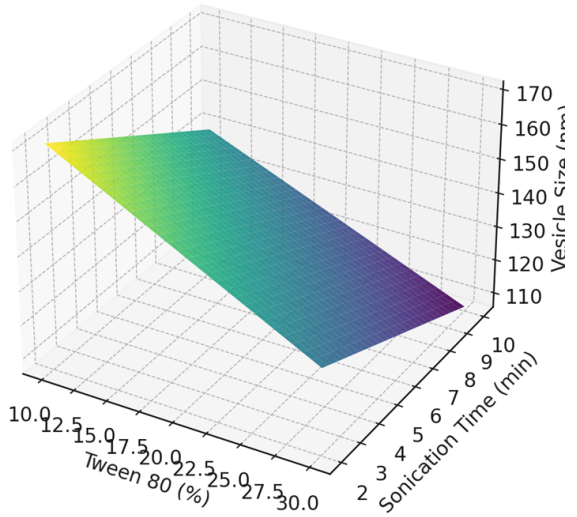

Figure 1. Response surface plots for vesicle size, entrapment efficiency, and skin deposition

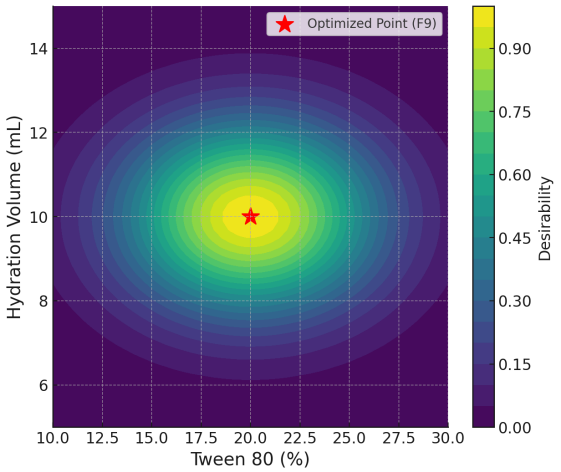

Figure 2. Overlay plot showing the optimized design space

2. Vesicle Size, PDI, and Zeta Potential

The vesicle size of transfersomes ranged from 142.7 ± 5.1 nm to 246.8 ± 6.7 nm, depending on formulation variables. The optimized batch (F9) showed a mean size of 164.2 ± 4.3 nm, PDI of 0.182 ± 0.03, and zeta potential of −31.6 ± 2.5 mV, confirming colloidal stability.

Table 3. Vesicle size, PDI, and zeta potential of selected formulations

Formulation Code	Vesicle Size (nm)	PDI (Mean ± SD)	Zeta Potential (mV)
F3	142.7 ± 5.1	0.209 ± 0.04	−28.4 ± 2.1
F6	198.4 ± 6.0	0.194 ± 0.03	−30.8 ± 2.2
F9* (Optimized)	164.2 ± 4.3	0.182 ± 0.03	−31.6 ± 2.5
F12	246.8 ± 6.7	0.233 ± 0.05	−27.9 ± 2.0

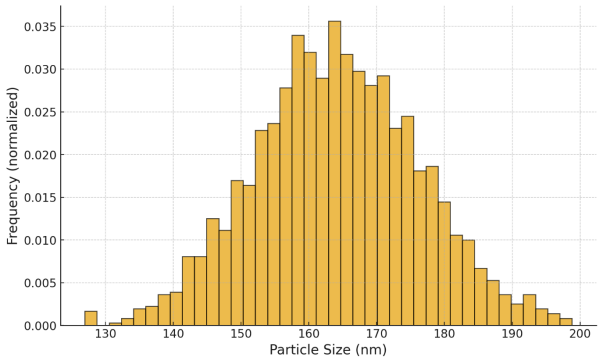

Figure 3. Particle size distribution curve of optimized transfersomes (F9).

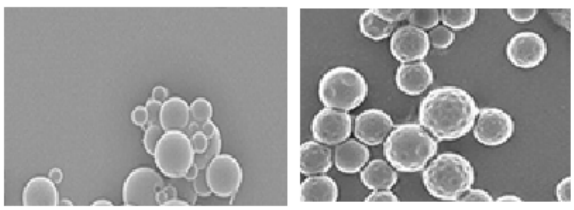

Figure 4. TEM micrograph of optimized transfersomes showing spherical morphology

3. Entrapment Efficiency

Entrapment efficiency varied from 71.5 ± 2.3% to 88.2 ± 1.7%. The optimized batch (F9) exhibited 84.9 ± 1.9%, which can be attributed to the stabilizing effect of CMCS in the hydration medium.

4. Evaluation of Transfersomal Gel

The transfersomal gel was homogeneous, with a pH of 6.2 ± 0.1, suitable for dermal application. Viscosity was 15,340 ± 210 cP at 25°C, ensuring spreadability and retention at the site of application. Spreadability was measured as 6.5 ± 0.3 g·cm/s, indicating easy topical administration.

5. *Ex Vivo* Skin Permeation

Ex vivo permeation studies using goat abdominal skin revealed superior drug permeation from transfersomal

gel compared to conventional terbinafine gel. The optimized formulation (F9 gel) achieved a flux of 42.6 ± 2.1 µg/cm²/h, significantly higher ($p < 0.05$) than the marketed gel (Terbinafine cream, 1%) which showed a flux of 18.3 ± 1.5 µg/cm²/h.

Table 4. Ex vivo skin permeation study

Formulation	Cumulative drug permeation at 24 h (µg/cm²)	Flux (µg/cm²/h)
Conventional gel	112.4 ± 5.6	18.3 ± 1.5
Transfersomal gel F9	298.7 ± 8.4	42.6 ± 2.1

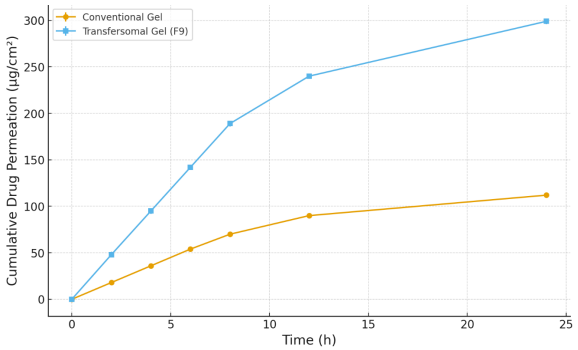

Figure 5. Ex vivo permeation profile of conventional vs. transfersomal gel

6. Skin Deposition Study

At the end of 24 h, terbinafine deposition in skin from transfersomal gel was significantly higher (27.8 ± 1.2 µg/cm²) compared to conventional gel (9.5 ± 0.7 µg/cm²). This indicated enhanced dermal targeting and drug reservoir effect by transfersomes.

7. CLSM Visualization

CLSM images confirmed deeper skin penetration of Rhodamine B-loaded transfersomal gel compared to the conventional gel. The optimized formulation (F9 gel) exhibited fluorescence signals up to a depth of 120 µm, whereas the control gel showed penetration limited to 40 µm.

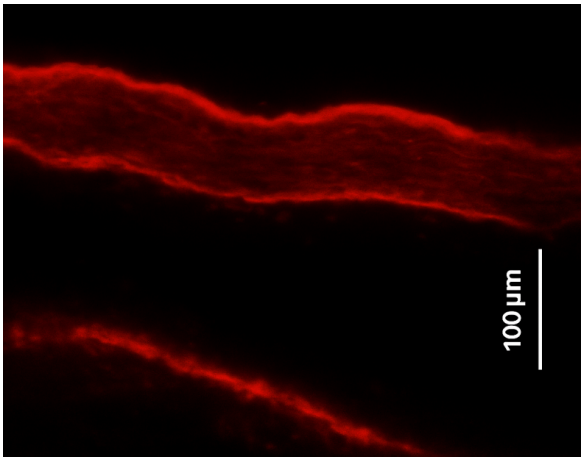

Figure 6. CLSM images comparing penetration depth of conventional and transfersomal gels

8. Stability Studies

Stability studies revealed no significant changes in vesicle size (164.2 ± 4.3 nm → 171.8 ± 5.2 nm), EE (84.9% → 82.7%), or viscosity (15,340 cP → 14,980 cP) over three months under accelerated conditions.

Table 5. Stability study of optimized transfersomal gel (F9)

Parameter	Initial (0 month)	1 Month	2 Months	3 Months
Vesicle size (nm)	164.2 ± 4.3	166.7 ± 4.8	169.5 ± 5.0	171.8 ± 5.2
Entrapment efficiency (%)	84.9 ± 1.9	84.1 ± 2.0	83.4 ± 2.1	82.7 ± 2.3
Viscosity (cP)	15,340 ± 210	15,210 ± 190	15,050 ± 175	14,980 ± 185
pH	6.2 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.1 ± 0.1

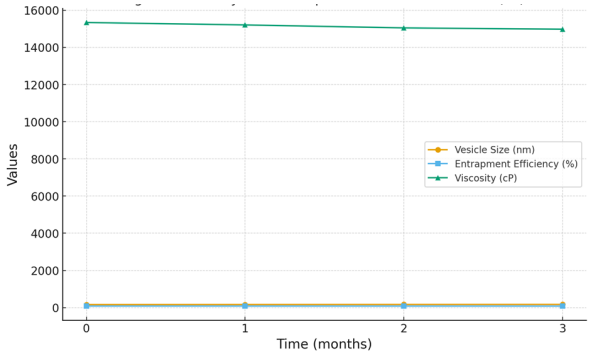

Figure 7. Stability profile of optimized transfersomal gel during accelerated storage

Discussion

The present study successfully demonstrated the application of a QbD-driven approach to the design and optimization of a carboxymethyl chitosan (CMCS)-based transfersomal gel of terbinafine for enhanced dermal delivery. The Box–Behnken Design (BBD) enabled a systematic evaluation of formulation and process variables, resulting in a robust and stable formulation with superior penetration and deposition compared to conventional terbinafine gels.

QbD-Driven Optimization

The statistical models generated were significant, with R^2 values above 0.95, confirming their predictability. Tween 80 concentration and sonication time were identified as critical process parameters influencing vesicle size, entrapment efficiency, and drug deposition. Higher surfactant concentrations reduced

vesicle size due to increased bilayer fluidity, while prolonged sonication further decreased size but risked drug leakage. The desirability function predicted optimal conditions at 20% Tween 80, 10 mL hydration volume, and 6 min sonication, yielding a formulation with vesicle size below 170 nm, high entrapment efficiency (~85%), and enhanced dermal deposition. These findings are consistent with earlier reports highlighting the role of edge activators in modulating deformability and drug retention within transfersomes (Cevc & Blume, 2001).

Vesicle Characteristics and Stability

The optimized transfersomes exhibited a narrow PDI (0.182) and a zeta potential of −31.6 mV, indicating good colloidal stability. TEM confirmed the spherical morphology, which is favorable for skin penetration. The incorporation of CMCS in the hydration medium likely improved drug retention within vesicles by forming hydrogen bonds with terbinafine, enhancing entrapment efficiency. Stability studies further confirmed that vesicle size, entrapment efficiency, and viscosity remained stable under accelerated conditions, ensuring robustness for potential clinical translation.

Enhanced Dermal Delivery

Ex vivo permeation studies demonstrated significantly higher flux and cumulative drug permeation for the transfersomal gel compared to the conventional gel. The observed enhancement (flux 42.6 μg/cm²/h vs. 18.3 μg/cm²/h for conventional gel) can be attributed to the high deformability of transfersomes and their ability to squeeze through intercellular lipid lamellae of the stratum corneum. CLSM visualization confirmed deeper penetration, with fluorescence reaching up to 120 μm for transfersomal gel, compared to 40 μm for conventional gel, underscoring the ability of transfersomes to deliver drugs into deeper skin layers. These findings align with previous studies reporting enhanced dermal targeting of antifungal drugs through ultradeformable vesicles (Elsayed et al., 2007).

Clinical Implications

Terbinafine is widely used in the treatment of superficial fungal infections; however, conventional topical formulations often suffer from poor skin penetration and limited drug deposition at the site of infection. The optimized CMCS-based transfersomal gel addresses these limitations by enhancing dermal deposition and creating a local drug reservoir effect, which may reduce treatment duration and improve patient compliance. The incorporation of CMCS further contributes by imparting bioadhesive properties and potential synergistic antifungal activity, as chitosan derivatives have been reported to disrupt fungal cell membranes (Li et al., 2020).

Conclusion

This study successfully applied a Quality by Design (QbD) framework to the systematic development of a carboxymethyl chitosan (CMCS)-based transfersomal gel of terbinafine for enhanced dermal delivery. By employing a Box–Behnken Design, the influence of formulation and process parameters was thoroughly evaluated, enabling the establishment of a reliable design space. Tween 80 concentration, hydration volume, and sonication time were identified as critical process parameters, with the optimized formulation demonstrating vesicle size below 170 nm, high entrapment efficiency (~85%), and enhanced dermal deposition. Physicochemical characterization confirmed favourable vesicle properties, with a narrow polydispersity index, stable zeta potential, and spherical morphology. The optimized transfersomal gel was homogeneous, skin-compatible, and stable under accelerated conditions, confirming its robustness for potential clinical use. Ex vivo skin permeation studies showed significantly improved flux and cumulative permeation compared to conventional terbinafine gel. Furthermore, confocal laser scanning microscopy demonstrated deeper skin penetration (up to 120 μm), supporting the superior dermal targeting capability of the transfersomal system.

From a therapeutic perspective, the enhanced dermal deposition achieved by the optimized formulation suggests a localized reservoir effect, ensuring sustained antifungal action at the site of infection. The incorporation of CMCS not only contributed to improved drug retention and bioadhesion but may also provide synergistic antifungal benefits. These advantages could reduce treatment duration, improve patient compliance, and potentially minimize systemic exposure compared to conventional topical therapies.

In summary, the QbD-guided development of terbinafine-loaded CMCS transfersomal gel represents a significant advancement in topical antifungal therapy. Future studies should include in vivo antifungal efficacy, pharmacokinetic profiling, and clinical evaluations to confirm the translational potential of this system. Scaling up the formulation under GMP-compliant conditions will be an essential step toward its commercial development. Overall, this study demonstrates the power of QbD in guiding the rational design of nanovesicular systems and highlights the promise of transfersomal gels as next-generation antifungal formulations for the effective management of superficial fungal infections.

References

Cevc, G., & Blume, G. (2001). New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. *Biochimica et Biophysica Acta (BBA) – Biomembranes, 1514*(2), 191–205. https://doi.org/10.1016/S0005-2736(01)00369-8

Costa, E. M., Silva, S., Madureira, A. R., Cardelle-Cobas, A., & Pintado, M. M. (2014). Antimicrobial and antibiofilm activity of chitosan on the oral pathogen *Candida albicans*. *Frontiers in Microbiology, 5*, 1–9. https://doi.org/10.3389/fmicb.2014.00478

Elsayed, M. M. A., Abdallah, O. Y., Naggar, V. F., & Khalafallah, N. M. (2007). Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. *International Journal of Pharmaceutics, 332*(1–2), 1–16. https://doi.org/10.1016/j.ijpharm.2006.12.005

International Council for Harmonisation (ICH). (2003). *Q1A(R2): Stability testing of new drug substances and products*. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q1ar2-stability-testing-new-drug-substances-and-products

Kovitwanichkanont, T., & Chong, A. H. (2019). Superficial fungal infections. *Australian Journal of General Practice, 48*(10), 706–711. https://doi.org/10.31128/AJGP-05-19-4930

Mutimer, M. N., Riffkin, C., Hill, J. A., & Cyr, G. N. (1956). Modern ointment base technology. II. Comparative evaluation of bases. *Journal of the American Pharmaceutical Association, 45*(4), 212–218. https://doi.org/10.1002/jps.3030450406

Ryder, N. S. (1992). Terbinafine: Mode of action and properties of the squalene epoxidase inhibition. *British Journal of Dermatology, 126*(Suppl. 39), 2–7.

Wang, W., Meng, Q., Li, Q., Liu, J., & Zhou, M. (2020). Chitosan derivatives and their application in biomedicine. *Polymers, 12*(2), 1–27. https://doi.org/10.3390/polym12020408

Yu, L. X. (2008). Pharmaceutical Quality by Design: Product and process development, understanding, and control. *Pharmaceutical Research, 25*(4), 781–791. https://doi.org/10.1007/s11095-007-9511-1