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DEVELOPMENT AND EVALUATION OF CLOTRIMAZOLE-LOADED NANOEMULGEL FOR ENHANCED TOPICAL ANTIFUNGAL DELIVERY

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ABSTRACT

Background: Clotrimazole is a widely used topical imidazole antifungal; however, its physicochemical properties and limited skin penetration can restrict effective drug delivery from conventional topical formulations. Nanoemulgel systems offer a promising approach by combining the nanoscale characteristics of nanoemulsions with the prolonged residence and improved application properties of a gel base. **Objective:** The present study aimed to develop and optimize a clotrimazole-loaded nanoemulgel for enhanced topical drug delivery, skin permeation, skin retention, and antifungal activity. **Methods:** Preformulation studies of clotrimazole included organoleptic characterization, solubility, melting point, and partition coefficient determination. Based on solubility screening, oleic acid, Tween 80, and Transcutol P were selected as the oil, surfactant, and co-surfactant, respectively. Pseudo-ternary phase diagrams were constructed, and six nanoemulsion formulations (F1–F6) were prepared using high-speed homogenization followed by ultrasonication. The optimized nanoemulsion was incorporated into Carbopol 934 gel to prepare four nanoemulgel formulations (NG1–NG4). Formulations were evaluated for physicochemical characteristics, in-vitro drug diffusion and release kinetics. The optimized nanoemulgel was further assessed for ex-vivo skin permeation and retention and antifungal activity against *Candida albicans* and *Aspergillus niger*. **Results:** Clotrimazole exhibited maximum solubility in oleic acid (82.45 ± 1.24 mg/mL), Tween 80 (95.64 ± 1.42 mg/mL), and Transcutol P (108.52 ± 1.63 mg/mL). Formulation F5 showed the smallest droplet size (98.4 ± 1.82 nm) and lowest polydispersity

index (0.164 ± 0.005), and was selected as the optimized nanoemulsion. Among the nanoemulgels, NG4 demonstrated superior performance, with 96.82 ± 0.58% drug diffusion at 12 h. Drug-release analysis showed the best fit with the Higuchi model (R^2 = 0.989), while the Korsmeyer–Peppas diffusion exponent (n = 0.684) indicated non-Fickian release. Compared with conventional clotrimazole gel, NG4 exhibited higher 12-h drug permeation (624.48 ± 1.58 vs. 436.26 ± 1.32 µg/cm²), flux (52.84 ± 1.06 vs. 36.42 ± 1.12 µg/cm²/h), and skin retention (246.82 ± 1.94 vs. 128.46 ± 2.08 µg/cm²). NG4 also produced larger zones of inhibition against *C. albicans* (31.8 ± 0.72 mm) and *A. niger* (28.4 ± 0.68 mm) than the conventional gel. **Conclusion:** The optimized clotrimazole-loaded nanoemulgel demonstrated favorable physicochemical characteristics, controlled drug release, enhanced skin permeation and retention, and improved antifungal activity compared with the conventional formulation. The developed nanoemulgel therefore represents a promising topical delivery system for improving the therapeutic performance of clotrimazole.

KEYWORDS: Clotrimazole; Nanoemulsion; Nanoemulgel; Topical drug delivery; Skin permeation; Skin retention; Antifungal activity; Carbopol 934.

INTRODUCTION

Superficial fungal infections constitute a major global dermatological burden and are among the most prevalent infectious diseases affecting humans. Fungal skin diseases were estimated to have a global incidence of approximately 21,277 cases per 100,000 population in 2019, with the overall number of incident cases continuing to increase over the preceding three decades.[1] Dermatophytoses alone affect approximately one-quarter of the global population, and persistent, recurrent, and treatment-refractory infections are increasingly recognized as important clinical problems.[2] The emergence and global dissemination of antifungal-resistant dermatophytes, particularly *Trichophyton indotineae*, have further complicated the management of superficial mycoses and underscore the need for effective therapeutic strategies and improved drug-delivery approaches.[3,4]

Topical administration remains an important therapeutic approach for localized superficial fungal infections because it enables drug delivery directly to the site of infection while potentially minimizing systemic exposure.[5] However, conventional topical formulations, including creams, ointments, and lotions, may provide inconsistent drug release, limited residence time, inadequate penetration across the stratum corneum, and variable drug concentrations at the site of infection. These limitations can compromise therapeutic

performance and may necessitate repeated application, potentially affecting patient adherence.[5,6] The stratum corneum represents the principal barrier to topical drug delivery, and optimization of formulation composition and drug–skin interactions is therefore critical for achieving adequate cutaneous drug deposition and therapeutic efficacy.

Clotrimazole is a broad-spectrum imidazole antifungal widely used for the treatment of superficial dermatophyte and *Candida* infections. Its antifungal activity is primarily associated with inhibition of ergosterol biosynthesis, resulting in disruption of fungal membrane integrity.[7] Despite its established therapeutic utility, clotrimazole presents important formulation challenges, particularly its poor aqueous solubility and highly lipophilic character. These physicochemical properties can limit dissolution and influence drug partitioning and penetration within the skin, thereby restricting effective delivery from conventional topical dosage forms.[8,9] Previous formulation studies have consequently investigated lipid-based and nanoscale systems to improve the cutaneous availability of clotrimazole.

Nanoemulsions are colloidal delivery systems composed of oil, aqueous phase, surfactant, and co-surfactant, with nanoscale droplets providing a large interfacial area for drug solubilization and interaction with the skin. Their composition can be optimized to improve the apparent solubility, dispersion, release, and cutaneous delivery of poorly water-soluble drugs.[10] Surfactants and co-surfactants may additionally influence the organization of stratum-corneum lipids and facilitate drug transport across the skin barrier. Consequently, nanoemulsion-based systems have attracted considerable interest for topical delivery of antifungal agents.[10,11]

However, the relatively low viscosity and fluid nature of nanoemulsions may limit their residence time and handling properties at the application site. Incorporation of an optimized nanoemulsion into a polymeric gel matrix produces a nanoemulgel, thereby integrating the drug-solubilizing and permeation-enhancing properties of the nanoemulsion with the viscosity, spreadability, stability, and prolonged residence characteristics of a gel.[11–13] Recent literature indicates that nanoemulgels are particularly promising for topical delivery of lipophilic drugs because nanosized droplets can facilitate drug transport while the gel matrix provides improved application characteristics and sustained release.[12,13]

Previous studies have demonstrated that advanced nanocarriers can improve the dermal delivery of clotrimazole. For example, clotrimazole-loaded nanoemulsions have been

investigated for optimization of droplet size, while vesicular systems such as invaethosomes and invaflexosomes have demonstrated enhanced skin permeability and antifungal performance compared with conventional formulations.[14,15] Nevertheless, there remains a need for systematically optimized clotrimazole nanoemulgel systems in which formulation composition, nanoscale characteristics, gel properties, drug diffusion, skin permeation, skin retention, and antifungal activity are evaluated as an integrated delivery strategy.

AIM AND OBJECTIVE

Therefore, the present study aimed to **develop and optimize a clotrimazole-loaded nanoemulgel for enhanced topical antifungal delivery**. The study specifically evaluated the formulation and optimization of clotrimazole nanoemulsions, incorporation of the optimized nanoemulsion into a Carbopol 934 gel matrix, physicochemical characteristics, in-vitro drug diffusion and release kinetics, ex-vivo skin permeation and retention, and comparative antifungal activity against *Candida albicans* and *Aspergillus niger*. This formulation strategy was designed to address the solubility, skin penetration, residence-time, and drug-release li.

3. MATERIALS AND METHODS

3.1 MATERIALS

Clotrimazole was used as the model antifungal drug. Oleic acid, Tween 80, and Transcutol P were selected as the oil, surfactant, and co-surfactant, respectively, based on preliminary solubility screening. Carbopol 934 was used as the gelling polymer, while propylene glycol served as a humectant and penetration enhancer. Triethanolamine was used for neutralization and pH adjustment, and methyl paraben was incorporated as a preservative. Methanol, ethanol, phosphate-buffered saline, distilled water, and analytical-grade reagents were used throughout the study. The selection of formulation components was consistent with established approaches for clotrimazole nanoemulsion development and topical nanoemulsion delivery.[16,17]

3.2 Preformulation Studies

3.2.1 Solubility Study

The solubility of clotrimazole was determined in different oils, surfactants, and co-surfactants to identify suitable components for nanoemulsion development. An excess amount of clotrimazole was added to each vehicle and equilibrated under controlled conditions with intermittent shaking. The samples were subsequently centrifuged and filtered, and the drug

concentration in the supernatant was quantified by UV–visible spectrophotometry using a previously established calibration curve. Components providing the highest drug solubilization were selected for further formulation development.

3.2.2 Melting Point

The melting point of clotrimazole was determined using a standard capillary method to confirm the identity and purity of the drug prior to formulation.

3.2.3 Partition Coefficient

The apparent partition coefficient of clotrimazole was determined using an appropriate immiscible organic solvent/water system. The drug was allowed to equilibrate between the two phases, followed by separation and spectrophotometric estimation of drug concentration in each phase. The partition coefficient was calculated as the ratio of drug concentration in the organic phase to that in the aqueous phase. The observed lipophilicity supported the need for a lipid-based delivery strategy.

3.3 Development and Optimization of Clotrimazole Nanoemulsion

3.3.1 Selection of Oil, Surfactant and Co-surfactant

The formulation components were selected on the basis of clotrimazole solubility and their ability to form a stable nanoemulsion. Oleic acid, Tween 80, and Transcutol P were selected as the oil, surfactant, and co-surfactant, respectively. The use of solubility screening followed by phase-diagram-based selection is an established strategy for developing clotrimazole nanoemulsions.[16]

3.3.2 Construction of Pseudo-ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed using the selected oil, surfactant/co-surfactant mixture (Smix), and aqueous phase. Different Smix ratios were investigated to identify the composition range capable of producing a clear and isotropic nanoemulsion region. The aqueous phase was progressively incorporated into mixtures of oil and Smix under continuous mixing, and the resulting systems were visually assessed for transparency, homogeneity, and phase separation. The nanoemulsion region was subsequently used to select appropriate formulation compositions.[16,18]

3.3.3 Preparation and Optimization of Nanoemulsion Formulations

Six clotrimazole-loaded nanoemulsion formulations (F1–F6) were prepared by varying the concentrations of oil, surfactant, and co-surfactant. Clotrimazole was initially dissolved in the selected oil phase, followed by incorporation of the surfactant and co-surfactant. The aqueous phase was subsequently added under continuous stirring. The resulting coarse emulsions were subjected to high-speed homogenization followed by ultrasonication to obtain nanosized droplets. Formulation optimization was based primarily on droplet size, PDI, zeta potential, drug content, physical appearance, and drug-release performance. F5 was subsequently selected as the optimized nanoemulsion for incorporation into the gel matrix.

3.4 Evaluation of Nanoemulsion

The prepared nanoemulsions were characterized for droplet size, polydispersity index (PDI), zeta potential, drug content, pH, viscosity, refractive index, percentage transmittance, surface morphology, and in-vitro drug release. These parameters are commonly used to establish the physicochemical characteristics and performance of nanoemulsions intended for topical delivery.[17,19]

3.4.1 Droplet Size, PDI and Zeta Potential

Droplet size, PDI, and zeta potential were determined using dynamic light scattering/electrophoretic light-scattering analysis. Samples were appropriately diluted with distilled water before measurement, and measurements were performed at controlled temperature. Droplet size and PDI were used to assess nanoscale characteristics and size distribution, whereas zeta potential was used as an indicator of interfacial charge and physical stability.[19] The optimized formulation demonstrated a droplet size of approximately 98 nm and a PDI below 0.2.

3.4.2 Drug Content

An accurately measured quantity of nanoemulsion was diluted with methanol and filtered through a 0.45-µm membrane filter. The drug concentration was determined spectrophotometrically at the predetermined λmax of clotrimazole using the calibration curve. Drug content was expressed as a percentage of the theoretical drug concentration.

3.4.3 pH, Viscosity and Percentage Transmittance

The pH was determined using a calibrated digital pH meter. Viscosity was measured using a Brookfield digital viscometer at 25 ± 1°C. Percentage transmittance was determined spectrophotometrically at 650 nm using distilled water as the blank. These parameters were

assessed to establish formulation uniformity, flow characteristics, transparency, and suitability for topical application.

3.4.4 Surface Morphology

The morphology of the optimized nanoemulsion was examined by scanning electron microscopy. The sample was appropriately diluted, dried, sputter-coated with gold, and examined at suitable magnification. Morphological characteristics including droplet shape, surface appearance, aggregation, and uniformity were evaluated.

3.4.5 In-vitro Drug Release

Drug release from the nanoemulsion was evaluated using a Franz diffusion cell fitted with a dialysis membrane. The receptor compartment contained phosphate buffer (pH 7.4) with a small quantity of methanol to maintain sink conditions and was maintained at 37 ± 0.5°C with continuous stirring. Samples were withdrawn at predetermined intervals, replaced with an equal volume of fresh medium, and analysed spectrophotometrically. Cumulative drug release was plotted against time.

3.5 Development of Clotrimazole Nanoemulgel

The optimized F5 nanoemulsion was incorporated into a Carbopol 934 gel base to develop the nanoemulgel. Carbopol 934 was dispersed in distilled water and allowed to hydrate, followed by addition of propylene glycol. The optimized nanoemulsion was incorporated gradually under continuous stirring. Triethanolamine was added dropwise to neutralize the Carbopol and adjust the formulation to a skin-compatible pH. Four formulations (NG1–NG4) containing increasing concentrations of Carbopol 934 (0.5–2.0% w/w) were prepared to optimize the gel characteristics.

The selection of Carbopol concentration was considered important because polymer concentration can substantially influence viscosity, drug diffusion, skin permeation, and drug retention from nanoemulsion-gel systems.[20]

3.6 Evaluation of Nanoemulgel

3.6.1 Appearance and Homogeneity

The formulations were visually examined for colour, transparency, consistency, smoothness, homogeneity, phase separation, grittiness, aggregates, and air bubbles.

3.6.2 pH and Viscosity

The pH was determined using a calibrated digital pH meter after dispersing approximately 1 g of formulation in distilled water. Viscosity was measured using a Brookfield viscometer at 25 ± 1°C.

3.6.3 Spreadability and Extrudability

Spreadability was determined using the glass-slide method and calculated as:

$$S = TM \times L$$

where *S* is spreadability, *M* is the applied weight, *L* is the distance travelled by the slide, and *T* is the time required. Extrudability was evaluated using a collapsible tube by measuring the quantity of formulation extruded under a specified load.

3.6.4 Drug Content and Swelling Index

Drug content was determined by extracting clotrimazole from an accurately weighed quantity of nanoemulgel using methanol, followed by filtration and UV–visible spectrophotometric analysis. The swelling index was determined by recording the weight increase of the formulation after exposure to phosphate buffer and calculated as:

$$Swelling\ Index\ (\%) = Wt - W0/W0 \times 100$$

where W_0 is the initial weight and W□ is the weight after swelling.

3.6.5 In-vitro Drug Diffusion

Drug diffusion from NG1–NG4 was investigated using a Franz diffusion cell with a dialysis membrane and phosphate buffer (pH 7.4) containing a small quantity of methanol as receptor medium. The receptor phase was maintained at 37 ± 0.5°C and continuously stirred. Samples were collected at predetermined intervals, replaced with fresh medium, and analysed spectrophotometrically.

3.6.6 Drug Release Kinetics

The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to characterize the release mechanism. The model showing the highest coefficient of determination (R^2) was considered the best-fitting model. The Korsmeyer–Peppas exponent was additionally used to characterize the release mechanism.

3.7 Ex-vivo Skin Permeation Study

Ex-vivo permeation of the optimized nanoemulgel was evaluated using excised abdominal skin of Wistar albino rats in a Franz diffusion cell. The skin was carefully separated from underlying adipose tissue, washed with normal saline, and mounted between the donor and receptor compartments with the stratum corneum facing the donor phase. The receptor medium was maintained at 37 ± 0.5°C under continuous stirring. At predetermined intervals, receptor samples were withdrawn and analysed spectrophotometrically. The cumulative amount of drug permeated per unit area was plotted against time.

The steady-state flux (Jss) was calculated from the linear portion of the permeation profile:

$$Jss = dQ/Adt$$

where *dQ/dt* represents the amount of drug permeated per unit time and *A* is the effective diffusion area. The permeability coefficient (Kp) was calculated as:

$$Kp = Jss/C0$$

where C_0 is the initial donor-phase drug concentration. At the end of the experiment, residual formulation was removed from the skin, the tissue was homogenized in methanol, and retained clotrimazole was extracted and quantified spectrophotometrically.

3.8 Antifungal Activity

The antifungal activity of the optimized nanoemulgel was evaluated against ***Candida albicans*** and ***Aspergillus niger*** using the agar-well diffusion method. Appropriate fungal cultures were inoculated onto Sabouraud dextrose agar, and wells containing the test formulation and conventional clotrimazole formulation were prepared under aseptic conditions. Following incubation under appropriate conditions, antifungal activity was quantified by measuring the diameter of the zones of inhibition in millimetres. The thesis specifically identifies these two organisms and the agar-well diffusion method for comparative antifungal evaluation.

The selection of *C. albicans* and *A. niger* is also consistent with previous clotrimazole topical-delivery research demonstrating comparative antifungal activity against these organisms.[21]

3.9 Statistical Analysis

All quantitative formulation and evaluation experiments were performed in triplicate wherever applicable, and results were expressed as **mean ± standard deviation (SD)**. Differences among formulation groups were analysed using **one-way analysis of variance (ANOVA)** followed, where appropriate, by a suitable post-hoc multiple-comparison test. For comparisons involving two groups, an appropriate two-tailed statistical test was used. A **p-value <0.05** was considered statistically significant. Statistical analysis was performed using appropriate statistical software. This approach is consistent with established pharmaceutical formulation optimization studies in which ANOVA is used to determine the statistical significance of formulation-dependent responses.[22,23]

RESULTS

4.1 Preformulation and Solubility Results

Clotrimazole was obtained as a white to pale-yellow crystalline powder, consistent with its reported physical characteristics. The drug exhibited poor aqueous solubility, confirming the need for a lipid-based delivery approach. Solubility screening demonstrated the highest drug solubilization in **oleic acid (82.45 ± 1.24 mg/mL), Tween 80 (95.64 ± 1.42 mg/mL), and Transcutol P (108.52 ± 1.63 mg/mL)**. These components were therefore selected as the oil, surfactant, and co-surfactant, respectively.

The partition coefficient of clotrimazole was **11.06 ± 0.28**, with a corresponding log P of **1.04 ± 0.03**, confirming its lipophilic nature and supporting the selection of an oil-based nanoemulsion system.

4.2 Optimization of Clotrimazole Nanoemulsion

Six nanoemulsion formulations (F1–F6) were developed by varying the concentrations of oil, surfactant, and co-surfactant. The formulations exhibited nanoscale droplet formation with differences in droplet size, PDI, zeta potential, drug content, pH, and optical characteristics.

Among the tested formulations, **F5 demonstrated the most favorable overall characteristics** and was therefore selected as the optimized nanoemulsion. F5 exhibited a mean droplet size of **98.4 ± 1.82 nm** and a PDI of **0.164 ± 0.005**, indicating a narrow and relatively uniform droplet-size distribution.

Table 1: Composition and physicochemical characterization of clotrimazole-loaded nanoemulsion formulations (F1–F6).

Parameter	F1	F2	F3	F4	F5	F6
Clotrimazole (% w/v)	1	1	1	1	1	1
Oil phase (% w/v)	5	7.5	10	5	7.5	10
Surfactant (% w/v)	20	20	20	25	25	25
Co-surfactant (% w/v)	10	10	10	12.5	12.5	12.5
Distilled water q.s. (%)	100	100	100	100	100	100
Droplet size (nm)	182.6 ± 3.24	165.8 ± 2.91	154.2 ± 2.47	132.5 ± 2.16	98.4 ± 1.82	118.7 ± 2.05
PDI	0.284 ± 0.012	0.251 ± 0.010	0.226 ± 0.008	0.198 ± 0.007	0.164 ± 0.005	0.181 ± 0.006
Zeta potential (mV)	−21.4 ± 0.84	−24.7 ± 0.76	−27.8 ± 0.69	−31.2 ± 0.58	−36.5 ± 0.52	−33.4 ± 0.61
Drug content (%)	91.42 ± 1.28	93.65 ± 1.16	95.18 ± 1.04	96.84 ± 0.92	98.76 ± 0.81	97.12 ± 0.88
pH	5.42 ± 0.06	5.58 ± 0.05	5.71 ± 0.04	5.86 ± 0.03	6.12 ± 0.02	5.94 ± 0.04
Viscosity (cP)	86.4 ± 1.82	92.7 ± 1.65	98.3 ± 1.48	104.6 ± 1.36	112.8 ± 1.24	108.2 ± 1.31
Refractive index	1.368 ± 0.004	1.372 ± 0.003	1.376 ± 0.003	1.381 ± 0.002	1.386 ± 0.002	1.383 ± 0.003

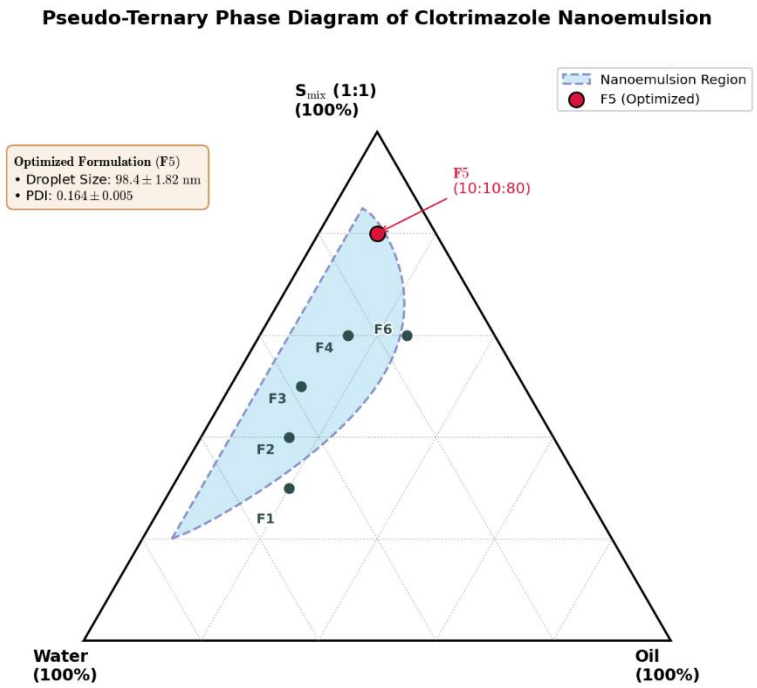


Figure 1. Pseudo-ternary phase diagram showing the nanoemulsion region.

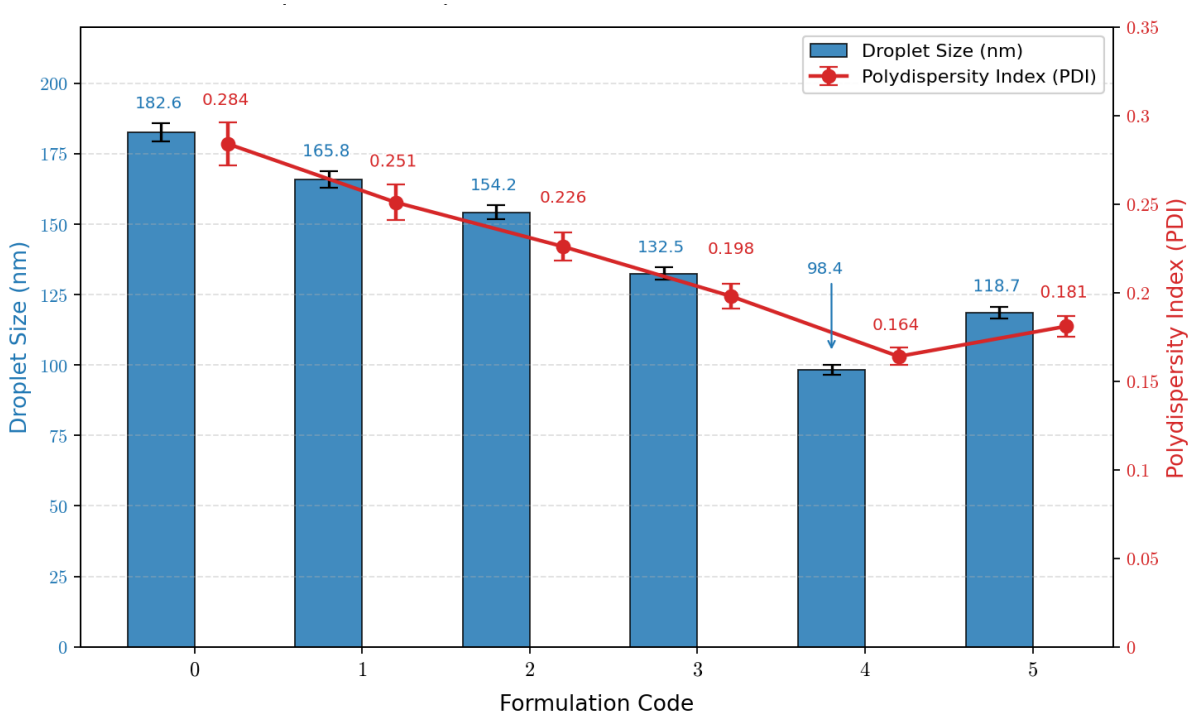


Figure 2: Comparative droplet size and PDI of F1–F6.

4.3 Optimization of Clotrimazole Nanoemulgel

The optimized F5 nanoemulsion was incorporated into Carbopol 934 gel to produce four nanoemulgel formulations (NG1–NG4), containing increasing concentrations of Carbopol 934 from 0.5 to 2.0% w/w. Increasing polymer concentration produced progressive changes in viscosity, spreadability, swelling behavior, and diffusion characteristics. **NG4**, containing 2.0% Carbopol 934, demonstrated the most favorable overall balance of formulation properties and was therefore selected as the optimized nanoemulgel. NG4 exhibited a viscosity of **11,986 ± 61 cP**, spreadability of **24.18 ± 0.98 g·cm/sec**, pH of **6.18 ± 0.02**, and swelling index of **172.58 ± 1.44%**.

Table 2: Composition and physicochemical characteristics of clotrimazole nanoemulgel formulations (NG1–NG4).

Parameter	NG1	NG2	NG3	NG4
Clotrimazole-loaded nanoemulsion (% w/w)	40	40	40	40
Carbopol 934 (% w/w)	0.5	1.0	1.5	2.0
Propylene glycol (% w/w)	5	5	5	5
Triethanolamine	q.s.	q.s.	q.s.	q.s.
Methyl paraben (%	0.1	0.1	0.1	0.1

w/w)				
Distilled water	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100
Appearance	White, smooth gel	Off-white, smooth gel	White, glossy gel	White, smooth glossy gel
Consistency	Good	Good	Very good	Excellent
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Highly homogeneous
Phase separation	Absent	Absent	Absent	Absent
pH	5.54 ± 0.05	5.72 ± 0.04	5.94 ± 0.03	6.18 ± 0.02
Viscosity (cP)	8425 ± 82	9658 ± 76	10842 ± 68	11986 ± 61
Spreadability (g·cm/sec)	32.48 ± 1.24	29.76 ± 1.12	26.84 ± 1.06	24.18 ± 0.98
Extrudability (g)	118 ± 3.2	132 ± 2.8	146 ± 2.4	158 ± 2.1
Extrudability grade	Excellent	Excellent	Good	Good
Drug content (%)	91.84 ± 1.26	94.36 ± 1.12	96.72 ± 0.96	98.42 ± 0.82
Swelling index (%)	118.42 ± 2.16	136.84 ± 1.92	154.26 ± 1.68	172.58 ± 1.44

4.4 Physicochemical Characterization

The optimized nanoemulsion F5 exhibited nanoscale droplet formation, low PDI, and acceptable physicochemical characteristics suitable for topical application. SEM analysis demonstrated predominantly spherical and uniformly distributed droplets without evident aggregation, supporting the nanoscale characteristics obtained by particle-size analysis.

The optimized nanoemulgel NG4 appeared homogeneous and smooth, without visible phase separation or coarse particles. Its skin-compatible pH and suitable viscosity indicated acceptable characteristics for topical administration. The drug content was within the acceptable formulation range, indicating relatively uniform drug distribution within the gel matrix.

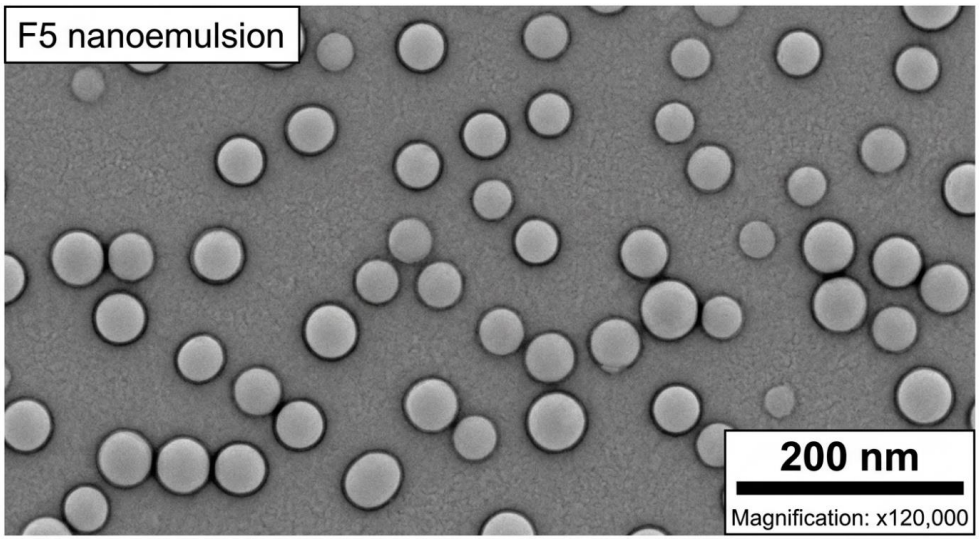


Figure 3. SEM image of optimized F5 nanoemulsion.

4.5 In-vitro Drug Diffusion and Release Kinetics

The in-vitro diffusion study demonstrated formulation-dependent drug release from NG1–NG4. Drug diffusion increased progressively with increasing Carbopol concentration, with NG4 demonstrating the highest cumulative diffusion. After 12 h, cumulative drug diffusion was:

Formulation	Drug diffusion (%)
NG1	75.36 ± 0.70
NG2	82.84 ± 0.66
NG3	90.26 ± 0.62
NG4	**96.82 ± 0.58**

Thus, NG4 exhibited the highest drug diffusion among the tested nanoemulgels.

Release-kinetic analysis showed that the optimized NG4 formulation followed the **Higuchi model**, with an R^2 value of **0.989**. The Korsmeyer–Peppas model yielded a diffusion exponent (**n**) of **0.684**, indicating a predominantly **non-Fickian/anomalous release mechanism**.

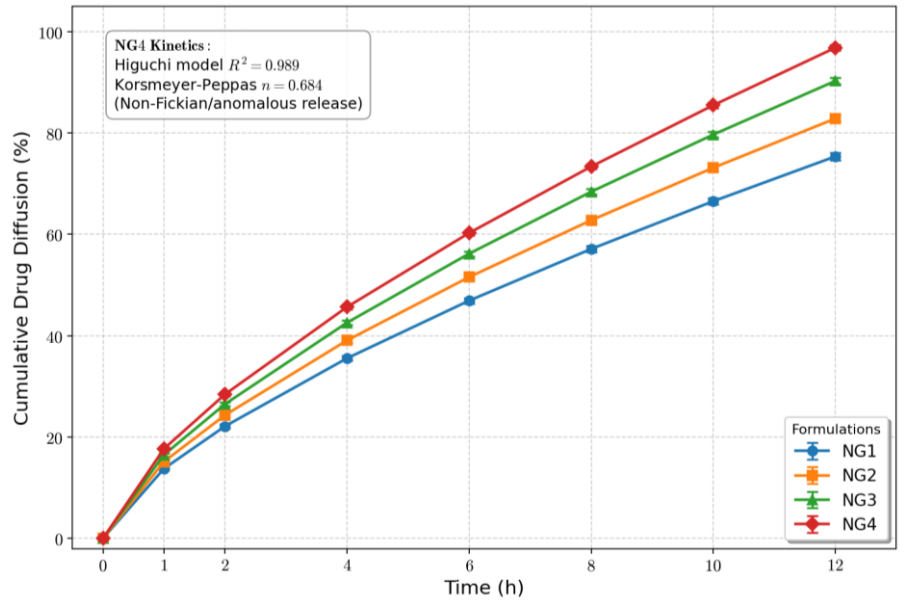


Figure 4: In-vitro drug-diffusion profiles of NG1–NG4.

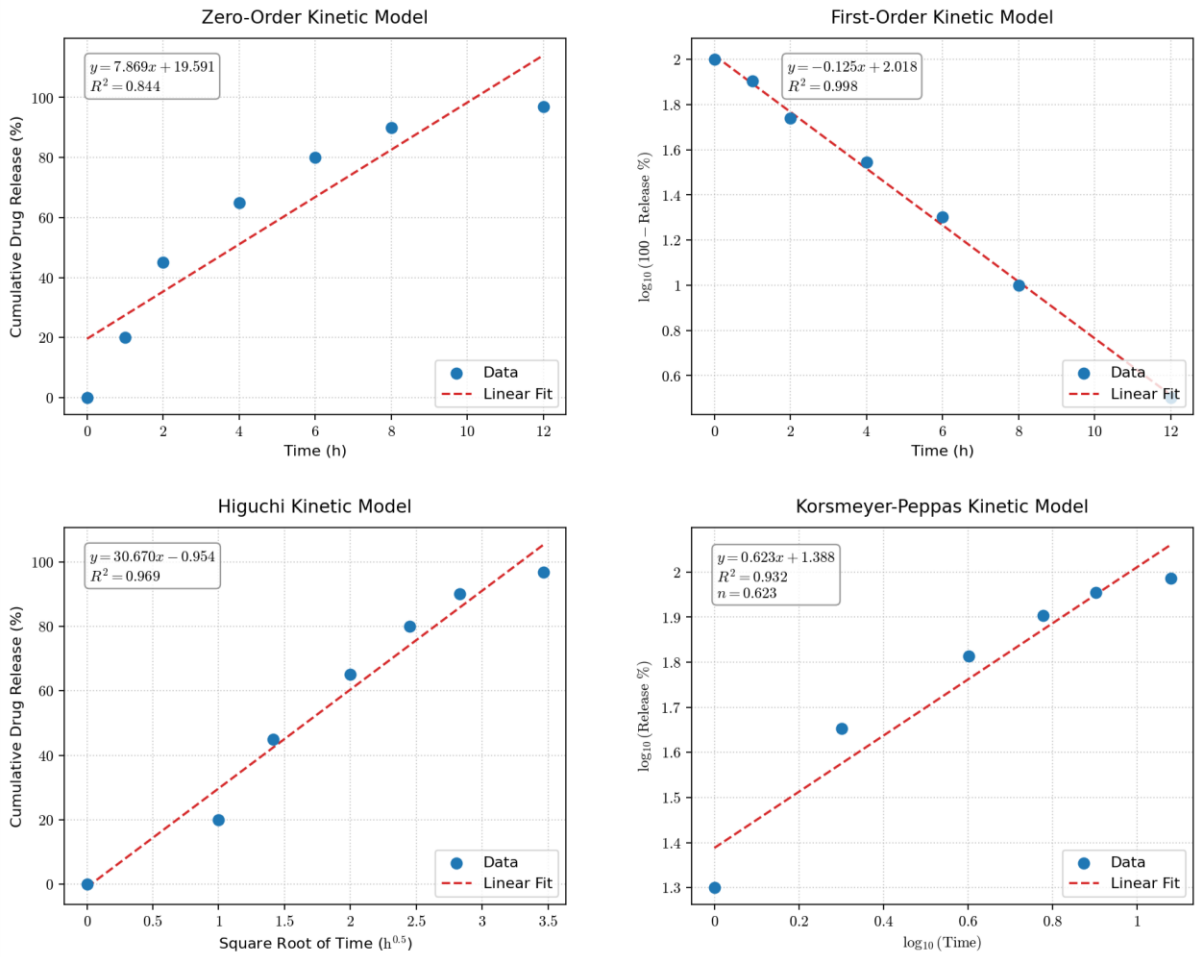


Figure 5: Release-kinetic plots of optimized NG4.

4.6 Ex-vivo Skin Permeation and Skin Retention

The optimized NG4 demonstrated enhanced ex-vivo skin permeation compared with the conventional clotrimazole gel. At 12 h, the cumulative amount of clotrimazole permeated through the skin from NG4 was **624.48 ± 1.58 µg/cm²**, compared with **436.26 ± 1.32 µg/cm²** for the conventional gel.

The steady-state flux of NG4 was **52.84 ± 1.06 µg/cm²/h**, compared with **36.42 ± 1.12 µg/cm²/h** for conventional gel. Similarly, the permeability coefficient increased from **2.84 ± 0.08 × 10^{-3} cm/h** for conventional gel to **4.12 ± 0.06 × 10^{-3} cm/h** for NG4. Importantly, NG4 demonstrated substantially greater skin retention (**246.82 ± 1.94 µg/cm²**) than the conventional formulation (**128.46 ± 2.08 µg/cm²**), indicating enhanced localization of clotrimazole within the skin.

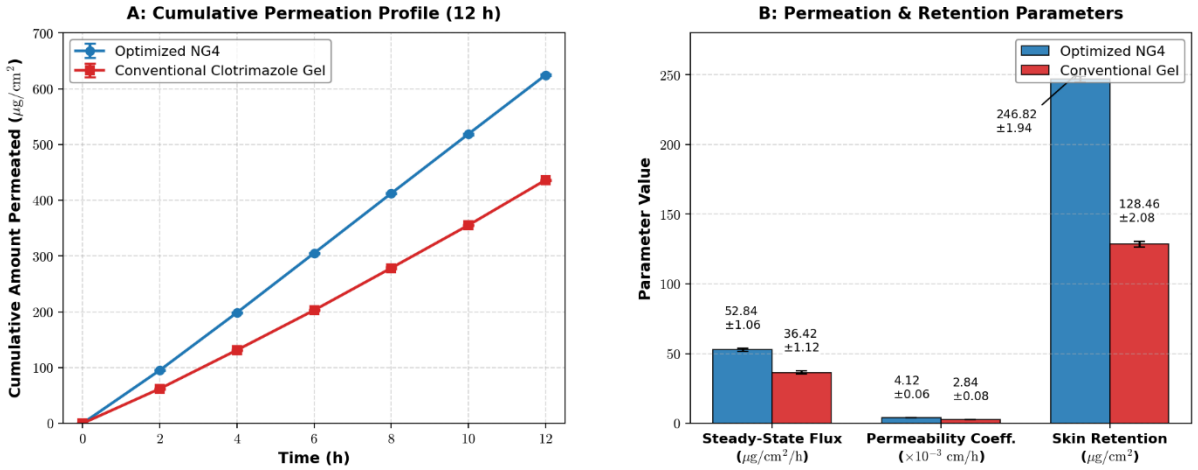


Figure 6: Comparative ex-vivo skin permeation profiles of NG4 and conventional clotrimazole gel.

Table 3: Comparative ex-vivo permeation, flux, permeability coefficient, and skin-retention parameters of optimized clotrimazole nanoemulgel (NG4) and conventional gel.

Parameter	Conventional Gel	Optimized Nanoemulgel (NG4)
Cumulative drug permeation at 1 h (µg/cm²)	46.32 ± 1.84	82.46 ± 2.14
Cumulative drug permeation at 2 h (µg/cm²)	88.74 ± 1.76	146.82 ± 2.06
Cumulative drug permeation at 4 h (µg/cm²)	162.48 ± 1.68	248.36 ± 1.94
Cumulative drug permeation at 6 h (µg/cm²)	238.16 ± 1.54	356.14 ± 1.82
Cumulative drug permeation at 8 h (µg/cm²)	312.84 ± 1.46	462.78 ± 1.74
Cumulative drug permeation at 10 h (µg/cm²)	378.52 ± 1.38	548.92 ± 1.66
Cumulative drug permeation at 12 h (µg/cm²)	436.26 ± 1.32	624.48 ± 1.58
Steady-state flux (µg/cm²/h)	36.42 ± 1.12	52.84 ± 1.06
Permeability coefficient (×10⁻³ cm/h)	2.84 ± 0.08	4.12 ± 0.06
Drug retention in skin (µg/cm²)	128.46 ± 2.08	246.82 ± 1.94

4.7 Comparative Antifungal Activity

The optimized NG4 exhibited greater antifungal activity than the conventional clotrimazole gel against both tested fungal organisms.

Against ***Candida albicans***, NG4 produced a zone of inhibition of **31.8 ± 0.72 mm**, compared with **22.4 ± 0.84 mm** for the conventional gel. Against ***Aspergillus niger***, NG4 produced a zone of inhibition of **28.4 ± 0.68 mm**, compared with **19.6 ± 0.76 mm** for the conventional formulation.

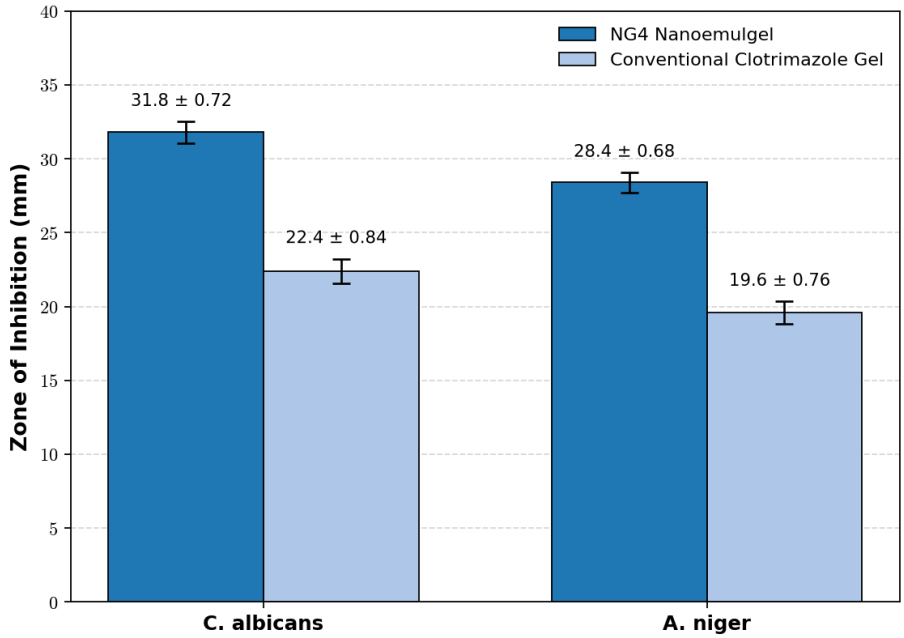


Figure 7: Comparative zones of inhibition of NG4 and conventional clotrimazole gel against *C. albicans* and *A. niger*.

5. DISCUSSION

The present study demonstrated a systematic formulation-development approach in which clotrimazole was incorporated into a nanoemulsion and subsequently converted into a Carbopol 934-based nanoemulgel. The optimized nanoemulsion (F5) and nanoemulgel (NG4) showed progressively improved formulation characteristics, drug diffusion, ex-vivo skin permeation and antifungal performance compared with the respective alternative formulations.

5.1 Effect of Formulation Variables

The composition of the nanoemulsion markedly influenced its physicochemical characteristics. Variation in the concentrations of oleic acid, Tween 80 and Transcutol P among F1–F6 produced corresponding changes in droplet size, PDI, zeta potential, drug content and optical characteristics. Increasing the surfactant/co-surfactant concentration generally favored reduction of droplet size, which can be associated with more efficient

reduction of interfacial tension during emulsification. The selected formulation components also provided high clotrimazole solubilization, supporting their use in the nanoemulsion system. The thesis identified oleic acid, Tween 80 and Transcutol P as the most suitable components based on the preliminary solubility studies.

The nanoemulsion optimization therefore depended on achieving an appropriate balance between drug solubilization, droplet size, size distribution, surface charge and drug content rather than maximizing a single formulation component. Such composition-dependent changes are consistent with the established behavior of nanoemulsion systems used for topical delivery.[16–19]

5.2 Why F5 Was Selected as the Optimized Nanoemulsion

Among the six formulations, F5 demonstrated the most favorable overall characteristics. It exhibited a droplet size of **98.4 ± 1.82 nm** and PDI of **0.164 ± 0.005**, indicating a relatively narrow and uniform droplet-size distribution. F5 also demonstrated the highest negative zeta potential among the formulations, supporting electrostatic stabilization of the dispersed droplets. The thesis consequently identified F5 as the optimized nanoemulsion based on its smallest droplet size, lowest PDI, favorable zeta potential, high drug content, transparency and drug-release characteristics.

The high percentage transmittance of F5 (**99.12 ± 0.64%**) further indicated its clear and transparent appearance, consistent with the formation of a nanosized dispersion. The combination of small droplet size and narrow size distribution provides a large interfacial area and may facilitate drug release and interaction with the skin, supporting the subsequent incorporation of F5 into the gel matrix.

5.3 Effect of Carbopol 934 Concentration

The concentration of Carbopol 934 was the principal formulation variable investigated during nanoemulgel development. NG1–NG4 contained **0.5, 1.0, 1.5 and 2.0% w/w Carbopol 934**, respectively, while the concentration of optimized nanoemulsion was maintained at 40% w/w.

Increasing Carbopol concentration resulted in a progressive increase in viscosity from **8425 ± 82 cP in NG1 to 11986 ± 61 cP in NG4**. Conversely, spreadability decreased from **32.48 ± 1.24 to 24.18 ± 0.98 g·cm/sec**, reflecting the increased consistency of the gel network.

A corresponding increase in swelling index was observed, from **118.42 ± 2.16% in NG1 to 172.58 ± 1.44% in NG4**, indicating greater water uptake with increasing polymer concentration. Although increasing polymer concentration generally increases resistance to drug diffusion, the optimized formulation NG4 demonstrated the highest cumulative diffusion in the present study. Thus, NG4 provided the most favorable overall balance between gel consistency, spreadability, swelling, drug content and drug diffusion.

5.4 Mechanism of Enhanced Drug Release

NG4 demonstrated **96.82 ± 0.58% drug diffusion at 12 h**, compared with 75.36 ± 0.70% for NG1. The progressive improvement in the observed diffusion profiles across the formulations indicates that the final nanoemulgel composition provided efficient drug release from the combined nanoemulsion–gel system.

The enhanced release may be related to the nanosized droplets of the optimized nanoemulsion, which provide a large interfacial area for drug transfer, together with the hydrated Carbopol network that provides a controlled diffusion pathway. Release-kinetic analysis showed the best fit with the **Higuchi model (R^2 = 0.989)**, while the Korsmeyer–Peppas exponent was **0.684**, indicating a non-Fickian/anomalous release mechanism.

These findings suggest that drug transport from NG4 was governed by a combination of diffusion and polymer-related matrix effects rather than simple concentration-gradient-driven release alone. This mechanism is desirable for topical therapy because it can support sustained drug availability at the application site.

5.5 Enhanced Skin Permeation and Retention

The optimized NG4 demonstrated substantially greater ex-vivo skin permeation than the conventional clotrimazole gel. At 12 h, cumulative permeation reached **624.48 ± 1.58 µg/cm²** for NG4 compared with **436.26 ± 1.32 µg/cm²** for the conventional formulation.

Similarly, the steady-state flux increased from **36.42 ± 1.12 µg/cm²/h** for conventional gel to **52.84 ± 1.06 µg/cm²/h** for NG4. The permeability coefficient increased from **2.84 ± 0.08 × 10^{-3} cm/h** to **4.12 ± 0.06 × 10^{-3} cm/h**, respectively.

Importantly, NG4 produced considerably greater skin retention of clotrimazole (**246.82 ± 1.94 µg/cm²**) than the conventional gel (**128.46 ± 2.08 µg/cm²**).

The improved permeation and retention can be attributed, within the formulation rationale supported by the thesis, to the nanosized droplets and the presence of penetration-enhancing components, which may increase interaction with skin lipids and facilitate drug transport into the skin. The combination of enhanced permeation with increased local skin retention is particularly relevant for topical antifungal therapy, where adequate drug concentration at the site of infection is essential.

5.6 Relationship Between Improved Drug Delivery and Antifungal Activity

The enhanced delivery characteristics of NG4 were accompanied by improved antifungal activity against both tested fungal strains. Against *Candida albicans*, NG4 produced a zone of inhibition of **31.8 ± 0.72 mm**, compared with **22.4 ± 0.84 mm** for conventional gel. Against *Aspergillus niger*, the inhibition zone increased from **19.6 ± 0.76 mm** with conventional gel to **28.4 ± 0.68 mm** with NG4.

6. CONCLUSION

The present study successfully developed a clotrimazole-loaded nanoemulgel using an optimized nanoemulsion incorporated into a Carbopol 934 gel matrix. Among the nanoemulsion formulations, **F5** demonstrated the most favorable nanoscale characteristics and was selected for nanoemulgel development. Among NG1–NG4, **NG4** exhibited the most favorable overall physicochemical properties and enhanced drug diffusion. The optimized formulation demonstrated Higuchi-type release with a non-Fickian diffusion mechanism, together with enhanced ex-vivo skin permeation, higher flux, increased permeability coefficient and greater skin retention compared with conventional clotrimazole gel. NG4 also demonstrated greater antifungal activity against *C. albicans* and *A. niger*. Collectively, these findings indicate that the developed nanoemulgel is a promising approach for improving the topical delivery and local performance of clotrimazole.

7. LIMITATIONS AND FUTURE PERSPECTIVE

Despite the encouraging formulation and in-vitro/ex-vivo findings, several limitations should be acknowledged. First, the therapeutic efficacy of NG4 was not established in an **in-vivo animal model of fungal infection**. Although enhanced diffusion, permeation, skin retention and antifungal activity were demonstrated, these findings require confirmation under physiological conditions. Future studies should therefore evaluate in-vivo antifungal efficacy, pharmacodynamic performance and cutaneous drug distribution.

Second, **skin safety and tolerability** require further investigation. Appropriate dermal-irritation, sensitization and histopathological studies should be conducted to establish the local safety of repeated nanoemulgel application.

Third, comprehensive **stability studies** under accelerated and long-term storage conditions should be performed to confirm maintenance of droplet size, PDI, drug content, physical appearance, rheological properties and antifungal performance during storage.

Finally, the formulation should undergo scale-up, process validation and clinical evaluation before its therapeutic applicability can be established. Well-designed clinical studies will be necessary to determine whether the enhanced drug delivery observed experimentally translates into improved clinical outcomes, patient acceptability and therapeutic effectiveness.

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