

Development and Evaluation of a Polymer-Based Topical Emulgel of Luliconazole for Effective Management of Superficial Fungal Infections Especially Tinea Pedis

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Abstract: ***Background:** Luliconazole is a strong topical imidazole antifungal with poor water solubility that may limit medication availability from traditional formulations. The objective of the present work was to create and analyze a polymeric luliconazole emulgel with enhanced physicochemical stability, topical performance, drug release, skin permeability and antifungal effectiveness. **Methods:** Drug-excipient compatibility was evaluated by FTIR and DSC and luliconazole was measured by UV spectrophotometry at 296 nm. Solubility screening and necessary hydrophilic-lipophilic balance (HLB) assessment were carried out to optimize the Tween® 20/Span® 80 emulsifier system. The physicochemical, rheological, mechanical and colloidal characteristics of HPMC and Sepineo™ P600 based emulgels were studied. The in vitro release was evaluated using Strat-M® membrane and the ex vivo penetration with pig ear skin. The antifungal effectiveness against Trichophyton rubrum and Epidermophyton floccosum and preliminary stability of 3 months were investigated. **Results:** The analytical technique was linear in the range of 2 to 12 µg/mL ($R^2 = 0.9999$). The stable emulgels were obtained using Sepineo™ P600 and S2 (3.5% w/w) was selected as the best formulation. The S2 exhibited pH 5.04, viscosity 3.89 Pa·s, drug content 100.9%, globule size 612 ± 34 nm, PDI 0.318 ± 0.041 and zeta potential -28.4 ± 2.1 mV. In vitro drug release and ex vivo permeability were found to be $96.19 \pm 1.12\%$ and $99.13 \pm 0.78\%$ at 8 h, respectively. Inhibition zones were 24.6 ± 1.1 and 22.9 ± 1.3 mm and MIC and MFC values were 0.98 and 1.95 µg/mL. S2 was steady physically for 3 months. **Conclusion:** The S2 based on Sepineo™ P600 showed good stability, release, penetration, and antifungal performance, enabling its continued development as a luliconazole topical delivery system.*

Keywords: Luliconazole, Emulgel, Sepineo™ P600, Skin permeation, Antifungal activity

1. Introduction

Superficial dermatophytosis is one of the most frequent dermatological disorders globe over and the Indian sub-continent in particular has had a large increase in incidence, chronicity and recurrence over the last decade [1], [2]. Tinea pedis (dermatophyte infection of the interdigital and plantar skin) mostly caused by *Trichophyton rubrum* and *Epidermophyton floccosum* is extremely common, difficult to clear up and prone to recurrence, with a major impact on the quality of life of patients.

Topical antifungal creams are still the first-line therapy for localized tinea pedis, and they avoid the systemic exposure associated with oral azoles [3]. However, conventional oil-in-water creams show poor penetration through the lipophilic stratum corneum and short cutaneous residence time, due to their low viscosity and weak bioadhesion. Therefore, sub-therapeutic drug concentrations may be reached at the site of infection, leading to clinical relapse.

The emulgel is an emulsion integrated in a hydrophilic gel network and has been found to be an attractive alternative topical carrier. It combines the drug loading capacity of an emulsion with the favorable rheology and bioadhesion of a

gel. Emulgels can boost percutaneous medication administration compared to a standard cream by lengthening cutaneous residence duration and improving application characteristics [4]–[9]. Luliconazole is an imidazole antifungal agent that inhibits lanosterol 14 α -demethylase and thereby blocks ergosterol manufacture and has dual fungistatic/fungicidal activity against dermatophytes [10]. It is a BCS class II molecule with poor water solubility and the efficacy of the drug topically is strongly dependent on the composition of the vehicle. Thus, rational design of formulation is particularly crucial.

A major problem in the design of emulgels is to achieve a stable emulsion at the lowest possible surfactant concentration, since traditional surfactants can damage the skin barrier at greater doses [11], [12]. Polymeric emulsifiers/gelling agents, hydroxypropyl methylcellulose (HPMC) and the acrylate-based emulsifying gel former Sepineo™ P600, provide a mechanistically different route to stabilization by increasing the viscosity of the continuous phase and creating a three-dimensional network around dispersed oil droplets.

In this study, a required-HLB (HLB) strategy was applied to create a Span® 80: Tween® 20 emulsifier blend for a

mineral oil/isopropyl myristate oil phase, and HPMC and Sepineo™ P600 were systematically evaluated as polymeric stabilizers for an otherwise unstable primary emulsion. The formulated emulgels were characterized physico-chemically and biopharmaceutically and the optimized emulgel was evaluated for in vitro antifungal activity and short-term physical/chemical stability to establish a mechanistic rationale for polymeric-emulsifier-stabilized emulgels as a topical platform for luliconazole.

2. Materials and Methods

2.1 Materials

Luliconazole was obtained as a gift sample from Glenmark Pharmaceuticals Ltd (Navi Mumbai, India). Carbopol 980 was procured from Lubrizol Advanced Materials (Cleveland, Ohio, USA), Transcutol® P from Gattefossé India Pvt. Ltd., Sepineo™ P600 from Seppic (France) and hydroxypropyl methylcellulose (HPMC) from Colorcon Asia Pvt. Ltd. All other excipients and reagents used were pharmaceutical or analytical grade.

2.2 Drug-Excipient Compatibility Studies

Compatibility of luliconazole with the selected excipients (mineral oil, isopropyl myristate, Transcutol® P, Tween® 20, Span® 80, HPMC and Sepineo™ P600) was evaluated by Fourier-transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). FTIR spectra of pure luliconazole and 1:1 w/w drug-excipient physical mixes were acquired spanning 4000–400 cm⁻¹ (KBr pellet method, resolution 4 cm⁻¹, 32 co-added scans) with an acceptable blank for baseline correction. DSC thermograms of pure luliconazole and physical mixes were acquired over 30–300 °C at a heating rate of 10 °C/min under a nitrogen purge (40 mL/min) using aluminum crimped pans, with an empty pan as the reference.

2.3 Development and validation of analytical method

A UV-spectrophotometric approach was developed and validated for measurement of Luliconazole. A standard solution in methanol was scanned in the range of 200–400 nm to determine the λ_{max} of luliconazole. A stock solution (100 µg/mL in methanol) was serially diluted to generate working standards from 2 to 12 µg/mL and the calibration curve was constructed by graphing the absorbance against concentration. The method was validated for linearity, precision (intra-day and inter-day, %RSD, n = 6), accuracy (recovery at 80%, 100% and 120% of the target concentration, n = 3 per level) and sensitivity (limit of detection [LOD] and limit of quantification [LOQ] were determined as 3.3σ/S and 10σ/S respectively, σ being the standard deviation of the intercept and S being the slope of the calibration curve) according to ICH Q2(R1) principles.

2.4 Screening of Oils, Surfactants and Co-Solvents

The shake-flask method was used to assess the saturation solubility of luliconazole in the selected oils (mineral oil, isopropyl myristate, castor oil, oleic acid), surfactants (Tween® 20, Span® 80) and co-solvents (Transcutol® P,

propylene glycol, ethanol). An excess of luliconazole was added to 2 mL of each vehicle in stoppered vials and shaken on an orbital shaker (100 rpm, 37 ± 1 °C, 24 h). The dispersions were centrifuged (4000 rpm, 30 min) and the supernatant filtered (0.45 µm) and properly diluted with methanol and measured spectrophotometrically at 296 nm against a reagent blank (n = 3).

2.5 Determination of Required HLB and Choice of Emulsifier Blend

The RHLB of the mixed oil phase (mineral oil and isopropyl myristate) was estimated using the traditional weighted-contribution method [13], [14]. For oil phase with 70% mineral oil (RHLB 11.8) and 30% isopropyl myristate (RHLB 11.5):

$$\text{RHLB}_{\text{oil phase}} = (0.70 \times 11.8) + (0.30 \times 11.5) \quad (1) \\ = 8.26 + 3.45 = 11.71$$

A combination of emulsifiers approaching this goal HLB was identified using mixtures of Span® 80 (HLB 4.3) and Tween® 20 (HLB 15.0):

$$\text{HLB}_{\text{blend}} = \frac{\% \text{Span } 80}{100} \times 4.3 + \frac{\% \text{Tween } 20}{100} \times 15.0 \quad (2)$$

A 30:70 ratio of Span 80:Tween 20 (HLB_{blend} = 11.79) was the closest practicable match to the desired RHLB of 11.71 and was used for all future emulsion formulations.

2.6 Preparation of the Primary Emulsion and Preliminary Stability Testing

Primary oil-in-water emulsions were prepared at three total emulsifier concentrations (Span 80:Tween 20, 30:70) - 1.0%, 1.5% and 2.0% w/w - at a fixed oil-phase loading (50% w/w; mineral oil 35% + isopropyl myristate 15%), with propylene glycol (5% w/w) and BHT (0.1% w/w) in the oil phase and purified water q.s. to 100%. The oil and aqueous phases were separately heated to 70 ± 2 °C, then the aqueous phase was slowly added to the oil phase under high-shear homogenization (Ultra-Turrax, 3000–4000 rpm, 10 min), and the emulsion was cooled to room temperature under continuous stirring. Each batch was visually inspected for color, appearance and beginning of phase separation (creaming/coalescence) for 30 min at room temperature.

2.7 Preparation of emulgel of luliconazole

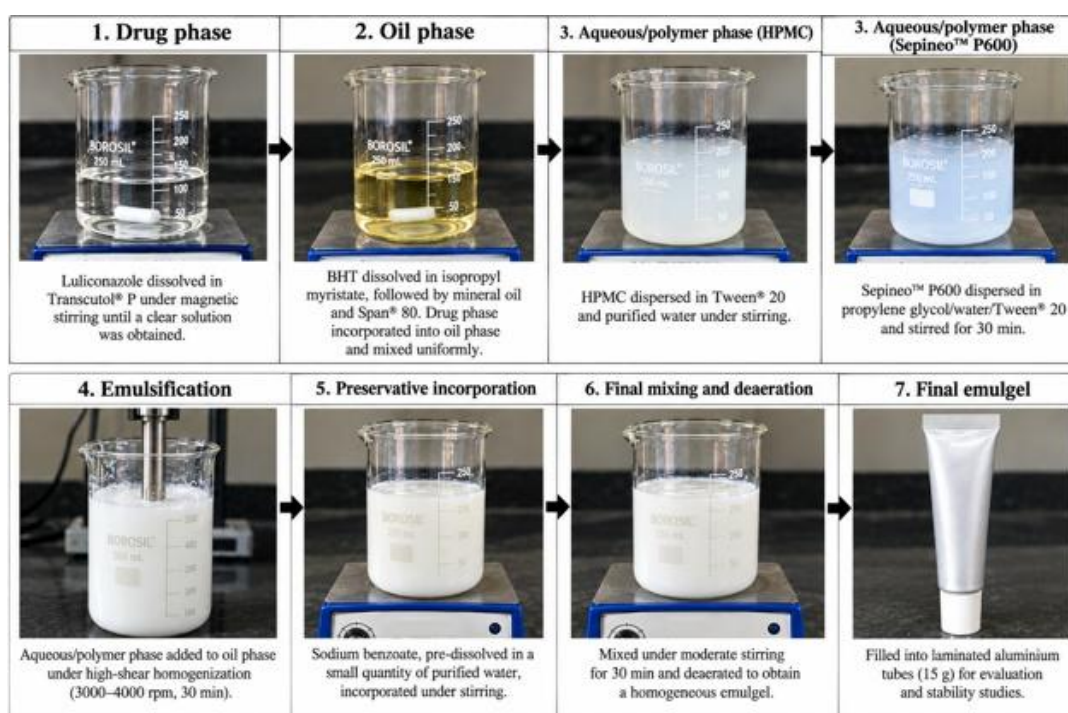
As the primary emulsions were kinetically unstable at all three emulsifier concentrations tested (Section 3.4), the ability of two polymeric gelling/emulsifying agents, HPMC (0.5% and 1.0% w/w; batches H1 and H2) and Sepineo™ P600 (4.5% and 3.5% w/w; batches S1 and S2) to convert the unstable emulsion into a physically stable emulgel was assessed. The final compositions are listed in Table 1.

Table 1: Composition (% w/w) of the HPMC-based (H1, H2) and Sepineo™ P600-based (S1, S2) luliconazole emulgel formulations

Ingredient (% w/w)	H1	H2	S1	S2
Luliconazole	1.00	1.00	1.00	1.00
Propylene glycol	5.00	5.00	20.00	20.00
Mineral oil	35.00	35.00	20.00	20.00
Isopropyl myristate	15.00	15.00	10.00	10.00
Tween® 20	1.00	1.00	10.00	10.00
Span® 80	0.50	0.50	1.00	1.00
Hydroxypropyl methylcellulose	0.50	1.00	—	—
Sepineo™ P600	—	—	4.50	3.50
Butylated hydroxytoluene	0.10	0.10	0.10	0.10
Sodium benzoate	0.15	0.15	0.15	0.15
Purified water, q.s. to	41.75	41.25	33.25	34.25
Total	100.00	100.00	100.00	100.00

2.8 Procedure Preparation

Batches of emulgel were prepared by three sub-phases: (i) drug phase: luliconazole was dissolved in Transcutol® P by magnetic stirring until clear; (ii) oil phase: BHT was dissolved in isopropyl myristate, followed by mineral oil and Span® 80, and the drug phase was incorporated under stirring for 10 min; and (iii) aqueous/polymer phase: HPMC or Sepineo™ P600 was dispersed in the propylene glycol/water/Tween® 20 phase under stirring for 30 min. Aqueous/polymer phase was introduced to the oil phase under high-shear homogenization (3000-4000 rpm, 30 min). Sodium benzoate (pre-dissolved in a small quantity of purified water) was incorporated under continued homogenization (30 min). The bulk was combined for 30 min with moderate stirring to achieve homogeneity, de-aerated and filled in laminated aluminum tubes (15 g fill) for assessment and stability experiments (Figure 1).

**Figure 1:** Preparation procedure of the luliconazole emulgel

2.9 Physicochemical properties evaluation

2.9.1 Sensory and physical qualities

Each formulation was subjectively evaluated in triplicate for color, appearance, homogeneity, consistency and phase separation immediately after preparation and after 24 h of equilibration at room temperature ($25 \pm 2^\circ\text{C}$).

2.9.2 Rheological (viscosity) research

Viscosity was determined with a Brookfield DV2T viscometer, using a spindle shape adequate for semi-solid formulations. Sufficient amount of each formulation was deposited in the sample holder without trapping air and allowed to equilibrate at $25 \pm 1^\circ\text{C}$ before measurement. Readings were taken at 100 rpm for 1 min after the response had settled. Measurements were performed in triplicate ($n = 3$) and are expressed as Pa·s, mean $\pm$ SD.

2.9.3 pH determination

The pH was determined at $25 \pm 1^\circ\text{C}$ with the use of a calibrated digital pH meter by both direct (electrode submerged in around 10 g of the formulation) and dilution method (10% w/w aqueous dispersion in filtered water). All measurements were performed in triplicate ($n=3$).

2.9.4 Texture spreadability analysis

The spreadability was measured using a texture analyzer equipped with a male/female cone probe assembly, and a 50-kg load cell. Formulation was consistently inserted in the female cone without trapping of air and tested in compression mode (test speed 3.0 mm/s; post-test speed 10.0 mm/s; penetration distance 15 mm; data-acquisition rate 10 points/s). The highest positive force (firmness) during compression and the maximum negative force during probe removal were recorded ($n=3$); lower firmness meant more ease of spreading and a bigger negative force meant greater cohesiveness/adhesiveness.

2.9.5 Bioadhesive force

The bioadhesive strength was measured with excised pig skin by a modified two-pan balance method. Two glass supports were prepared each carrying a skin piece of the same size with the appropriate surface of the tissue facing out. 1.0 g of emulgel was put between the two skin surfaces and kept in contact for 5 min. Then, the counter-pan was loaded with weights stepwise until full separation. The bioadhesive strength (g/cm^2) was measured by dividing the detachment weight by the contact area ($n = 3$).

2.9.6 Extrudability

The formulations were filled in laminated aluminum tubes, which were placed between two glass slides under a constant load of 500 g and the amount of emulgel extruded through the tube orifice was recorded in a fixed observation time. Extrudability was assessed qualitatively as poor (+), good (++) or excellent (+++) based on ease and consistency of extrusion. Uniform tube diameters, nozzle opening, applied load and observation period were maintained between formulations.

2.9.7 Size of globule, polydispersity index and zeta potential

The mean globule size, polydispersity index (PDI) and zeta potential of the improved formulation was assessed by particle-size/zeta-potential analyzer. An aliquot of the emulgel was diluted roughly 1:100 with double-distilled water and gently vortex-mixed to create a homogeneous dispersed sample while minimizing disruption of the interior droplets. Size and PDI of globules were determined by dynamic light scattering and zeta potential was determined by electrophoretic light scattering. The measurement was performed in controlled temperature settings (mean $\pm$ SD, $n = 3$).

2.9.8 Content of drug (assay)

Weigh exactly 1.0 g of emulgel and distribute in methanol in a 100 mL volumetric flask. Sonicate for 15 min to ensure complete extraction and make up to the volume with methanol. The solution was filtered and a portion of the filtrate was further diluted with methanol to a concentration within the validated analytical range. The absorbance was measured at 296 nm using the validated method (Section 2.3) and the drug content was represented as % of the theoretical label claim ($n = 3$).

2.10 In Vitro Studies of Drug Release

In vitro release was carried out in Franz diffusion cells (effective diffusion area 3.56 cm^2) with a Strat-M® synthetic membrane, a validated non-animal-based surrogate for human skin diffusion [15], [16]. A measured amount of emulgel (corresponding to a nominal loading of about 10 mg formulation/ cm^2 membrane area, i.e. roughly 0.1 mg luliconazole/ cm^2) was spread evenly onto the donor compartment. The receptor compartment was filled with pH 7.4 phosphate buffer, kept at $35 \pm 0.5^\circ\text{C}$ and swirled at 300 rpm. Aliquots (1 mL) were collected at 15, 30 and 45 min and 1, 2, 3, 4, 5, 6, 7 and 8 h and immediately replaced by an equivalent volume of fresh medium, equilibrated to the temperature of the culture at the time of sampling. Samples were examined at 296 nm and the cumulative percentage

drug release was determined after adjusting for medication removal during prior sample ($n = 3$ per formulation).

2.11 Ex-vivo Permeation Studies

Ex vivo permeation of the two Sepineo P600 batches (S1, S2) was investigated using excised pig ear skin (effective diffusion area 3.56 cm^2) with the stratum corneum oriented toward the donor compartment. A weighed quantity of emulgel containing 1% w/w luliconazole was applied uniformly on the exposed surface of skin. The receptor compartment (pH 7.4 phosphate buffer, $37 \pm 0.5^\circ\text{C}$, 100 rpm) was sampled (1 mL) at 1-h intervals for 8 h with immediate replacement of withdrawn volume and analyzed at 296 nm. The total amount penetrated per unit area was estimated after correction for consecutive sample ($n = 2$).

2.12 Kinetics of Drug Release/Permeation

Release/permeation data obtained for optimized formulation S2 was fitted to zero-order, first-order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models to define the major drug-transport mechanism [17]–[19]. $\log(M_t/M_\infty)$ vs $\log(\text{time})$ for the Korsmeyer-Peppas model was depicted using the first part of the release profile (up to about 60% cumulative release). The main indicator of goodness of fit was the coefficient of determination (R^2). The release exponent (n) was used as a secondary, exploratory indicator of the transport mechanism. Since the conventional n -value thresholds derived for polymeric controlled-release systems are not necessarily transferable to the Franz-cell skin-permeation geometries, they were interpreted with caution and in combination with the Higuchi and Hixson-Crowell fits.

2.13 Antifungal Activity In Vitro

The improved emulgel (S2) was assessed for in vitro antifungal activity against clinical isolates of *Trichophyton rubrum* and *Epidermophyton floccosum*. A drug free placebo emulgel was used as a formulation control and commercialized luliconazole cream was used as a comparator. Zone-of-inhibition testing was carried out on Sabouraud dextrose agar by the well-diffusion method. Inoculated plates were prepared with uniform diameter wells, which were loaded with the test materials and incubated under conditions suitable for the dermatophytes being tested. The diameter of the zone of inhibition was then determined. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined by broth microdilution according to the CLSI document M38 for filamentous fungi [20]; MFC was determined by subculture of the aliquots of wells showing inhibition on drug free agar and assessment of viable growth.

2.14 Studies of Stability

The optimized formulation (S2) was filled in laminated aluminum tubes (nominal fill 15 g) and stored under long-term ($25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$), intermediate ($30 \pm 2^\circ\text{C}/65 \pm 5\% \text{ RH}$) and accelerated ($40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$) conditions. Samples were removed at 1, 2 and 3 months and assessed for colour, appearance, homogeneity, phase separation, pH,

viscosity, spreadability, extrudability, drug content, content distribution (top/middle/bottom of the tube), and in vitro drug release, using the procedures indicated above. This 3-month study is reported as a preliminary/short-term stability study under long-term, intermediate and accelerated settings and does not constitute completion of a full ICH Q1A(R2) long-term stability study.

2.15 Statistical Analysis

Unless otherwise indicated (ex vivo permeation, $n = 2$), all quantitative data are expressed as mean $\pm$ standard deviation (SD) of at least three independent assessments. Statistical analysis of the differences between formulations was done by one-way ANOVA followed by Tukey's post hoc test and $p < 0.05$ was regarded statistically significant.

3. Results and Discussion

3.1 Drug-Excipient Compatibility Studies (FTIR and DSC)

Drug-excipient compatibility is one of the major preformulation considerations since the physicochemical interactions will take place between the active pharmaceutical ingredient (API) and formulation components and affect the drug stability, release characteristics and product performance. The FTIR spectrum of pure luliconazole showed a characteristic band at around 2210 cm^{-1} ($\text{C}\equiv\text{N}$ stretching of the acetonitrile group), absorption between $1590\text{--}1480\text{ cm}^{-1}$ ($\text{C}=\text{N}$ /aromatic ring vibrations) and characteristic bands near 1090 cm^{-1} and 740 cm^{-1} ($\text{C}-\text{Cl}$ and $\text{C}-\text{S}$ stretching respectively) which is in agreement with the molecular framework.

In all the seven drugs-excipients physical combinations, the main characteristic bands were seen with little differences in location ($\leq 3\text{ cm}^{-1}$) and intensity (Table 2, Figure 2) and no new conspicuous absorption bands indicating novel chemical functionalities were detected. The small variations in the peak intensity and broadening, mainly in the mixtures with the hydrophilic polymers HPMC and Sepineo™ P600 are caused by the overlapping absorption bands of excipients, dilution of the drug in the physical mixture and non-covalent interactions and do not prove the chemical incompatibility alone.

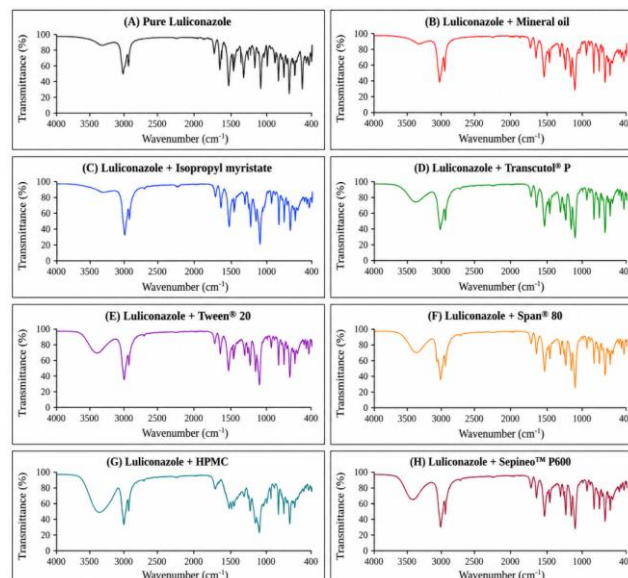


Figure 2: FTIR spectra of luliconazole and certain physical combinations of medication and excipients

The DSC thermogram of pure luliconazole demonstrated a pronounced endothermic event at $151.2\text{ }^{\circ}\text{C}$ correlating to the melting transition of the crystalline substance and compatible with its stated melting range (about $150.6\text{--}151.6\text{ }^{\circ}\text{C}$). In all the physical mixes this endothermic process was still observed, with moderate decreases in the peak temperature ($146.8\text{--}149.8\text{ }^{\circ}\text{C}$) and some broadening (Table 2, Figure 3). The changes are consistent with dilution of luliconazole by the excipients, partial dissolution of the drug in liquid or semi-solid components during heating or dispersion of the drug within the excipient/polymer matrix rather than chemical degradation and no new major thermal event attributed to incompatible drug-excipient complex has been identified.

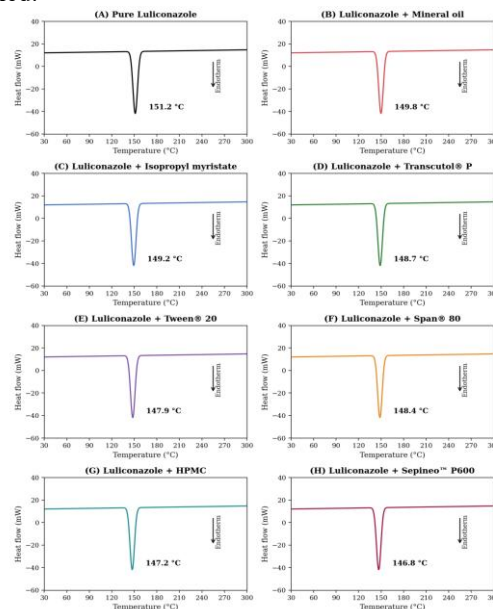


Figure 3: DSC thermograms of luliconazole pure and respective physical combinations

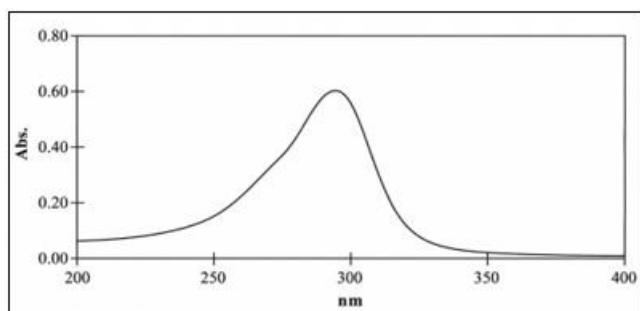
Table 2: Compatibility study of luliconazole with various excipients by FTIR and DSC

Sample	C≡N (cm ⁻¹)	C=N/aromatic (cm ⁻¹)	C-Cl (cm ⁻¹)	C-S (cm ⁻¹)	DSC peak (°C)
Pure luliconazole	2210	1589	1091	741	151.2
luliconazole + Mineral oil	2209	1588	1090	740	149.8
luliconazole + Isopropyl myristate	2211	1587	1092	742	149.2
luliconazole + Transcutol® P	2209	1588	1089	740	148.7
luliconazole + Tween® 20	2210	1586	1090	739	147.9
luliconazole + Span® 80	2208	1587	1089	741	148.4
luliconazole + HPMC	2211	1588	1091	740	147.2
luliconazole + Sepineo™ P600	2209	1586	1090	742	146.8

The FTIR and DSC results demonstrated that there was no evidence of a major chemical incompatibility between luliconazole and the excipients selected in the present study, in turn supporting the use of mineral oil, isopropyl myristate, Transcutol® P, Tween® 20, Span® 80, HPMC and Sepineo™ P600 for further formulation development. The lack of a severe incompatibility with Sepineo™ P600 was particularly important as this polymer was later used for the production of the physically stable S1 and S2 formulations.

3.2 Development and Validation of the Analytical Procedure

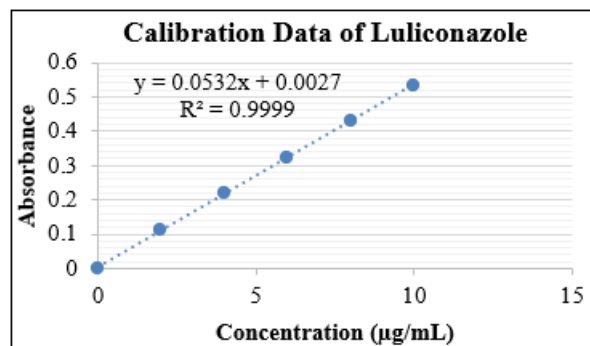
The UV-visible spectra of luliconazole in methanol exhibited a characteristic absorption peak at 296 nm (Figure 4), which was used as the analytical wavelength for subsequent drug-content, in vitro release and ex vivo permeation studies.

**Figure 4:** UV-visible absorbance spectra of luliconazole ($\lambda_{\max}$ = 296 nm) in methanol

In the range of 2-12 $\mu\text{g/mL}$, the absorbance of luliconazole increased in a dose-dependent manner (Figure 5). Linear regression analysis gave the equation $y = 0.0528x + 0.0057$ ($R^2 = 0.9999$), which shows that the linearity was excellent in the concentration range studied.

Table 3: Calibration data of Luliconazole at 296 nm

S. No.	Concentration ($\mu\text{g/mL}$)	Absorbance at 296 nm
1	0	0
2	2	0.1113
3	4	0.2169
4	6	0.3225
5	8	0.4281
6	10	0.5337
7	12	0.6393

**Figure 5:** Calibration curve of luliconazole in methanol (2-12 $\mu\text{g/mL}$) at 296 nm**Table 4:** Validation parameters of luliconazole UV-visible spectrophotometric technique

S. No	Validation parameter	Result
1	$\lambda_{\max}$	296 nm
2	Linearity range	2-12 $\mu\text{g/mL}$
3	Regression equation	$y = 0.0528x + 0.0057$
4	Correlation coefficient (R^2)	0.9999
5	Intraday precision (% RSD, n = 6)	0.42-0.91%
6	Interday precision (% RSD, n = 6, 3 days)	0.58-1.16%
7	Accuracy (% recovery, 80-120% levels)	99.21-100.54%
8	LOD	0.20 $\mu\text{g/mL}$
9	LOQ	0.61 $\mu\text{g/mL}$

Table 5: Accuracy (recovery) of the UV method for Luliconazole

Recovery level	Amount added ($\mu\text{g/mL}$)	Mean recovery (%) $\pm$ SD (n = 3)	% RSD
80%	4	99.21 $\pm$ 0.48	0.48
100%	5	99.86 $\pm$ 0.57	0.57
120%	6	100.54 $\pm$ 0.63	0.63

3.3 Screening of Oils, Surfactants and Co-Solvents

The saturation solubility of luliconazole in the screened vehicles is presented in Figure 6. Among the oils, oleic acid showed the highest apparent solubilization capacity (11.64 ± 0.44 mg/mL) followed by isopropyl myristate (9.87 ± 0.31 mg/mL); mineral oil (3.42 ± 0.18 mg/mL) and castor oil (2.15 ± 0.12 mg/mL) showed significantly lower solubility. Transcutol® P as co-solvent demonstrated significantly better solubilizing capacity (68.92 ± 1.85 mg/mL) than ethanol (42.17 ± 1.02 mg/mL) and propylene glycol (18.46 ± 0.61 mg/mL). The solubility of Tween® 20 was 24.30 ± 0.72 mg/mL, and the solubility of Span® 80 was 14.08 ± 0.53 mg/mL.

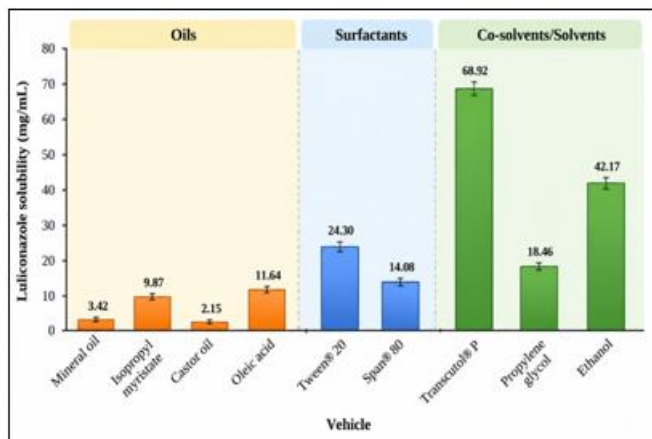


Figure 6: Equilibrium solubility of luliconazole in different oils, surfactants and co-solvents (mean $\pm$ SD, n = 3)

Based on these results, mineral oil and isopropyl myristate were selected as oil-phase components, Tween® 20 and Span® 80 as the surfactant system and Transcutol® P as the principal drug-solubilizing co-solvent/permeation enhancer for subsequent formulation development, due to their GRAS status and compatibility with the intended o/w emulgel architecture.

3.4 Required HLB Determination and Preparation of Primary Emulsion

The HLB needed for the mineral oil/isopropyl myristate oil phase was calculated to be 11.71 (Section 2.5) and a blend of Span® 80:Tween® 20 30:70 ($HLB_{blend} = 11.79$) was

selected. While the calculated RHLB was matched, primary emulsions generated at total emulsifier concentrations of 1.0%, 1.5% and 2.0% w/w all showed apparent phase separation within 20–30 minutes (Table 6). The slightly longer separation time with increasing emulsifier content indicates better interfacial coverage, but was not enough to avoid droplet coalescence and creaming. The results show that HLB matching and standard surfactant stabilization alone were not enough to confer adequate physical stability under the formulation conditions studied—viscosity, droplet-size distribution, and interfacial film strength are equally important—and justified the following polymer-assisted stabilization approach.

Table 6: Composition of trial batches of emulsions (Span 80:Tween 20, 30:70) and preliminary stability screening

Ingredient (% w/w)	E-1.0	E-1.5	E-2.0
Luliconazole	1	1	1
Propylene glycol	5	5	5
Mineral oil	35	35	35
Isopropyl myristate	15	15	15
Tween® 20	0.7	1	1.4
Span® 80	0.3	0.5	0.6
Butylated hydroxytoluene	0.1	0.1	0.1
Purified water, q.s. to	41.9	41.4	40.9
Onset of phase separation	20 min	30 min	30 min

3.5 Preparation of Emulgel and Its Physico-Chemical Analysis

Table 7: Physical appearance, homogeneity and phase-separation behavior of emulgel formulations (n = 3)

Batch	Polymer	Description	Homogeneity	Phase separation
H1	HPMC 0.5%	White, smooth, non-uniform emulgel	No	Yes
H2	HPMC 1.0%	White, smooth, apparently uniform	No	Slight
S1	Sepineo P600 4.5%	White, smooth, uniform emulgel	Yes	No
S2	Sepineo P600 3.5%	White, smooth, uniform emulgel	Yes	No

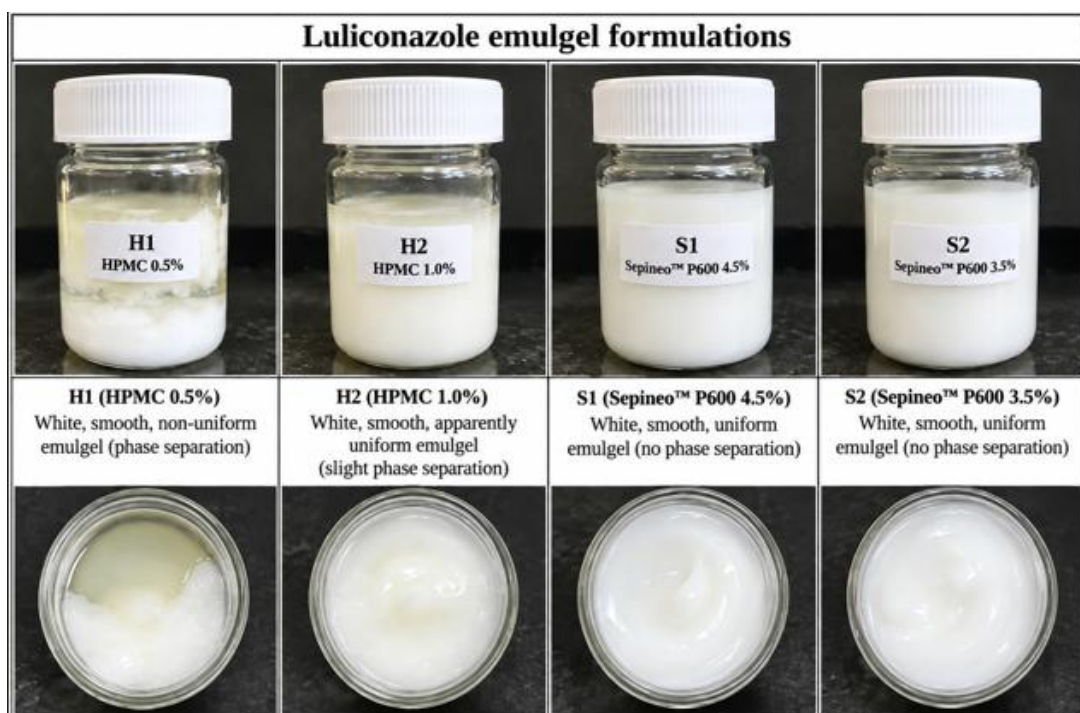


Figure 7: Visual appearance of luliconazole emulgel formulations (H1, H2, S1 and S2)

HPMC and Sepineo™ P600 both operate as hydrophilic polymeric emulsifiers, increasing the viscosity of the continuous phase and producing a three dimensional network around the dispersed oil droplets. The lower concentration of HPMC tested, (H1, 0.5%) was not adequate to prevent phase separation and resulted in a non-uniform product. Increasing the HPMC to 1.0% (H2) resulted in an improvement in apparent uniformity, but still allowed small phase separation. Conversely, both batches of Sepineo P600 (S1, S2) yielded smooth, homogeneous emulgels with no apparent phase separation, suggesting that this acrylate-based polymeric emulsifier formed a stronger, immobilizing network around oil droplets than did the cellulose ether at the concentrations tested, confirming that polymer-assisted stabilization, as opposed to escalation of surfactant concentration, was the better approach to a physically stable luliconazole emugel.

3.5.1 Rheological Behavior and pH

Table 8: Viscosity and pH of emugel formulations (mean $\pm$ SD, n=3)

Batch	Polymer (conc.)	Viscosity (Pa·s)	pH
H1	HPMC 0.5%	1.5	5.16
H2	HPMC 1.0%	1.68	5.21
S1	Sepineo P600 4.5%	4.58	5.1
S2	Sepineo P600 3.5%	3.89	5.04

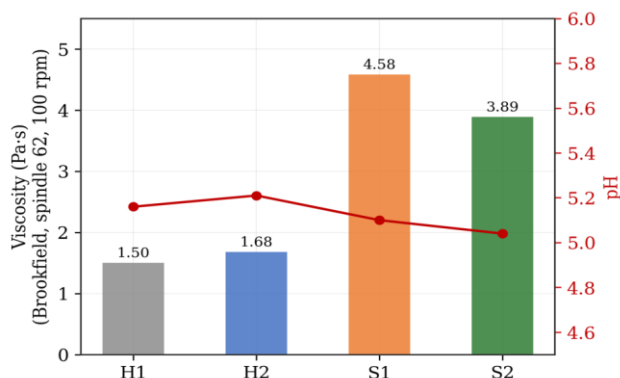


Figure 8: Viscosity and pH of created emugel formulation

The viscosity was concentration dependent in each series (H1<H2; S2<S1) confirming the formation of a gel-network as a function of concentration. The Sepineo P600 batches were significantly more viscous than the HPMC batches, which is consistent with a better thickening efficiency and its function in immobilizing oil droplets to reduce creaming and coalescence. All the formulations showed a narrow pH range (5.04-5.21), which is suitable to skin and near to the physiological pH of normal skin, indicating a good chemical compatibility of the formulation ingredients and a low expected risk of irritation at the site of application.

3.5.2 Mucoadhesive Strength and Spreadability

Table 9: Emugel formulations: Spreadability (firmness) and bioadhesive strength (mean, n = 3)

Batch	Firmness (g)	Bioadhesive strength (g/cm ²)
H1	30.57	6.89
H2	31.67	8.01
S1	40.12	9.32
S2	38	8.12

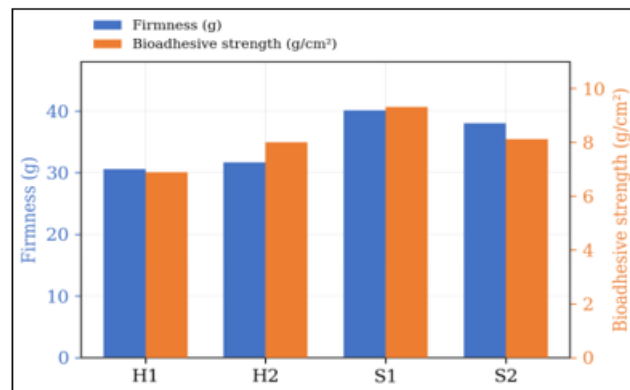


Figure 9: Spreading (firmness) and bioadhesive strength of emugel formulations

Firmness varied from 30.57 g (H1) to 40.12 g (S1) and increasing polymer content resulted in stronger gel network, higher firmness and lower spreadability, respectively. The bioadhesive strength values varied from 6.89 to 9.32 g/cm² and increased with the concentration of polymer, which indicates a stronger entanglement of the polymer chain with the biological substrate; S1 demonstrated the strongest bioadhesion. Higher hardness slightly reduces spreading but also increases cutaneous residence duration, a favorable trade-off for a topical antifungal designed to remain at the site of infection, as long as spreadability remains acceptable for application by the patient, as shown for S2.

3.5.3 Extrusion property

Table 10: Emugel formulations: Extrudability grade

Batch	Extrudability
H1	+ (poor)
H2	+ (poor)
S1	+ (poor)
S2	++ (good)

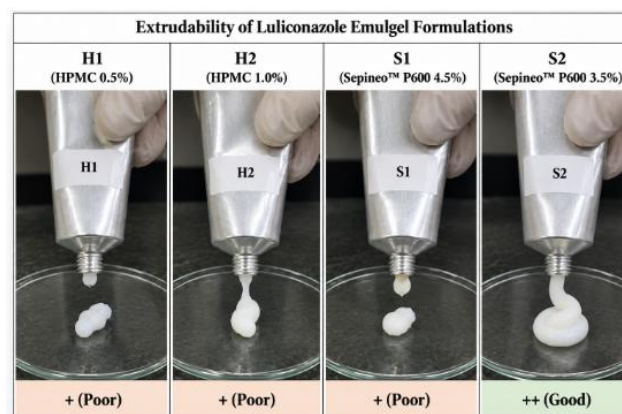


Figure 10: Representative photos of extrudability of the emugel formulations (grading scale: + bad, ++ good, +++ outstanding)

H1 formulation showed poor extrudability as per its non-uniform phase split structure. H2 and S1 were more homogeneous but their higher viscosity/firmness led to only fair extrudability. S2, the batch of the physically stable formulations with the lower Sepineo concentration, showed adequate structural integrity and good extrudability, indicating a favorable balance of viscosity and consistency for practical tube-based dispensing.

3.5.4 Particle size, polydispersity index and zeta potential

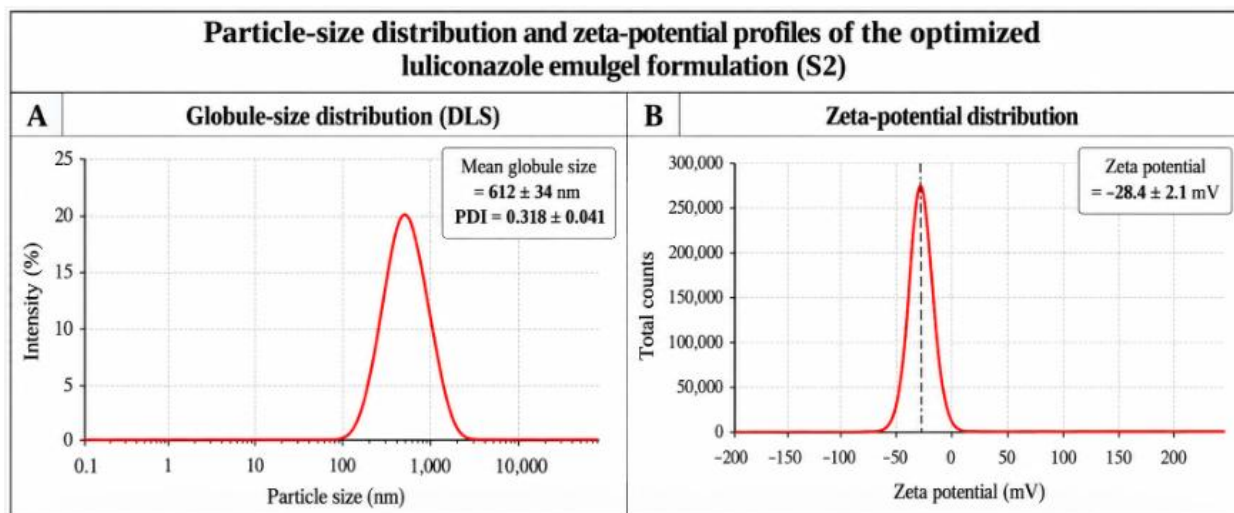


Figure 11: Globule size distribution (A) and zeta potential profile (B) of the improved formulation (S2)

The optimized formulation (S2) presented a mean globule size of 612 ± 34 nm and a polydispersity index (PDI) of 0.318 ± 0.041 , indicating the formation of a sub-micron oil-droplet dispersion with an acceptably narrow size distribution, which was consistent with the fine and homogeneous appearance observed macroscopically (Section 3.5). The zeta potential for S2 was -28.4 ± 2.1 mV. This moderately negative value suggests some electrostatic contribution to colloidal stability, but cannot be considered as a stand-alone proof of overall formulation stability; the physical stability of S2 is more likely the result of the combined contribution of interfacial (surfactant), polymeric/steric (Sepineo™ P600 network) and rheological stabilization mechanisms acting together, in agreement with the macroscopic and rheological findings reported above.

3.5.5 Assay (Drug Content)

Table 11: Assay of emulgel formulations (mean, n = 3)

Batch	Drug content (% of label claim)
H1	96.7
H2	97.9
S1	99.5
S2	100.9

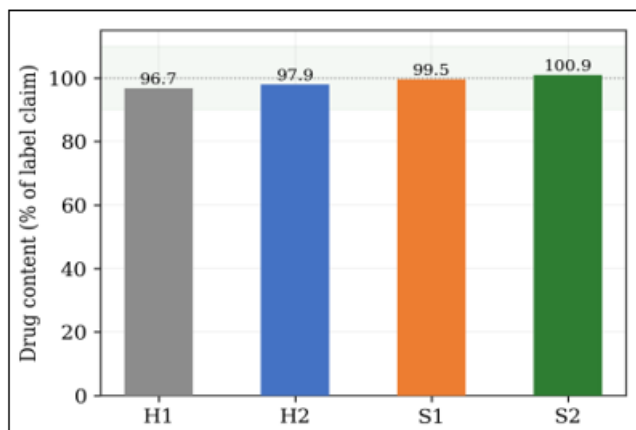


Figure 12: Drug content (assay) in emulgel formulations

Drug content in all the batches was found to be in the range

of 96.7 to 100.9% which was within the usually accepted 90-110% pharmacopeial range for semisolid dosage forms. This indicated efficient integration of drug and minimum loss during processing irrespective of the polymer system employed. The somewhat higher assay readings for the Sepineo P600 batches seem to indicate a slightly better drug retention within this polymeric matrix during the processing steps.

3.6 In-vitro Drug Release Studies

Table 12: In vitro cumulative % drug release of emulgel formulations over Strat-M® membrane (mean $\pm$ SD, % CDR, n = 3)

Time	H1	H2	S1	S2
15 min	14.67 ± 0.76	12.13 ± 1.78	13.78 ± 1.35	20.87 ± 1.23
30 min	20.87 ± 0.91	22.45 ± 1.00	18.12 ± 1.05	28.90 ± 0.90
45 min	28.45 ± 0.82	30.40 ± 1.58	29.81 ± 1.50	39.89 ± 0.85
1 h	36.89 ± 0.99	34.78 ± 0.35	40.20 ± 1.85	55.60 ± 1.05
2 h	45.35 ± 0.67	43.76 ± 1.15	48.97 ± 0.59	71.98 ± 1.14
3 h	53.47 ± 1.45	51.54 ± 0.65	57.12 ± 1.15	82.34 ± 0.88
4 h	60.78 ± 1.34	59.67 ± 0.45	68.78 ± 1.50	90.90 ± 1.12
5 h	63.98 ± 0.56	71.23 ± 0.29	73.34 ± 0.89	95.12 ± 0.87
6 h	70.45 ± 0.28	74.57 ± 0.12	78.28 ± 0.95	95.89 ± 0.90
7 h	75.50 ± 0.89	78.90 ± 1.05	82.90 ± 1.12	96.10 ± 0.55
8 h	78.98 ± 0.91	85.16 ± 0.28	90.25 ± 0.75	96.19 ± 1.12

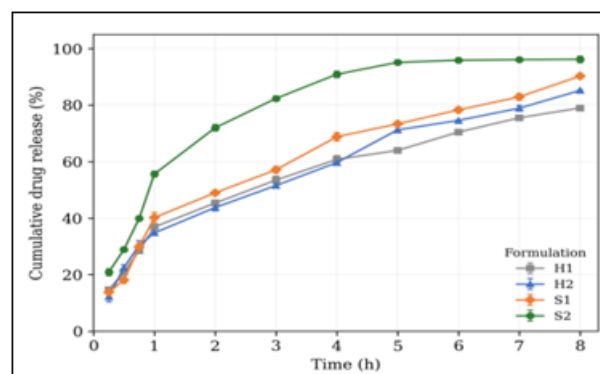


Figure 13: In vitro cumulative drug release profile of luliconazole emulgel formulations (mean $\pm$ SD, n = 3)

All formulations showed a time-dependent sustained release of medication over 8 h, with S2 having the fastest and most complete release ($96.19 \pm 1.12\%$) followed by S1 ($90.25 \pm 0.75\%$), H2 ($85.16 \pm 0.28\%$) and H1 ($78.98 \pm 0.91\%$). The increased release from S2 is consistent with a smaller cross-linked gel network, giving a shorter diffusional channel length, and with the greater Transcutol® P/propylene-glycol ratio utilized in the S-series formulations (Table 12).

3.7 Ex Vivo Permeation Study

Table 13: Ex vivo cumulative percentage drug release of formulations S1 and S2 through porcine ear skin (mean $\pm$ SD, % CDR, n = 2)

Time (h)	S1	S2
1	25.66 ± 0.15	21.77 ± 1.20
2	45.65 ± 0.34	49.45 ± 0.98
3	60.06 ± 0.67	63.23 ± 1.01
4	71.48 ± 0.85	70.12 ± 0.20
5	86.17 ± 0.23	83.45 ± 0.56
6	89.12 ± 0.37	98.90 ± 0.23
7	90.37 ± 0.12	99.11 ± 1.23
8	91.47 ± 0.98	99.13 ± 0.78

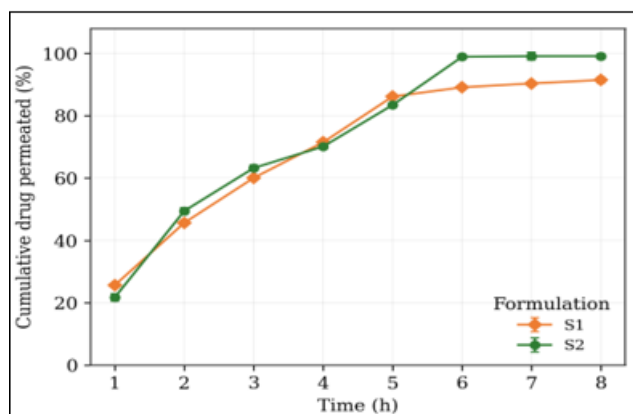


Figure 14: Permeation profile of formulations S1 and S2 through porcine ear skin (mean $\pm$ SD, n = 2)

The sustained permeation for 8 h was exhibited by both S1 and S2. The cumulative permeation was almost complete ($99.13 \pm 0.78\%$) for S2 compared to S1 ($91.47 \pm 0.98\%$) which was in line with the in vitro release trend and indicated that the medication was efficiently available at the target site. The use of two replicates for this test is acknowledged as a restriction (Section 4.3), and a greater replicate number ($n \geq 3$) is advised for a confirmatory data set.

3.8 Kinetics of Drug Release

Table 14: Drug release kinetic modeling for improved formulation S2 (using ex-vivo permeation data)

Kinetic model	R ²	Rate constant / exponent
Zero-order	0.9296	$k_0 = 10.347 \% \cdot h^{-1}$
First-order	0.8303	$k_1 = 0.0753 h^{-1}$
Higuchi	0.9792	$k_H = 41.393 \% \cdot h^{-0.5}$
Hixson–Crowell	0.8701	$k_{HC} = 0.2229 h^{-1}$
Korsmeyer–Peppas	0.9804	$n = 0.656$

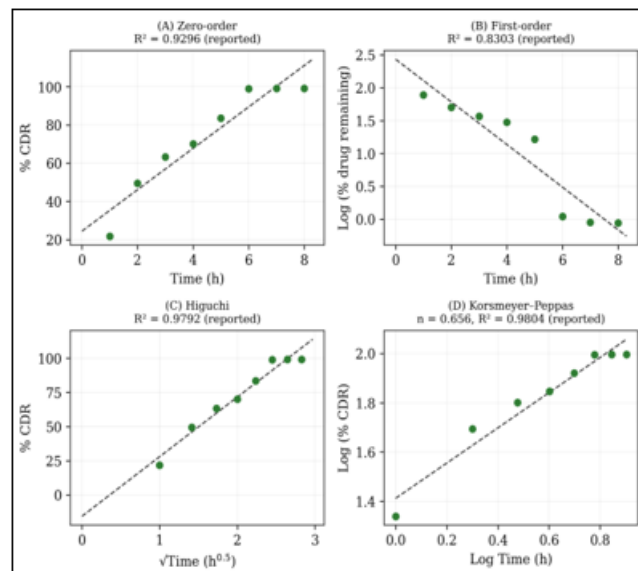


Figure 15: Kinetic modeling of drug release for optimized formulation S2 (ex vivo permeation data)

The Korsmeyer–Peppas ($R^2 = 0.9804$) and Higuchi ($R^2 = 0.9792$) models better fit the release data of S2 compared to the first-order ($R^2 = 0.8303$) and Hixson–Crowell ($R^2 = 0.8701$) kinetics. The strong Higuchi correlation indicates that drug release was mostly a diffusion process through the hydrated Sepineo P600 matrix and not mainly a matrix erosion or dissolution-controlled process, in agreement with the considerably lower Hixson–Crowell fit. The Korsmeyer–Peppas release exponent ($n = 0.656$) is consistent with anomalous (non-Fickian) transport according to the assumptions of that model. Since this classification is based on polymeric controlled-release geometries rather than Franz-cell skin-permeation systems, it is included here as a supplementary, exploratory indicator in combination with the Higuchi fit, rather than as a definitive mechanistic assignment. The overall fit models support a combination diffusion-associated release process and polymer relaxation, a pattern usually favoring reduced burst release and sustained drug availability at the application location.

3.9 Laboratory Antifungal Activity

The improved emulgel (S2) was studied for in vitro antifungal activity against *Trichophyton rubrum* and *Epidermophyton floccosum* and was compared with marketed luliconazole cream and drug free placebo emulgel utilizing zone of inhibition, MIC and MFC as comparison metrics (Table 15).

Table 15: Antifungal susceptibility in vitro of optimized emulgel (S2) versus commercialized cream

Organism	Test material	Zone of inhibition (mm)	MIC ($\mu\text{g/mL}$)	MFC ($\mu\text{g/mL}$)
<i>T. rubrum</i>	Placebo emulgel	–	–	–
<i>T. rubrum</i>	Optimized emulgel (S2)	24.6 ± 1.1	0.98	1.95
<i>T. rubrum</i>	Marketed cream (Luzu®)	21.3 ± 0.9	1.95	3.91
<i>E. floccosum</i>	Placebo emulgel	–	–	–
<i>E. floccosum</i>	Optimized emulgel (S2)	22.9 ± 1.3	0.98	1.95
<i>E. floccosum</i>	Marketed cream (Luzu®)	19.8 ± 1.0	1.95	3.91

The drug-free placebo emulgel did not demonstrate any discernible zone of inhibition against either organism, indicating that the emulgel base itself did not possess any antifungal activity and that the activity observed with S2 was mostly attributed to luliconazole. The mean inhibition zone of S2 against *T. rubrum* was 24.6 ± 1.1 mm, while the marketed cream gave an inhibition zone of 21.3 ± 0.9 mm. The MIC and MFC values of S2 were 0.98 and 1.95 $\mu\text{g/mL}$, respectively, and the values for the marketed formulation were 1.95 and 3.91 $\mu\text{g/mL}$, respectively, which was a difference of about two-fold for both parameters. Comparable results were reported against *E. floccosum* (S2: 22.9 ± 1.3 mm, MIC/MFC 0.98/1.95 $\mu\text{g/mL}$; marketed cream: 19.8 ± 1.0 mm, MIC/MFC 1.95/3.91 $\mu\text{g/mL}$).

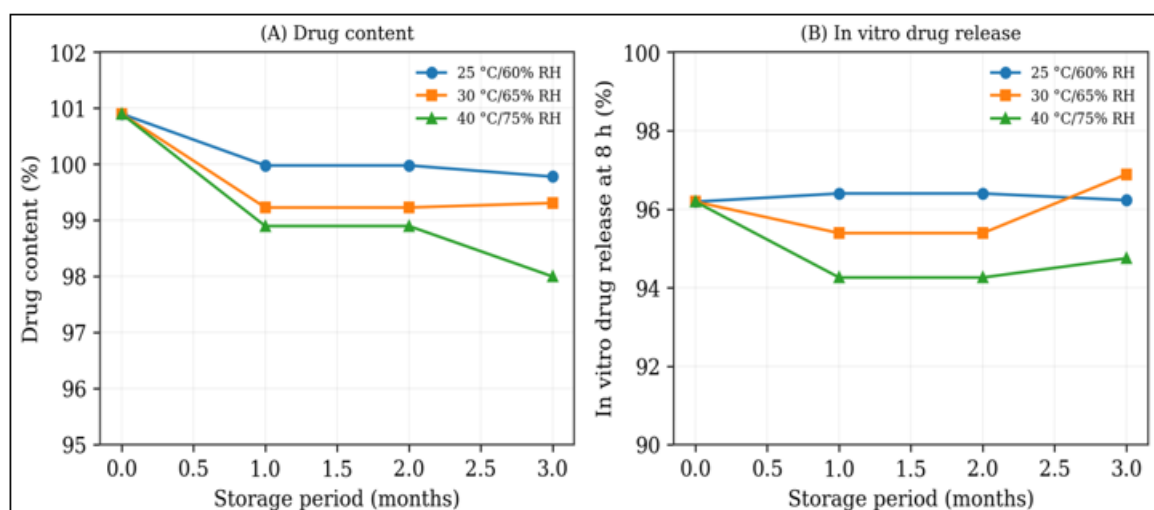
attributed to better release and diffusion of luliconazole from the emulgel matrix to the surrounding agar, aided by the finely dispersed oil phase and solubilizing/permeation enhancing excipients. The sub-micron globule size of S2 (612 ± 34 nm) could provide a relatively large interfacial area for drug partitioning. The wider inhibition zones should not be taken as evidence for greater intrinsic drug activity because agar diffusion is also influenced by formulation viscosity and drug release. Nevertheless, the concordant ~two-fold reduction in both MIC and MFC compared to the marketed comparator, obtained independently by broth microdilution, adds to the conclusion that the optimized formulation provided effective in vitro delivery of luliconazole to both dermatophytes tested.

The larger inhibition zones observed for S2 may be

3.10 Accelerated and extended storage stability

Table 16: Stability profile of improved formulation S2 for period of 3 months at 25 °C/60% RH, 30°C/65% RH and 40°C/75% RH

Parameter	Month	25 °C/60% RH	30 °C/65% RH	40 °C/75% RH
Colour & appearance	1–3	No change	No change	No change
Phase separation	1–3	None	None	None
pH	3	4.48	4.56	4.58
Spreadability (g/s)	3	15.89	15.67	16.12
Extrudability	1–3	+++	+++	+++
Drug content (%)	3	99.78	99.31	98
Content uniformity, top/mid/bottom (%), month 3	3	97.90 / 99.82 / 100.82	97.54 / 99.95 / 100.54	100.80 / 97.78 / 99.70
In vitro drug release at 8 h (%)	3	96.23	96.89	94.75

**Figure 16:** Stability profile of the improved formulation S2 under the studied storage circumstances (drug content and in vitro release at 8 h)

Formulation S2 was physically stable throughout the 3-month testing at all three storage conditions with no phase separation and no clinically relevant change in color, appearance, homogeneity, pH, spreadability or extrudability. The drug concentration was in the range of 98.0–99.8% and

in vitro release at 8 h was over 94% throughout including under accelerated conditions (40°C/75% RH). Data on content uniformity (top/middle/bottom of the fill within each condition) at month 3 further indicate sufficient production and storage homogeneity along the tube length. These results

are the first proof of the short-term physical and chemical stability of the improved emulgel under the conditions studied, and the long-term stability would be confirmed by extending the study to the time required by ICH Q1A(R2).

3.11 Selection of the optimization formulation

Taking into account the physicochemical, biopharmaceutical, microbiological and stability results shown above, formulation S2 (Sepineo™ P600, 3.5% w/w) was chosen as the optimal luliconazole emulgel. Among the other three batches, S2 exhibited excellent homogeneity and physical stability, good viscosity/firmness balance with good extrudability, the highest drug content (100.9%), the fastest and most complete in vitro release (96.19% at 8 h) and ex vivo permeation (99.13% at 8 h), a diffusion-associated release mechanism, and superior in vitro antifungal potency against both *T. rubrum* and *E. floccosum* compared to the marketed cream, while remaining physically and chemically stable for 3 months under long-term, intermediate and accelerated conditions. To summarize, the presented data suggest a mechanistically justified S2 topical antifungal emulgel that should be further evaluated in confirmatory ex vivo permeation studies with larger replicate numbers, extended (ICH duration) stability testing and in vivo efficacy studies that were beyond the scope of the present investigation.

4. Summary and Conclusion

The present study effectively formulated and assessed the polymer based luliconazole emulgel for topical administration. The preformulation experiments by FTIR and DSC confirmed no major incompatibility between luliconazole and the specified excipients. The new UV spectrophotometric technique showed remarkable linearity at 296 nm in the range of 2–12 µg/mL ($R^2 = 0.9999$). Based on solubility and HLB screening, mineral oil, isopropyl myristate, Transcutol® P, Tween® 20 and Span® 80 were selected as formulation components. However, the traditional emulsions prepared merely by optimizing HLB underwent phase separation, revealing the need for stabilization through the use of polymers.

The physical stability of the polymer-based formulations was higher for Sepineo™ P600 than for HPMC. The optimized formulation S2 with 3.5% w/w Sepineo™ P600 showed satisfactory physicochemical and application properties such as viscosity of 3.89 Pa.s, pH 5.04, good extrudability, drug content of 100.9%, mean globule size of 612 ± 34 nm, PDI of 0.318 ± 0.041 and zeta potential of -28.4 ± 2.1 mV. S2 showed in vitro drug release of $96.19 \pm 1.12\%$ at 8 h and ex vivo permeation of $99.13 \pm 0.78\%$ through pig ear skin. The drug release was best explained by the Korsmeyer-Peppas and Higuchi models, and the n value of 0.656 indicated anomalous transport.

The improved emulgel also shown promising in vitro antifungal efficacy against *Trichophyton rubrum* and *Epidermophyton floccosum* with inhibition zones of 24.6 ± 1.1 and 22.9 ± 1.3 mm respectively. The S2 showed homogeneity with no evident phase separation throughout the 3 month preliminary stability assessment and had a

satisfactory drug content and release characteristics.

The optimal luliconazole emulgel was found to be S2 (3.5% w/w Sepineo™ P600) with ideal physical stability, topical application, drug release, ex vivo skin penetration and antifungal activity. These results suggest Sepineo™ P600-based emulgel as a potential topical delivery platform for luliconazole. Further extended stability, in vivo efficacy and clinical research are needed to demonstrate its long-term performance and therapeutic potential.

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