RESEARCH ARTICLE

Formulation and Evaluation of Nanoparticle Loaded Hydrogel Containing Antifungal Agent for the Treatment of Onychomycosis Using Factorial Design

Fiza Farheen[1]*, Hemant Kumar Yadav[1], Abhay Raizaday[2]

[1]*School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India*
[2]*Department of Pharmaceutics, College of Pharmacy, JSS Academy of Technical Education, Noida, Uttar Pradesh, India.*

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ABSTRACT

SLNs are hopeful carriers for the topical delivery of antifungal drugs. This research aimed to develop a topical gel combining Efinaconazole-loaded SLNs with fluconazole to enhance the treatment of onychomycosis. The objective was to formulate a nail gel that capitalizes on the localized action and improved adherence of efinaconazole, a crucial agent in treating nail disorders like onychomycosis. The formulation utilized an alcohol-based system with specialized ingredients to ensure low surface tension and optimal wetting properties. Fluconazole, a broad-spectrum third-generation triazole antifungal, was included for its systemic and superficial antifungal activity. It works by inhibiting the formation of ergosterol, a key component of fungal cell membranes, by blocking the fungal cytochrome P-450 enzyme. The SLN-based delivery system allows for the slower release of Efinaconazole, while fluconazole diffuses more rapidly. The optimized gel formulation showed sustained drug release, with Efinaconazole releasing 32.85% of the drug by the 8th hour, compared to 74.05% for fluconazole during the same period. A stability study on the F4 formulation, which utilized Carbopol 934, was conducted according to ICH guidelines and demonstrated that the gel maintained its quality attributes, such as color, odor, homogeneity, pH, and viscosity over 6 months of testing. These results indicate that the F4 topical gel formulation is stable and suitable for long-term use.

Keywords: Onychomycosis, Antifungal agent, Efinaconazole, Fluconazole, Solid lipid nanoparticles, Antifungal gel.

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INTRODUCTION

Fungal infection of the nail unit, known as onychomycosis, may be due to dermatophytes, yeasts, & non-dermatophyte molds. It affects 10 to 12% of the population in USA.[1,2] The primary treatment goal is to eliminate the fungal infection and restore a normal, healthy nail. Four classes of antifungal drugs are currently approved for treating onychomycosis: allylamines, azoles, morpholines, and hydroxypyridinones.[3] Onychomycosis is not only widespread but also challenging to treat, often leading to nail discoloration, thickening, and detachment from the nail bed. These symptoms can cause physical discomfort as well as psychological and social distress. Traditional treatments, including both oral and topical antifungal therapies, often suffer from limitations such as lengthy treatment periods, variable efficacy, and potential systemic side effects. Efinaconazole, the first FDA-approved azole specifically for the topical treatment of onychomycosis, has shown effectiveness against a broad range of fungal species.[4] As a triazole antifungal, efinaconazole's therapeutic potential extends beyond just treating onychomycosis, suggesting possible future applications. The causative agents of this infection are primarily dermatophytes, leading to superficial fungal infections in the nail bed. Onychomycosis and tinea unguium are usual types of fungal nail infections, often resulting in thickened, darkened, disfigured, and split nails, conditions collectively known as mycotic nails.[5,6]

Triazole antifungal agents like fluconazole are often utilized to treat onychomycosis, candidiasis, and systemic mycoses, among other fungal illnesses. The way it works is by blocking an enzyme termed lanosterol 14-α-demethylase. This enzyme is essential for making ergosterol, an element of fungal cell membranes. The fungal cell membrane becomes compromised and eventually dies as a result of fluconazole's disruption of ergosterol synthesis. Among the many frequent

*Author for Correspondence: fizafarheen31@gmail.com

infections in onychomycosis, fluconazole is highly prized for its wide range of action, which includes effectiveness against the majority of *Candida* species, *Cryptococcus neoformans*, and dermatophytes. Oral pills, capsules, and intravenous solutions are among its numerous forms, giving it versatility in treating both systemic and localized infections. Fluconazole has a favorable pharmacokinetic profile, which includes broad distribution in human tissues, a lengthy half-life, and great oral absorption. Because of its ability to efficiently reach the nail bed when taken orally, it's favored for the treatment of onychomycosis.[7,8]

Recent advances in nanotechnology have opened new avenues for enhancing drug delivery, particularly in treating challenging infections like onychomycosis. Nanoparticle-based drug delivery systems offer the potential for improved penetration through the nail plate, sustained release of active drugs, and increased local drug concentration at the site of infection. Incorporating these nanoparticles into a hydrogel matrix further enhances their efficacy by providing a controlled and localized drug release, thereby minimizing systemic absorption and reducing side effects. In this study, we aim to formulate and evaluate a nanoparticle-loaded hydrogel containing an antifungal agent specifically designed for the treatment of onychomycosis. In order to find the best formulation, we will use the factorial design technique. This will let us systematically investigate how different formulation factors affect hydrogel's properties and effectiveness. This method will add to what is already known about drug delivery systems utilizing nanoparticles and can also assist in the search for a cure for onychomycosis.[9,10]

MATERIALS AND METHODS

Materials

Efinaconazole was sourced from Vigour Pharmaceuticals Ltd., Mumbai, India. Cyclolab, R & D Ltd., Hungary, provided a complimentary sample of Epi-β-CD. Lipid excipients, Compritol® 888 ATO and Precirol® ATO5, were supplied by Gattefosse, France. Reagents, including methanol, acetonitrile (HPLC grade), soya lecithin, acetone, Carbopol 934, and Poloxamer 188, were obtained from Sigma Aldrich, Mumbai. All chemicals and solvents used were of analytical reagent grade, and an ultra-pure water system ensured experiment integrity.

Methods

Identification and confirmation of drugs

Identification and Confirmation of drugs were carried out by analyzing melting point, UV and physical properties.[10]

Preparation of solid lipid nanoparticle loaded with cyclodextrins complexes Efinaconazole

This method is also known as paste/slurry complexation. This method is widely acceptable for complexation. A homogeneous paste was achieved by soaking mixtures of efinaconazole and selected CDs in equimolar amounts (1:1 molar ratio) in a mortar with a solvent that contained an equivalent volume of methanol and water (1:1v/v). The mixture was then dried beneath a vacuum at 40°C. Prior to their subsequent usage in analysis or assessment, dried complexes were ground in fine powder and preserved in an airtight container.[10]

Preparation of solid lipid nanoparticles (SLN)

SLN containing efinaconazole complexed with cyclodextrin were formed utilizing a modified emulsification-ultrasonication technique. Efinaconazole was dissolved in acetone at 75°C and combined with a lipid (Compritol 888 ATO) and the inclusion complex [Efinaconazole-Epi-β-CD (KD)] formed by the kneading technique. The Poloxamer-188 and soya lecithin were liquefied in water at 80°C to create the aqueous phase. The lipid phase was homogenized at 75°C as well as then mixed with the aqueous phase utilizing an UltraTurrax T10 at 500 rpm for 10 minutes. The resulting pre-emulsion was ultrasonicated at 50W for 5 minutes and then added to cold water (2–4°C) with stirring. The crystallized SLNs were saved for further research.[10,11]

Formulation development of efinaconazole SLN-loaded fluconazole hydrogel

Carbopol 934 was liquefied in 60 mL of water with stirring for 1-hour. Separately, disodium edetate and triethanolamine were dissolved in 10 mL of water and stirred for 10 minutes. A PEG solution was prepared by mixing 4.83 mL of PEG with 12 mL of water. The pH of the carbopol solution was adjusted to 7.4 using the edetate-triethanolamine mixture, followed by the addition of the PEG solution, forming a clear, uniform gel base. Four topical gel formulations (F1-F4) containing fluconazole and efinaconazole were prepared utilizing carbopol 934 at concentrations ranging from 1.5 to 2.5%. Specific ingredient details are provided in Table 1.[10]

Optimization of cyclodextrin-based nanocarriers

A preliminary analysis was conducted utilizing response surface methods with Box-Behnken design to optimize generated solid lipid nanoparticles for chosen variables. Experimental design, which included 17 runs with 3 variables and 3 stages, was performed utilizing Design-Expert software. A collection of points located at midpoints of every edge of 3D cube and multiple copies of centre points made up design. This establishes a study area that will be utilized to optimize formulation by assessing its primary, interaction, and quadratic impacts on constituents. The design produced a quadratic model, which was:

$$Y = A0 + A1X1 + A2X2 + A3X3 + A4X1X2 + A5X2X3 + A6X1X3 + A7X1^2 + A8X2^2 + A9X3^2 \text{ --- (1)}$$

The dependent variable Y represents the response estimated for every factor level, with regression coefficients A0 to A9 derived from these responses. Independent variables X1, X2, and X3 were coded as −1, 0, and +1 for lower, medium, and higher levels. Responses Y1 (particle size) and Y2 (entrapment efficiency %EE) were analyzed. A 3D response surface graph illustrated the effects of parameters on these responses.

Table 1: Gel formulations with carbopol 934

Gel code	*Efinaconazole SLN (eq. to 20 mg Drug)*	*Fluconazole (20 mg)*	*Carbopol- 934 (g) (%)*	*Triethanolamine (g)*	*Disodium EDTA*	*Propylene glycol*	*DM Water (100 g)*
F1	20	20	1%	1.5	0.005	5	QS
F2	20	20	1.50%	1.5	0.005	5	
F3	20	20	2%	1.5	0.005	5	
F4	20	20	2.50%	1.5	0.005	5	

Model reliability was indicated by % bias, calculated utilizing Equation 2.

%Bias = Predicted Value-Experimental Value/Predicted Value X 100 ---- (2)

Existing in Table 2 are coded values of independent and dependent variables that were utilized in the experimental design.[11]

Evaluation parameters cyclodextrins-based nanoparticles

MPS, polydispersity index (PDI) and zeta potential (ZP)

DLS utilizing a Malvern Zetasizer ZS nano 90 (Malvern Instruments, USA) was utilized to assess the ZP, PDI, and MPS distribution of cyclodextrins-based SLNs. The PDI indicates the distribution wideness of MPS, while the ZP helps predict the physical stability of the nanoparticles.[11]

%Yield, %drug loading (%DL) and %EE

To evaluate %DL and %EE of cyclodextrins-based nanoparticles, the nanoparticles were added to 10 mL of methanol, filtered through a 0.2 µm Millipore® membrane, and centrifuged at 12,500 rpm for 45 minutes. The filtrate was diluted with DMF and examined for efinaconazole content utilizing a Systronics UV spectrophotometer at 268 nm.[12]

%Yield, %DL and %EE = Weight of NP/Weight of Polymer + Weight of Hydrogel X 100 – (3)

Transmission electron microscopy (TEM)

TEM was utilized to examine the morphology and drug distribution of the nanoparticles. A drop of diluted sample was placed on a carbon-coated copper grid, stained with 2% phosphotungstic acid for 30 seconds, and dried at room temperature for 10 minutes. The excess stain was detached with filter paper.[13]

Table 2: Independent variables with their coded value and actual values for optimization of SLNs

Variables	*Levels*		
	-1 (Low)	*0(Medium)*	*+1 (Higher)*
Independent variables			
X_1=Amount of lipid (w/v)	1.0	2.0	3.0
X_2 = amount of surfactant (%)	0.5	1.0	1.5
X_3=Stirring speed	300	500	700
Dependent variables			Constraints
Y1=Particle size (MPS)			Minimize
Y2=Entrapment efficiency (%EE)			Maximize

Differential scanning Calorimetry (DSC)

DSC examination was directed in the direction of determining the state of efinaconazole within the carrier system and assessing interactions between the drug, cyclodextrins, and polymers. DSC curves of the pure drug, Epi-β-CD, Compritol ATO 888, and lyophilized drug-loaded nanoparticles were documented utilizing a DSC. The sample was heated from 50 to 300°C at 10°C per minute in a sealed aluminum pan, with alumina powder as a reference and dry nitrogen gas flow throughout.[14]

X-ray diffraction study (XRD)

The produced nanoparticles' crystalline or amorphous nature was determined by XRD analysis. A powder X-ray diffractometer was utilized to conduct X-ray powder diffraction experiments on lyophilized drug-loaded nanocarriers, as well as on the pure drugs Efinaconazole, Epi-βCD, and Compritol ATO 888. The samples were run through the apparatus utilizing an existing of 30 mA with an operating voltage of 40 kV, scanning from 2^{θ} to 60^{θ}.[15]

Gel Evaluation Procedure

Spreadability test

Two sets of uniformly sized glass slides were prepared. A layer of gel was applied between them, creating a 7.5 cm gap. The slides were compressed to form a thin layer covered with 100 g of gel, and excess was removed. A 20 g weight was then attached to the top slide, and the time it took to move 7.5 cm was recorded. The experiment was recurrent thrice to determine the average time.[11]

Spreadability deliberated as: S= m × l/t wherein, S= Spreadability, m-weight tied to upper slides (20 g), l-glass slide length (7.5cm), t-time taken in sec.

Extrudability

A clamp was utilized to prevent any rollback while forcefully squeezing a closed, collapsible tube containing approximately 20 g of gel from the crimped end. Afterward removing the cap, the gel was extruded, then weighed and measured. The amount of gel extruded was then calculated.

pH measurement

A digital pH meter was utilized to test gel's pH. To do this, the glass electrode was dipped all way in the gel system, covering it. We took 3 separate measurements of measurement and averaged them.

Appearance and homogeneity

Produced gels were visually assessed for their homogeneity and physical appearance.

Viscosity

A Brookfield viscometer (S-62, model LVDV-E) was utilized for measuring the gel's viscosity at 25ºC and 12 rpm spindle speed.[12-15]

In-vitro diffusion profile

All formulations underwent *in-vitro* diffusion utilizing custom-built Franz diffusion cells with a surface area of 3.8 cm^2. A phosphate buffer (pH 4.9) served as the receptor medium, and a dialysis membrane made from rat abdominal skin was utilized. The skin, with its stratum corneum facing the formulation, was positioned in the donor cell containing 100 mL of buffer. After applying 1 g of gel-like formulation, the setup was sustained at 37 ± 1°C. Samples were withdrawn at regular interims over 8 hours and examined spectrophotometrically at 255 nm for fluconazole and 268 nm for efinaconazole.[12]

Stability study

Stability testing for the efinaconazole SLN-loaded fluconazole gel was conducted over 6 months in a stability chamber following ICH criteria. The gel was tested at three humidity levels: 25 ± 2°C/60% ± 5%RH, 32 ± 2°C/60% ± 5%RH, and 40 ± 2°C/75% ± 5%RH. Samples were assessed for changes in organoleptic proprieties, pH, viscosity, and net content at regular intervals.[15]

In-vivo Study

Animals

Male Indian white rabbits, 14 weeks old, were obtained from PBRI, Bhopal. Each of the three groups (5-rabbits/group) was used for an onychomycosis model, and each of the three groups (4-rabbits/group) for treatment effectiveness.

Test organism

Trichophyton mentagrophytes TIMM2789, isolated from a guinea pig, was obtained from Bluemaarlin Engineering Pvt. Ltd, Pune.

Inoculum preparation

T. mentagrophytes was suspended in saline with 0.05% Tween 80, cultured on Sabouraud dextrose agar at 28°C for 2 weeks, and the microconidia suspension was adjusted to 10^8 conidia/mL.

Onychomycosis production

Rabbits received intramuscular methylprednisolone acetate (4 mg/kg) for 4 weeks. After 2 weeks, 0.2 mL of microconidia suspension was applied to a nail, and toes were bound and covered. 0.5 mL sterile water was added to create an ideal fungal environment. After 2 weeks, the coverings were removed, and the post-infection phase (0, 2, or 6 weeks) was observed. Animals were euthanized, and nails were collected for analysis.

Histopathological examination

Nails were fixed in formalin, decalcified, and embedded in paraffin. Sections were stained with periodic acid-Schiff and examined microscopically as shown in Figure 15. Fungal growth was graded (0 to 3) and examined in six nail regions to determine infection rate and intensity.

Drugs and treatment

The gel formulation F4 contained 0.02% efinaconazole SLN and 2.5% fluconazole with carbopol 934 as the gelling agent due to its favorable properties. Nails were cleaned with water, and 3.6µl of each drug was applied topically daily for 28 days post-infection. Control animals were infected but not treated.

Therapeutic efficacy evaluation

Infected nails were divided for analysis. One half underwent histopathology, while the other was used for culture recovery on Sabouraud dextrose agar with cycloheximide, chloramphenicol, and sisomicin. Plates were incubated at 28°C for 2 weeks. Fungal presence was indicated by growth on one or more samples, and the fungal load was scored from +10 to 0 based on positive nail fragments out of 10.

Statistical analysis

Histological infection rate & frequency of fungus-positive nails in every group were examined utilizing Fisher's exact test. The severity of fungal load on affected nails in every group was evaluated utilizing Student's t-test. Significance was calculated by $p < 0.05$ values.[10-15]

RESULTS AND DISCUSSION

Identification and Confirmation of Drugs

Physical properties, melting point (MP) and solubility studies

Fluconazole and Efinaconazole were evaluated against Indian Pharmacopoeia, 1996 standards and found to meet appearance, color, taste, and odor benchmarks. Fluconazole had an MP of 139°C, matching the reference range of 138 to 140°C, while efinaconazole had an MP of 193°C, within the range of 192 to 195°C. Solubility tests showed fluconazole was, to some extent soluble in water, soluble in DMSO and ethanol, and highly soluble in DMF. Efinaconazole was somewhat soluble in water, methanol, and ethanol, soluble in acetone and dichloromethane, and highly soluble in DMF.

UV spectrophotometer study

The UV spectra of fluconazole and efinaconazole were examined utilizing a Shimadzu UV 1800 double-beam spectrophotometer, scanning the range of 200 to 400 nm. The maximum absorption for fluconazole was identified at 255 nm, as shown in Figure 1. Similarly, the maximum absorption for efinaconazole was detected at 268 nm, as depicted in Figure 2.

Optimization of solid lipid nanoparticles (SLNs)

In addition, the findings of first research informed the selection of certain opalescent characteristics as critical factors. These variables included the optimal concentrations of lipid (1.0–

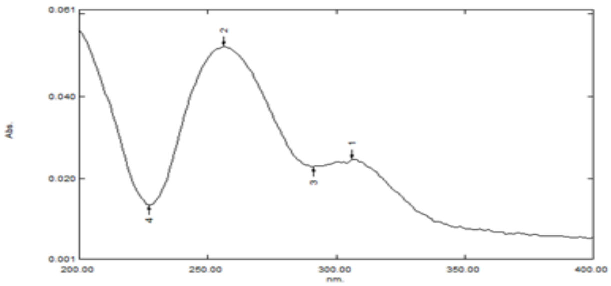

Figure 1: UV spectra of fluconazole

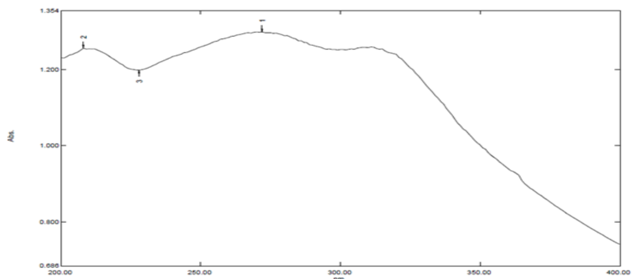

Figure 2: UV spectra of efinaconazole

3.0% w/v) and surfactant (0.5–1.5% w/v) for the production of solid lipid nanoparticles. Table 3 displays the entrapment effectiveness and particle size of seventeen different SLN formulations.

Effects on particle size (Y1)

In 17 different batches of SLNs loaded with an efinaconazole inclusion complex, particle size varied between 112 and 163 nm for different combinations of factors. A quadratic equation may be utilized to describe the impact of independent factors on particle size:

$$Y1\,[PS\,(nm)] = 151.11 + 5.34X_1 - 0.20X_2 - 3.36X_3 - 0.83X_1X_2 + 3.27X_1X_3 - 1.21X_2X_3 + 14.26X_1^2 + 23.69X_2^2 + 13.82X_3^2 \quad (3)$$

A satisfactory match was observed, as the aforementioned r^2 was determined to be 0.994. Figures 3, 4, and 5 show 3D response surface plots of the impact of SS-SC, LC-SS, and SC-LC, correspondingly.

Impact on %EE (Y2)

Between 58.12 and 77.10%, for different combinations of variables, was the %EE of 17 batches of SLNs. The following quadratic equation describes the relationship between the effectiveness of entrapment and a number of independent variables:

$$Y2\,[EE\,(\%)] = 75.77 + 3.13X_1 + 1.51X_2 + 1.07X_3 - 0.98X_1X_2 + 1.28X_1X_3 - 1.37X_2X_3 + 0.80X_1^2 - 4.38X_2^2 - 3.60X_3^2 \quad (4)$$

Figures 6, 7, and 8 indicated 3D surface plots of the impact of SS-SC, LC-SS, and SC-LC correspondingly. For further optimization of SLNs, the SLN-9 and SLN-12 formulations were chosen since they were deemed optimal. Table 4 shows the impacts of different stirring speeds on optimized SLN formulation (Table 5).

The SLN-9 formulation, with an MPS of 153.6 nm and a

Table 3: Particle size and entrapment efficiency of 17 formulations of SLNs

SLN Formulation	*Dependent variables*	
	Particle size (nm)	*Entrapment efficiency (%)*
SLN1	160.12	74.23
SLN2	112.51	58.11
SLN3	124.10	67.19
SLN4	139.27	63.18
SLN5	145.09	58.01
SLN6	158.23	59.24
SLN7	137.19	66.15
SLN8	148.06	75.98
SLN9	153.60	79.25
SLN10	139.18	65.10
SLN11	116.31	62.48
SLN12	155.84	78.36
SLN13	150.3	73.91
SLN14	138.62	64.12
SLN15	143.78	70.25
SLN16	163.16	69.28
SLN17	129.21	63.39

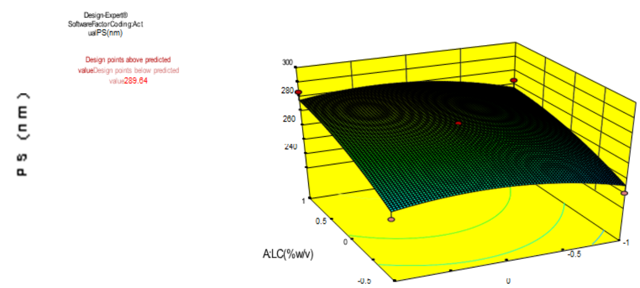

Figure 3: 3D surface plot for result of SS–SC interaction terms on MPS of SLNs

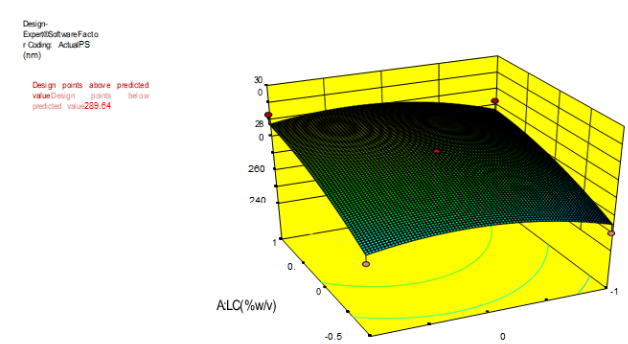

Figure 4: 3D surface plot for result of LC–SS interaction terms on MPS of SLNs

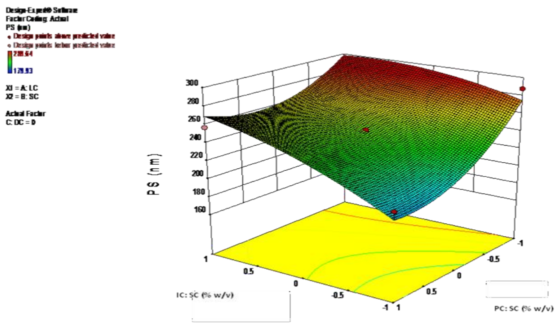

Figure 5: 3D surface plot for result of SC–LC interaction terms on MPS of SLNs

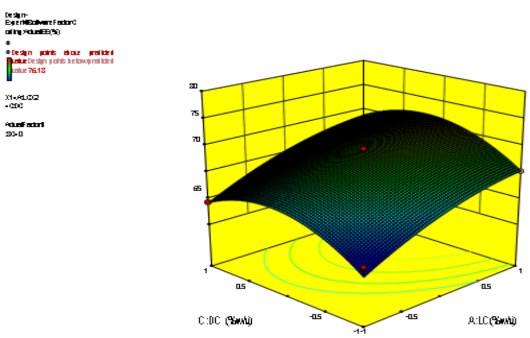

Figure 6: 3D surface plot for result of SS–SC interaction terms on %EE of SLNs

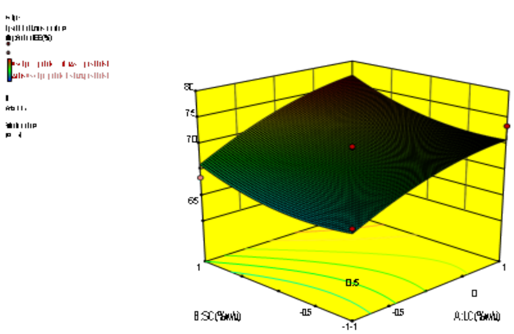

Figure 7: 3D surface plot for result of SS–LC interaction terms on %EE of SLNs

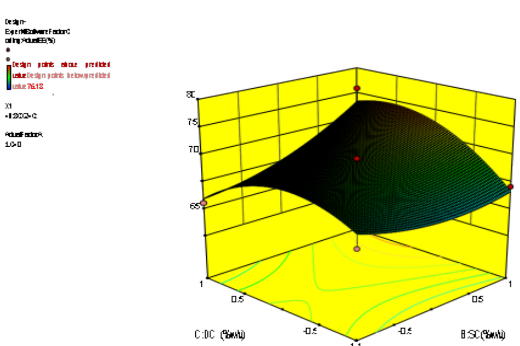

Figure 8: 3D surface plot for result of LC–SC interaction

%EE of 79.25%, was the most effective. The experimental formulation closely matched expected values with an MPS of 151.54 ± 2.53 nm as well as %EE of 77.81 ± 0.15%. The optimal formulation, achieved by mixing 2.0% w/v LC with 1.5% w/v SC at 500 rpm, showed a 1.30% bias for particle size and a 1.80% bias for %EE, indicating successful optimization.

Dependent variables had a substantial value of R2 and ANOVA findings, indicating that observed response variables were also significant. Optimal SLNs' analysis of variance (ANOVA) outcomes are portrayed in Table 6.

Characterization of SLNs

MPS, PDI and ZP

The optimized efinaconazole-loaded nanoparticles had a size of 151.54 ± 2.53 nm with a narrow PDI, slightly larger than the blank SLNs (148.7 ± 1.09 nm). The drug-loaded SLNs showed a higher zeta potential of -21.3 ± 0.05 mV compared to -19 ± 0.22 mV for the blank nanoparticles, indicating increased stability. The ZP and size distribution of the efinaconazole-cyclodextrin complexes are illustrated in Figures 9 and 10.

%Yield, %drug loading and %EE

The optimized SLNs had a %yield of 96.71 ± 0.53%. The optimal SLN formulation %EE was determined to be 77.81 ± 0.15%, while the percentage drug loading was 48.61%. Because the drug has a strong affinity for the chosen lipid matrix, its lipophilic nature accounts for the high entrapment.

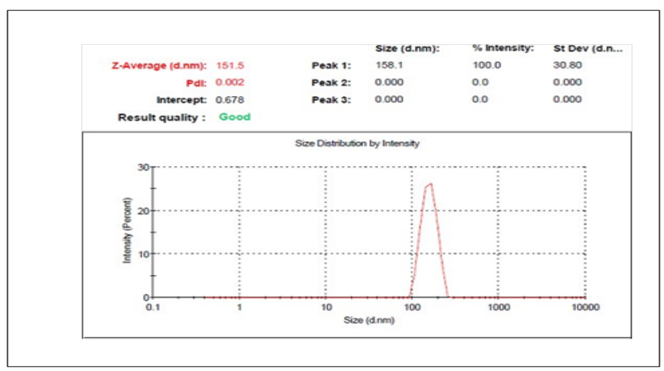

Figure 9: MPS of optimized SLNs loaded with Epi-β-CD KD complex

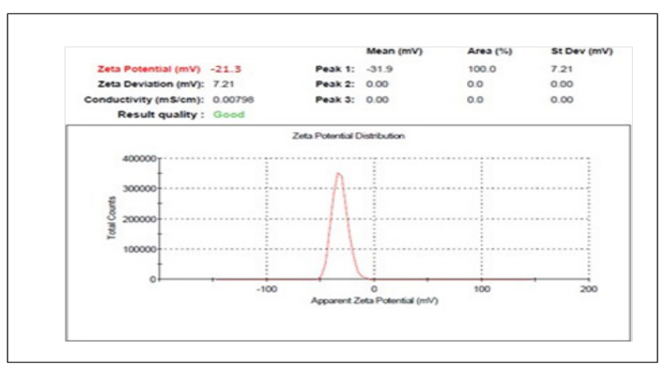

Figure 10: ZP of optimized SLNs loaded with efinaconazole-Epi-β-CD KD complex

Table 4: Effect of varying stirring speed on optimized SLNs formulations

Formulation	*Compritol ATO 888 (%w/w)*	*Poloxamer-188 (% w/w)*	*Stirring speed (rpm)*	*Particle size (nm)*	*Entrapment efficiency (%)*
SLN-9	2.0	1.5	300	412 ± 18	83.3 ± 6
			500	153 ± 11	79.5 ± 4
			700	140 ± 09	44.2 ± 1
SLN-12	3.0	1.5	300	289 ± 22	86.1 ± 5
			500	155 ± 10	78.9 ± 3
			700	148 ± 72	47.3 ± 6

Table 5: % bias between observed and predicted values of SLNs

Response variable	*Predicted values*	*Experimental/Observed values*	*%Bias**
MPS (nm)	153.60	151.54	1.30
(%EE)	79.25	77.81	1.80

TEM

Optimized SLNs were examined utilizing TEM for drug distribution, shape, and size. SLNs' TEM pictures verified that the nanoparticles had a restricted size distribution and an oval or nearly spherical shape. They went on to say that the nanoparticles didn't clump together. The scan also shows that particles are not very polydispersity and have a fairly consistent size distribution. In Figure 11, you can see the TEM picture.

DSC

DSC analysis revealed that pure Efinaconazole melted at 193°C, while Compritol 888 ATO showed a peak at 75°C, indicating no chemical interaction between them. The DSC Thermogram of Efinaconazole-loaded SLNs displayed endothermic peaks at 74°C for lipid and 155°C for mannitol, with no peak for Efinaconazole itself. The Compritol 888 ATO peak shifted to a lower temperature, likely due to reduced particle size and increased surface area, suggesting that efinaconazole is entrapped in the SLNs in an amorphous state as shown in Figure 12.

XRD study

XRD revealed intense peaks for Efinaconazole at 5.2, 11.6, 21.4, 32.4, and 36.9° 2θ, and peaks for Compritol ATO 888 at 4.3, 21.3, and 23.5° 2θ. In the SLN preparation, efinaconazole peaks

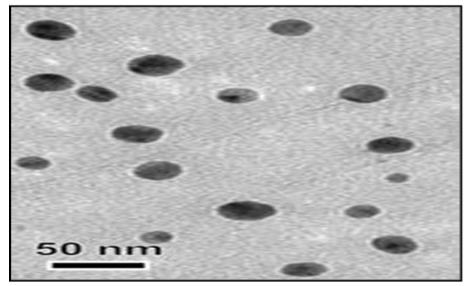

Figure 11: TEM image of optimized SLNs

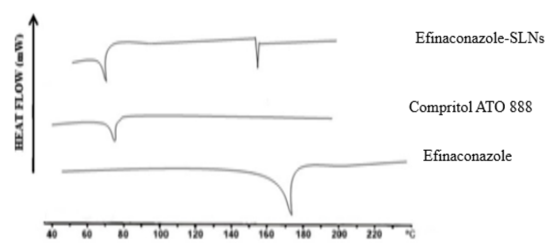

Figure 12: DSC graph of pure efinaxonazole, compritol ATO 888 and SLNs

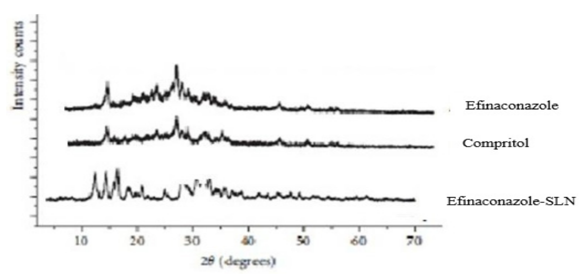

Figure 13: XRD of efinaconazole, compritol ATO 888 and SLNs

Table 6: Summary of ANOVA, R2 and *p-values* of optimized parameters of SLNs

Parameters	*DF*	*SS*	*MS*	*F*	*P value*	*R^2*	*SD*	*%CV*
MPS (nm)								
Model	9	168.8	4825.01	4.8	0.0253 Significant	0.994	10.57	9.74
Residual	7	1.64	781.92					
Total	16	170.5						
(%EE)								
Model	9	267.8	29.76	4.4	0.0309 Significant	0.997	2.59	2.88
Residual	7	46.83	6.69					
Total	16	314.68						

Table 7: Estimation parameters of gel

Code	*pH*	*Viscosity (Poise)*	*Spreadability (gcm/sec)*	*Extrudability*	*Physical appearance*
F1	7.51	0.385	64	Excellent	Creamish, smooth, translucent
F2	7.5	0.394	56.39	Excellent	Creamish, smooth, translucent
F3	7.53	0.419	45.05	Good	Creamish, smooth, translucent
F4	7.6	0.431	32.19	Good	Creamish, smooth, translucent

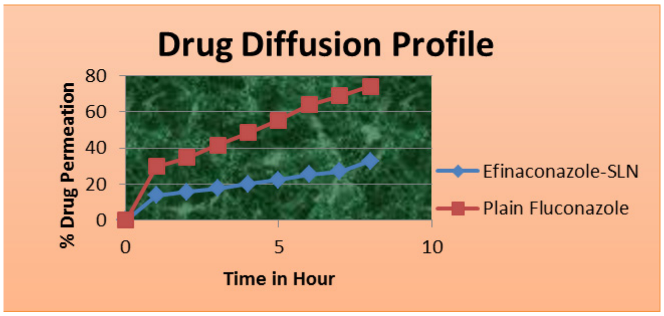

Figure 14: *In-vitro* diffusion profile

Table 8: *In-vitro* diffusion profile

S. No.	*Time (Hours)*	*%CDR*	*%CDR*
1	1	13.9	29.6
2	2	15.65	34.66
3	3	17.72	41.55
4	4	20.05	48.32
5	5	22.41	55.21
6	6	25.32	63.95
7	7	27.02	68.85
8	8	32.85	74.05

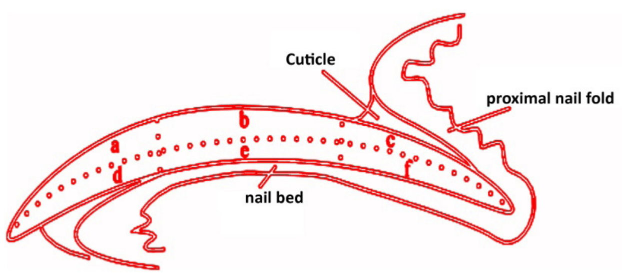

Figure 15: Histopathology of rabbit

Figure 16: In a study to develop a model of onychomycosis, the infected nails were observed at 6 weeks post-infection for their gross appearance. Some of the affected nails really looked hazy, like human onychomycosis

were deformed, indicating reduced diffraction intensities. This suggests a transformation from crystalline to amorphous form, as evidenced by the disappearance of major drug peaks and reduced signals in the SLNs XRD pattern. X-ray diffractograms are shown in Figure 13.

Gel evaluation

The physical appearance, pH, viscosity, spreadability, extrudability, and *in-vitro* diffusion of four gel formulations (F1–F4) using carbopol polymers were evaluated. Table 7 shows the results, all of which met ICH standards.

In-vitro diffusion study

We present the *in-vitro* diffusion study of formulations F1-F4 in Table 8 and Figure 14. The gel formulations were tested for *in-vitro* permeability utilizing phosphate buffer saline pH 4.9, as the membranes utilized had pH values ranging from 4.5 to 7.8. All three of the carbopol 934 formulations, with the exception of formulation F4, had *in-vitro* release profiles that achieved an 85% release from the formulation after 8 hours. Since formulation F4 demonstrated sustained release of the drug from the preparation, it takes almost 8 hours to release 33% of efinaconazole and 74.05% of fluconazole, making it the best-performing formulation among the others.

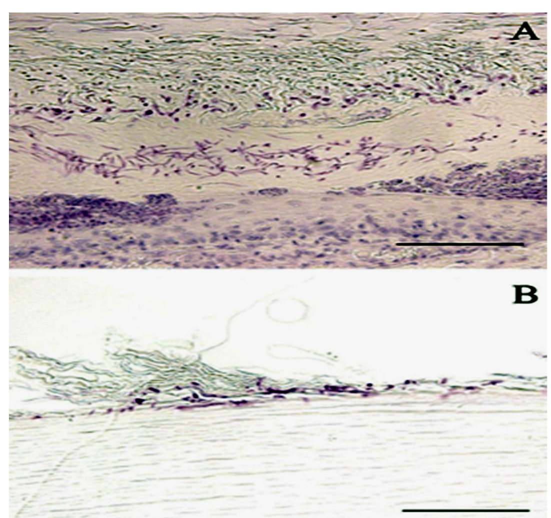

Figure 17: A and B is histological results from infected nails six weeks and two weeks after infection, correspondingly. A cluster of necrotic tissue and several fungi were seen in the nail's deep layers (A), and B, chains of spores were seen at the nail's surface in a few of the infected nails. Scale bar = 100 µm

Table 9: Stability study results

S.No.	*Parameter*	*Gel contain 0.05% w/v efinaconazole SLN & fluconazole*														
		storage condition														
		Months														
		0	*1*	*2*	*3*	*6*	*0*	*1*	*2*	*3*	*6*	*0*	*1*	*2*	*3*	*6*
1	Color	No change														
2	Odor	No change														
3	Homogeneity	Smooth														
4	pH	7.49–7.57														
5	Viscosity (poise)	0.398–0.431														

Table 10: *In-vivo* parameters

Post-infection period (wk) and location[e]	*Infection rate[a] (%)*		*Total infection rate[b] (%)*	*Appearance rate of subungual abscesses[c] (%)*		*Total appearance rate of subungual abscesses[d] (%)*
	Dorsal side	*Ventral side*		*Dorsal side*	*Ventral side*	
^{0}D	0.0 (0.00)	12.1 (0.21)		0.0	0.0	
M	6.6 (0.05)	22.3 (0.33)	53.4	0.0	22.1	36.7
P	45.6 (0.68)	35.3 (0.54)		6.2	15.7	
^{2}D	0.0 (0.00)	61.0 (1.32)		0.0	13.8	
M	16.1 (0.32)	35.7 (1.01)	83.4	0.0	36.8	40.0
P	42.2 (0.4)	29 (0.41)		2.3	12.2	
^{6}D	39 (0.94)	89 (2.12)		32.2	89	
M	49 (1.1)	45.6(1.12)	93.3	32.2	46.7	93.3
P	18 (0.5)	12.1(0.35)		5.6	6.3	

Stability study

According to Table 9, the study's results show that topical gel F4, which was created, is stable. The F4 formulation, which was made utilizing carbopol 934, had higher quality features, thus, a stability study was conducted according to ICH requirements to assure quality, safety, and effectiveness throughout the shelf life. Subsequently, 0, 1, 2, 3, and 6 months of stability testing, the topical produced gel formulation showed no changes in color, odor, homogeneity, pH, or viscosity. The research proved that the topical gel F4, as prepared, is stable.

In-vivo study

Figure 15 shows the Histology of the rabbit, Following a 2-week infection phase, post-infection periods of 0, 2, and 6 weeks were examined. Microscopic examination showed opaque nails similar to human onychomycosis, with T. mentagrophytes invading the nail bed and plate. The infection rates were 53.4% at 0 weeks, 83.4% at 2 weeks, and 93.3% at 6 weeks. The highest infection rates were observed in the proximal/dorsal (45.6%), distal/ventral (61.0%), and distal/ventral areas (89.0%) as shown in Figure 16 and Table 10. The most severe infections were found on the proximal/dorsal side (0.68), distal/ventral side (1.32), and (2.12). Subungual abscesses and necrosis of the nail bed were observed near the fungus. The rates of subungual abscesses increased over time, with 36.7% at 0 weeks, 40.0% at 2 weeks, and 93.3% at 6 weeks, as shown in Figure 17.

[a]Infection rate deliberated by formula (number of histologically fungus-positive nails in each region/number of nails tested in each region) ×100.[b] Total infection rate was deliberated by formula (number of histologically fungus-positive nails/number of nails tested) ×100. [c] To find out how often certain areas showed up, we divided total number of nails examined in each area by total number of nails with subungual abscesses region)×100. [d] Total appearance rate was deliberated by formula (number of nails with subungual abscesses/number of nails tested) × 100.

[e] D, distal; M, middle; P, proximal.

[f] Numbers in parentheses are averages of infection intensity grades in each region (Same for Table 11).

Histopathological study

The F4 formulation, applied topically once daily, reduced the infection rate to 45.5% (10/22) compared to 65.3% (15/23) in the control group. The highest infection rates were seen in the medium/ventral (18%), distal/ventral (64.3%), and distal/ventral (32.2%) areas. Subungual abscesses occurred in 54.2% of the control group and 39.1% of the F4 group. No significant difference in overall infection rates was found between the drug-treated and control groups.

Microbiological evaluation

A microbiological study of the two reference drugs' antifungal efficacy in the nail was conducted utilizing a culture recovery technique. Infected nail healing time and fungal load are shown in Table 12. In the control group, only 17 out of 24 patients made a full recovery. On the other hand, 62.5 (15/24) were associated with the F4 formulation.

Table 11: Histopathological study

Post-infection period (wk) and location[e]	*Infection rate[a] (%)*		*Total infection rate[b] (%)*	*Appearance rate of subungual abscesses[c] (%)*		*Total appearance rate of subungual abscesses[d] (%)*
	Dorsal side	*Ventral side*		*Dorsal side*	*Ventral side*	
None						
D	0.0 (0.00)	64.3 (1.72)		0.0	46.7	
M	25.3 (0.44)	33.6 (0.66)	63.3	20.6	42.4	54.2
P	12.2 (0.12)	3.9 (0.11)		4.1	4.0	
F4 Formulation						
D	4.5 (0.12)	32.2 (0.8)		4.3	32.2	
M	18.5 (0.28)	18 (0.32)	45.5	8.4	13.1	39.1
P	13.3 (0.12)	8.3 (0.07)		4.2	0.0	

Table 12: Microbiological evaluation

Treatment	*Period of treatment (days)*	*No. of fungus-positive nails/ total no. of nails (%)*	*Avg. fungal burden of infected nails*
None	28	17/24 (70.8)	+ 4.2
F4 Formulation	28	15/24 (62.5)	+ 2.6

DISCUSSION

The current investigation aims to improve skin absorption and delivery of medicinal substances while preserving skin homeostasis. Nano-sized colloidal particles like micelles, liposomes, and nanoparticles have been developed to enhance drug permeability, protect against degradation, and control release without damaging the skin. In this study, we enhanced the solubility of efinaconazole by forming a cyclodextrin complex with its chemical structure, which was then utilized towards create solid lipid nanoparticles (SLNs). Finally, the efinaconazole-SLN was incorporated into a topical fluconazole gel. Initially, the drugs were identified utilizing standard infrared spectra and ultraviolet absorption maxima. Subsequently, they underwent melting point determination, partition coefficient assessment, and solubility studies to produce the efinaconazole-SLN and fluconazole gel. Following this, a standard curve was generated utilizing a 4.9 pH phosphate buffer, and the drug was examined utilizing a UV spectrophotometer. It was confirmed that the drug met pharmacopeial standards concerning its melting point, partition coefficient, solubility, wavelength of maximum absorption, and IR spectral characteristics.

CONCLUSION

Fluconazole diffuses more rapidly than efinaconazole-loaded SLNs, which release the drug at a slower rate. The optimized formulation containing both fluconazole and efinaconazole showed sustained drug release, with fluconazole releasing 74.05% after 8 hours and efinaconazole releasing 32.85% over the same period. The F4 formulation with carbopol 934 demonstrated superior quality and stability per ICH guidelines. Subsequently, up to 6 months of testing, the topical gel showed no changes in color, odor, homogeneity, pH, or viscosity, confirming its stability.

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