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Original Article

Design, Optimization, and Chemical Characterization of a Terbinafine Nanosponge-Based Nanogel Using a Factorial Methodological Approach

Addanki Anusha, Annammadevi Govardhini Sayam*

GITAM School of Pharmacy, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India

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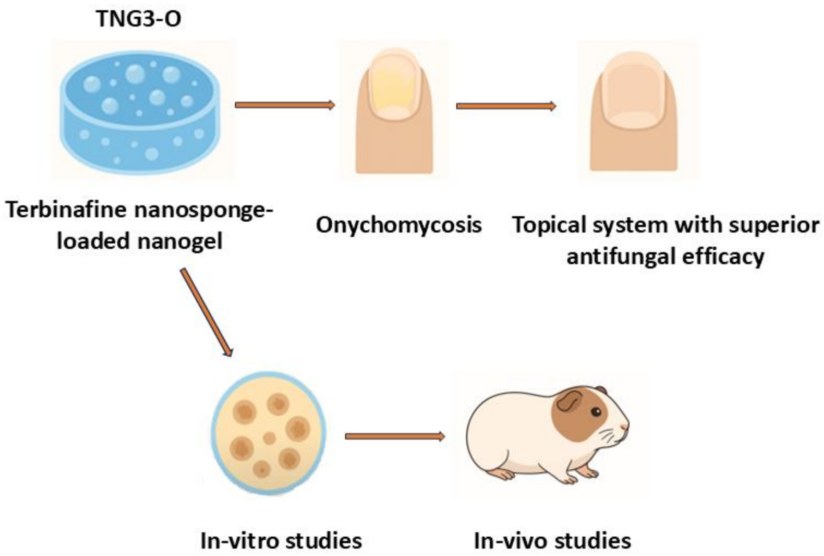
ABSTRACT

Terbinafine exhibits poor nail permeability, which limits its effectiveness against onychomycosis. To enhance topical delivery, a terbinafine nanosponge-loaded nanogel (TNG3-O) has been developed for sustained antifungal action. Pre-formulation studies validated drug purity and compatibility using FTIR and XRD analyses. A 3^2 factorial design improved the nanosponge formulation by using Eudragit RS100 and PVA. Nanosponges prepared by emulsion solvent diffusion were added to a Carbopol 940-based nanogel and tested for their physical and chemical properties, antimicrobial activity, and *in vivo* activity. The TNG3-O had a particle size of 177.2 nm, a zeta potential of –25.7 mV, and a drug loading of 67.5%. It had a pH of 5.71, a viscosity of 35,100 mPa.s, a spreadability of 36.5 g.cm/s, and a drug content of 93.12%, and it was stable. SEM confirmed that the nanosponges were spherical and evenly spread. TNG3-O created inhibition zones of 29 ± 0.00 mm (*Candida albicans*) and 30 ± 0.00 mm (*Trichophyton rubrum*), which was better than the marketed formulation. *In vivo*, TNG3-O significantly diminished fungal invasion and formation of subungual abscesses in infected guinea pigs. TNG3-O offers a stable and effective topical system with superior antifungal efficacy and is a promising approach for the management of onychomycosis.

* Corresponding author: Annammadevi Govardhini Sayam
E-mail: mannam@gitam.edu

GRAPHICALABSTRACT



Introduction

Onychomycosis is a prevalent fungal infection of the nails, whichis characterized by nail discoloration, thickening, and brittleness, frequently leading to discomfort and subsequent bacterial infection. This continues to be a therapeutic challenge because the nail plate is a dense keratinized structure that greatly limits drug penetration into the infected area [1]. The need for safer and more effective topical delivery systems is highlighted by the fact that oral terbinafine is highly effective in the treatment of onychomycosis. However, its use is restricted due to the possibility of systemic adverse effects, such as hepatotoxicity, taste disturbances, gastrointestinal complaints, and drug-drug interactions [2].

The nail plate is a heavily keratinized barrier that inhibits the diffusion of most antifungal drugs, making transungual medication administration a substantial challenge. As both human nails and bovine hoof membranes are made of α-keratin and exhibit similar diffusion behavior, comparative *in vitro* permeability experiments have demonstrated that the latter may be used as a suitable substitute. In contrast, the former is typically more permeable because of its less thick keratin matrix [3]. Hence, enhancing the topical penetration and retention of terbinafine using nanocarrier-based systems represents a promising strategy.

Nanotechnology-based carriers, like nanosponges, are a promising way to give transcutaneous and topical medications. Nanosponges can bind hydrophilic and lipophilic drugs because they are porous and polymerize. This allows for controlled release, better solubility, and longer skin (or nail) retention [4]. Improved patient compliance is the result of nanosponge systems, which merge the benefits of nanocarriers (such as increased solubility, controlled release, and extended retention) with the spreadability and simplicity of gel use (among other application attributes) [5].

Polymers such as Eudragit RS100 and stabilizers such as polyvinyl alcohol (PVA) are often used to make nanosponges (and nanoparticles) because changes in their concentrations can have a significant effect on important formulation properties such as particle size, zeta potential, and entrapment efficiency [6]. Factorial design and other statistical optimization techniques facilitate the systematic assessment of formulation parameters and their interactions, thereby enabling the development of robust and reproducible nanosponge systems [7].

The present study enhanced terbinafine-loaded nanosponges using a 2-factor, 3-level factorial design. Nanosponges were prepared using the emulsion solvent diffusion method with Eudragit RS100 and PVA. A nanogel (TNG3-O) based on

Carbopol 940 was improved for topical use and drug residence time by adding a better nanosponge formulation. The created system was tested for its physicochemical properties, structural features, stability, antifungal activity against *Candida albicans* and *Trichophyton rubrum, in vitro* diffusion using bovine hoof membranes, and *in vivo* effectiveness in an onychomycosis model. The goal of this study was to reduce the systemic exposure while increasing the topical absorption and therapeutic effectiveness of terbinafine.

Materials and Methods

Melting Point Determination

The melting point of terbinafine was measured using the capillary technique, which is in line with pharmacopeial standards. A small amount of the material was placed into a capillary tube, and a melting point apparatus was used. To determine the melting point, the temperature was gradually increased until the substance was completely liquid [8].

Determination of Absorption Maximum (λ_{max})

To prepare a series of dilutions, a stock solution of terbinafine (1,000 µg/mL) in methanol was prepared and mixed with phosphate buffer (pH = 5.0) to make it less concentrated. A UV-Visible spectrophotometer (LabIndia 3000+) was used to scan the solution from 200 nm to 400 nm. The λ_{max} or maximum absorption wavelength was recorded.

Linearity Study

The volumes of the working solution ranging from 5 to 25 µg/mL were examined at 222 nm using the same spectrophotometric technique. By graphing the absorbance against concentration, a calibration curve can be generated and the parameters for the regression analysis can be determined.

Solubility Studies and Selection of Polymer

To find a polymer that would work well in nanosponge formulations, the solubility of terbinafine was tested in water, methanol, and 1% (w/v) solutions of several polymers such as ethylcellulose, Eudragit RS100, hyper-cross-linked polystyrene, methyl *β*-cyclodextrin, and hydroxypropyl *β*-cyclodextrin.

In microcentrifuge tubes, one milliliter of each solvent or polymer solution was mixed with approximately 20 mg terbinafine. To get to equilibrium, the mixture was shaken in a vortex for 72 h at 25 °C. After centrifugation at 18,000 rpm, the supernatants were diluted with methanol according to the manufacturer's protocol, and then examined using a spectrophotometer. They were filtered using a 0.20 µm syringe filter [9].

Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR (Jasco FT/IR-4X) was used to determine whether terbinafine was compatible with other ingredients. The KBr pellet method was used to obtain spectra for solid materials in the range of 500–4,000 cm^{-1}, and the ATR mode was used for liquid samples. To assess potential interactions, the obtained spectra were compared with those of the pure drug [10,11].

X-Ray Diffraction (XRD)

The XRD was used on a Shimadzu diffractometer operating at 40 kV and 30 mA to examine the crystalline properties and compatibility of terbinafine with other drugs. The scan rates of 4°/min was used to scan the samples in reflection mode over a 2θ range of 10–40°. Diffractograms were obtained for the formulation mixtures, specific excipients, and pure medications [12].

Design of Experiments (DoE)

A 2-factor, 3-level factorial design (3^2 design) was employed using Design-Expert v12 (Stat-Ease Inc., USA) to optimize the terbinafine-loaded nanosponges (Figures S5 and S6).

Eudragit RS100 (X_1) and PVA (X_2) were selected as independent variables, while particle size (Y_1), zeta potential (Y_2), and drug loading (Y_3) were chosen as responses. Nine formulations were prepared according to the software design matrix [13].

Formulation of Terbinafine-loaded Nanosponges

The emulsion solvent diffusion method was used to prepare terbinafine nanosponges based on the nine runs produced by the factorial design.

Methanol-dissolved Eudragit RS100 was used for the dispersed phase, and water-dissolved PVA for the continuous phase. A magnetic stirrer set to 40 °C mixed the polymeric solution into the PVA solution at 22,000 rpm until all of the solvent had evaporated (Figure 1, Table S2).

The mixture was then agitated at 2,000 rpm for 2 h after the addition of terbinafine, which was dissolved in a small quantity of methanol. After a 12 h pre-freezing period at -80 °C, the nanosponge dispersion was lyophilized under vacuum at -48 °C for 48 h, resulting in a liberated nanosponge powder [14].

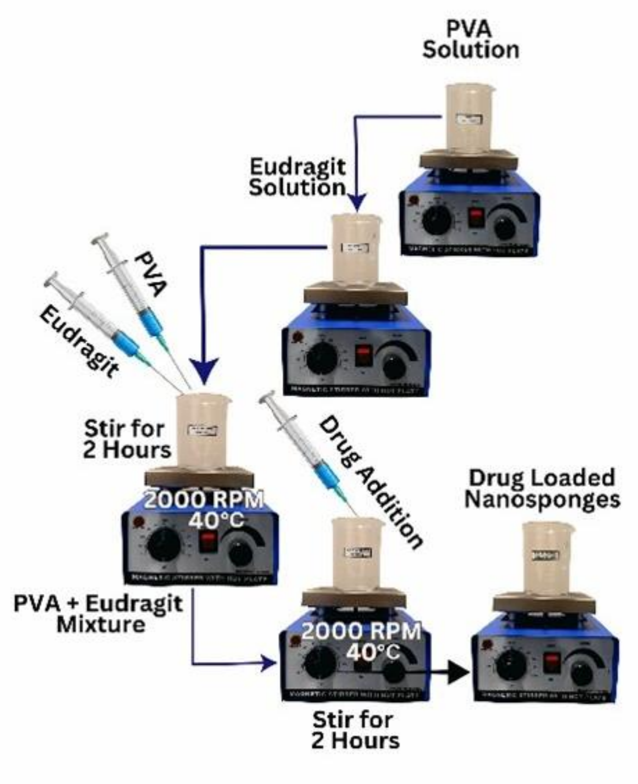


Figure 1: Terbinafine method of preparation

Characterization of Nanosponges

A nanoparticle analyzer (Horiba SZ-100) was used to measure the PDI, particle size, and zeta potential of terbinafine nanosponges at 25 °C. After the drugs were separated by centrifugation, established methods were used to calculate the entrapment efficiency and drug loading using spectrophotometry [15].

Formulation of Nanosponges-Loaded Nanogels

The nanosponge suspension was prepared in water. Carbopol 940 was slowly added to the nanosponge suspension and mixed using a magnetic mixer set to 500 rpm until the mixture was completely smooth. To neutralize the homogeneous dispersion, a certain amount of triethanolamine was added. This amount was based on the concentrations shown in Table S9. To stop the formation of air bubbles and ensure that the neutralizing ingredient was spread out evenly, the gel was gently but thoroughly stirred by hand [16].

Characterization and Evaluation of Nanogels

The prepared nanogel was evaluated for its viscosity, spreadability, pH determination, and drug content.

Viscosity

The viscosities of nanogel formulations (TNG-1 to TNG-5) were determined using a Brookfield viscometer (C50-1 spindle) at 25 and 37 °C. Measurements were performed in triplicate.

Spreadability

Spreadability was evaluated using the glass slide method, and calculated using the Equation 1:

$$S = M \times L / T \quad (1)$$

Where, S is the spreadability (g.cm/s), M is the applied weight (g), L is the distance moved (cm), and T is time (s).

pH Determination

The pH of each gel formulation was measured at room temperature using a calibrated digital pH meter.

Drug Content

To prepare the solution, 1 mg of terbinafine gel was mixed with phosphate buffer (pH=5.5), stirred for 2 h, filtered, and then analyzed at 222 nm using a UV-Vis spectrophotometer. The drug content (%) was calculated accordingly [17].

Scanning Electron Microscopy (SEM)

SEM was used to examine the surface morphology of the optimized nanosponge-loaded nanogel formulation (TNG-3). A small piece of lyophilized material was placed on an aluminum stub, sputter-coated with gold, and observed under vacuum at different magnifications [5,18].

In Vitro Diffusion Study

Bovine hoof membranes attached to Gummer-type diffusion cells were used to test the *in vitro* permeation of terbinafine nanosponges (TNG1-TNG5). Prior to use, the membranes were carefully hydrated for 12 h. A 5 mL receptor chamber was kept at 32 °C and swirled phosphate buffer (pH=5.5, 0.003% NaN_3) at 600 rpm. A 250 µL sample of each formulation was placed on the donor's side. A Waters Alliance e2690 high-performance liquid chromatography system (RP-18 column, acetonitrile: phosphoric acid: methanol = 52:28:20, flow 1 mL/min, and 300 nm) was used for analyzing the samples that were taken up to 36 h [19,20].

Stability and DSC

The optimized terbinafine nanogel (TNG3-O) was put through to months of short-term stability tests at 4±1 °C and 25±1 °C. The samples were evaluated for physical appearance, viscosity, spreadability, and drug content. Differential Scanning Calorimetry (DSC; Mettler Toledo DSC 3) was used to study thermal stability. Its indium calibration can result in interactions or deterioration [21].

Determination of Antimicrobial Activity

The cup-plate (agar diffusion) method to test the efficacy of the optimized nanogel (TNG3-O) against *Candida albicans* and *Trichophyton rubrum*. After 72 h of incubation, the fungal suspensions were added to Sabouraud's agar plates. Afterward, 0.05 mL TNG3-O, a commercial formulation (TNF-MF), and standard terbinafine were added to 10 mm wells. The plates were kept at 30 °C for 48 h, and then the size of the inhibitory zones (mm) was measured [22].

In Vivo Studies

In vivo evaluation was performed in accordance with the ethical guidelines approved by the Animal Ethics Committee of the organization (Ref. No. CCSEA/IAEC/JLS/19/02/23/178).

Animals: Male Dunkin–Hartley strain guinea pigs (300–400 g) were procured from a certified laboratory and acclimatized under standard animal house conditions for 7 days before the study. Each of the three groups (5-Guinea pigs per group was used for an onychomycosis model) for treatment effectiveness.

Test Organism: *Trichophyton rubrum* isolated from a guinea pig was obtained from KN Laboratories, Hyderabad.

Inoculum Preparation: *Trichophyton rubrum* was cultured on Sabouraud dextrose agar at 28 °C for 2 weeks. A suspension of macroconidia (10^8 conidia/mL) in saline containing 0.05% Tween-80 was prepared for inoculation.

Onychomycosis Induction: 4 mg/kg intramuscular methylprednisolone acetate was administered to guinea pigs in the experiment. After two weeks, each nail was treated with 0.2 mL of *Trichophyton rubrum* suspension, and then sealed to maintain moisture. After two weeks, the covers were removed and the infection was checked at 0, 2, and 6 weeks. To perform histopathological and cultural studies, animals were euthanized and their claws were collected [23].

Drug Treatment: After infection, each nail was treated once a day for 28 days with 3.6 µL of optimized TNF-OF (TNG3-O) formulations that used Carbopol 940 as the gelling agent. Control animals were infected, but left untreated.

Evaluation of Therapeutic Efficacy: Half of the infected nail samples were used for histology and the other half for culture recovery on Sabouraud's dextrose agar with cycloheximide, chloramphenicol, and azinomycin. The plates were maintained at 28 °C for two weeks. The fungal load was rated from +10 to 0, based on the number of good nail pieces.

Histopathological Studies: After fixation in formalin and decalcification, the nail samples were placed in paraffin. After staining with periodic acid-Schiff (PAS) reagent, the samples were examined under a microscope. the fungal growth was scored in six different areas of the nail from 0 to 3 to determine the infection rate and intensity.

Statistical Analysis: The data on histological infection rate and culture recovery were examined using Fisher's exact test, whereas the severity of fungal loads was assessed using Student's t-test. Statistical significance was defined as $p < 0.05$.

Results

Melting Point

The melting point of terbinafine was found to be 205 °C, which is within the reported range of 195–214 °C, indicating purity and consistency with standard values.

UV Spectroscopic Analysis

The λ_{max} of terbinafine in the buffer (pH 5.0) was 222 nm (Figure S1). The calibration curve showed linearity over 5–25 µg/mL, with the equation Y = 0.045X + 0.002 and correlation coefficient R^2 = 0.9999 (Figure S2).

Solubility and Polymer Selection

Among the polymers tested (Table S1), terbinafine exhibited the highest solubility in Eudragit RS100 (0.523 mg/mL). Therefore, Eudragit RS100 was selected as the primary polymer for nanosponge formulation.

The concentration ranges of Eudragit RS100 and PVA were selected based on preliminary screening experiments (data not shown).

Compatibility Study

The FT-IR spectrum of pure terbinafine exhibited characteristic peaks at 3,445 cm^{-1} (aromatic C–H stretching), 2,968 cm^{-1} (aliphatic C–H stretching), 1,454 cm^{-1} (C–N stretching), and 1,384 cm^{-1} (–CH_3 bending) (Figure S3). The overlay spectra of terbinafine with individual excipients and the optimized formulation showed no significant shifts or disappearance of peaks, indicating the absence of any chemical interactions between the drug and excipients (Figure 2).

Figure S4 shows that pure terbinafine was crystalline, with clear diffraction peaks at 2θ = 6.08°, 18.16°, 20.18°, and 24.26° in the XRD pattern. There was no significant change or loss of peaks because the equivalent peaks were almost in the same places in the different formulations. The results demonstrated that terbinafine remained in a crystalline state, with no significant interaction or polymorphism alteration during the formulation (Figure 3).

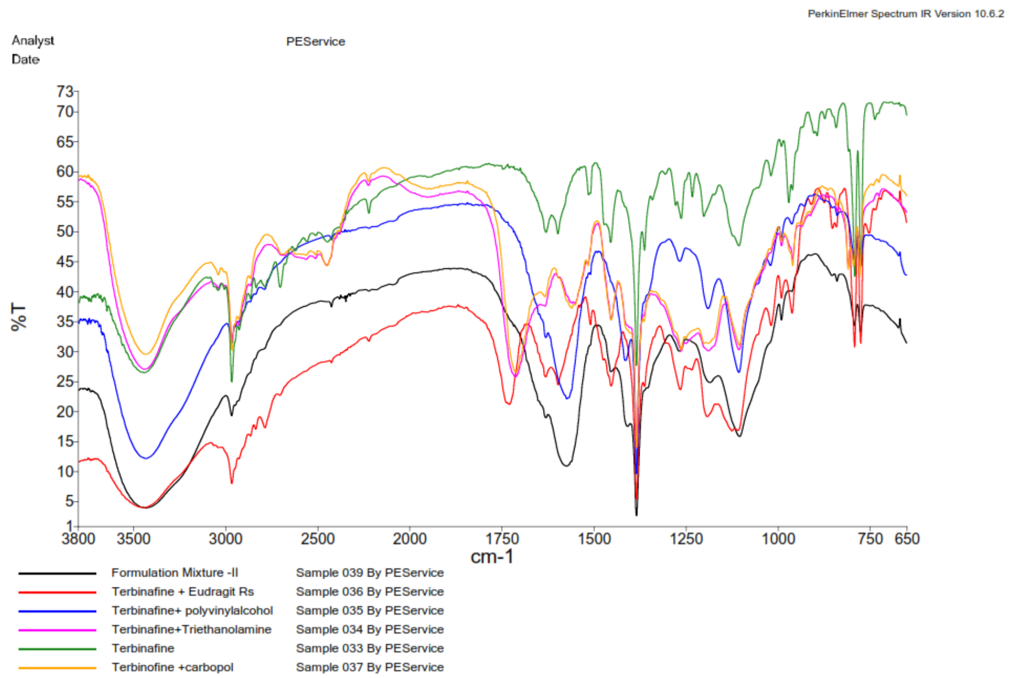


Figure 2: Overlay spectra of Terbinafine pure drug with individual excipients and TNG formulation mixture

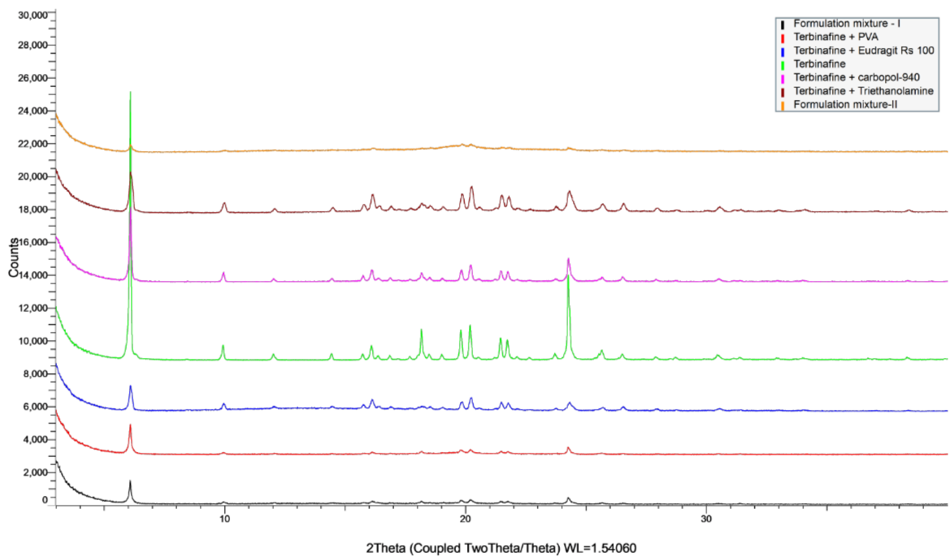


Figure 3: Overlay X-ray diffraction of pure drug along with individual excipients. Formulation mixture-I & formulation mixture-II

Characterization of Terbinafine-Loaded Nanosponges

Terbinafine nanosponges were successfully formulated as free-flowing porous powders with uniform particle distributions (Figures S5 and S6 and Table S2).

A 3^2 factorial design was applied using *Design-Expert® software* to evaluate the effects of Eudragit RS100 (factor A) and PVA (factor B) concentrations on the key responses: particle size (Y_1), zeta potential (Y_2), and drug loading (Y_3) (Figures 4-6, Tables S3 and S4).

All nine formulations (TN1–TN9) showed particle sizes ranging from 98.6 to 290.4 nm, zeta potential between −41.4 and −11.3 Â mV, and PDI values between 0.252 and 0.534. Drug loading ranged from 49.26 to 75.15 %.

The optimized formulation contained 57.5 mg Eudragit RS 100 and 0.12 % PVA, it had a particle size of 177.2 nm, zeta potential −25.7 mV, and drug loading 67.5 %, closely matching predicted values (Tables S5-S10, Figures S7 and S8).

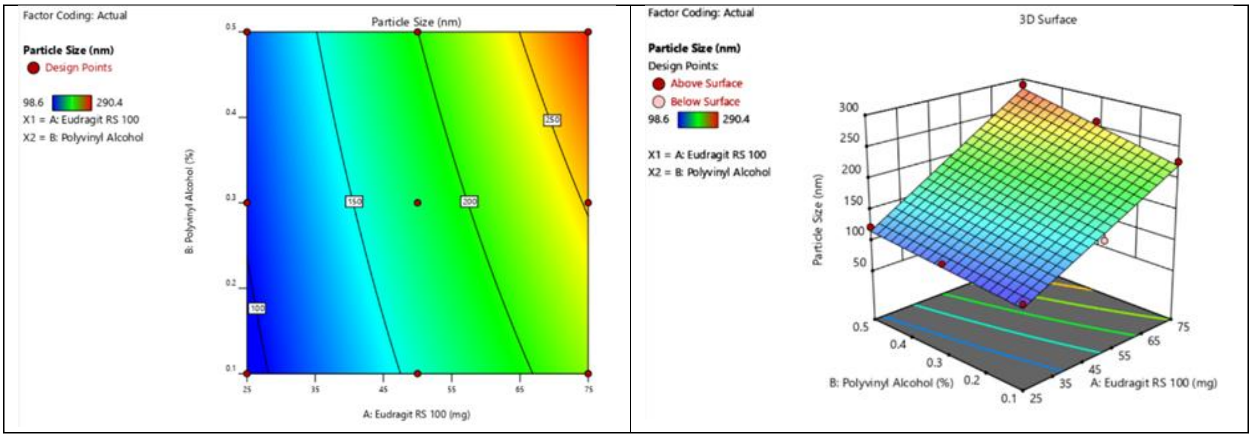


Figure 4: Response surface plot for terbinafine nanosponges particle size

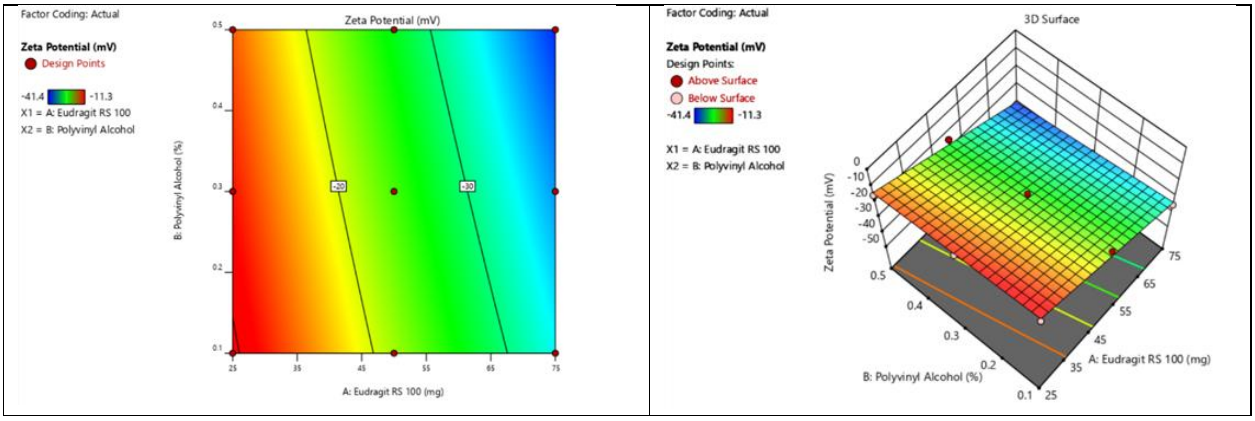


Figure 5: Response surface plot for nanosponges zeta potential

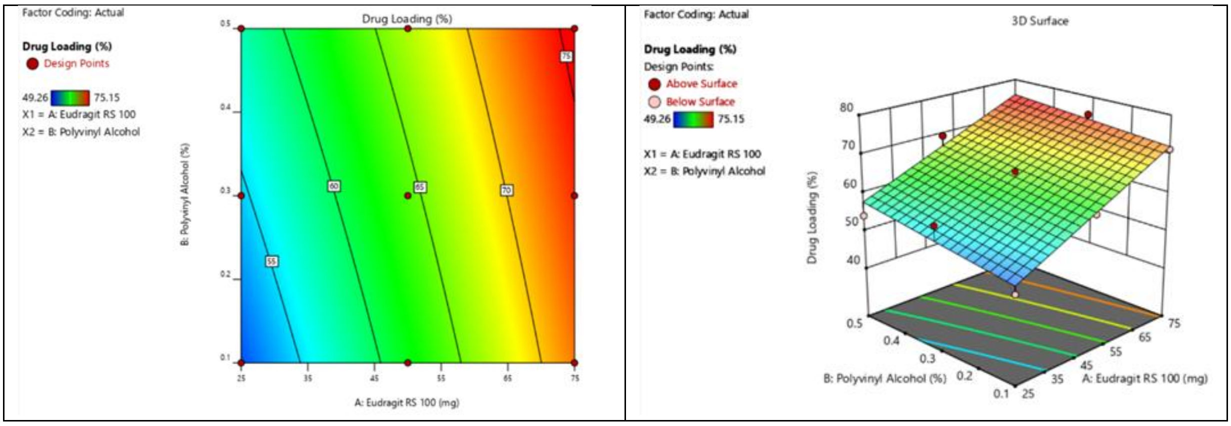


Figure 6: Response surface plot for nanosponges drug loading

Evaluation of TNG3-O

The pH of the nanogels used was between 5.26 and 5.74. The viscosity was between 15,500 and 48,100 mPa·s, which made it easy to spread and achieve consistency. TNG-3 had a balanced spreadability of 36.2 g·cm/s and a high drug content of 93.74%. Its viscosity is in the ideal range of 35,000–40,000 mPa·s.

SEM

SEM images of the optimized formulation (TNG-3) revealed discrete spherical particles with a smooth surface and uniform morphology (SEM images of the optimized formulation (TNG-3) at various magnifications are shown in Figure 7.

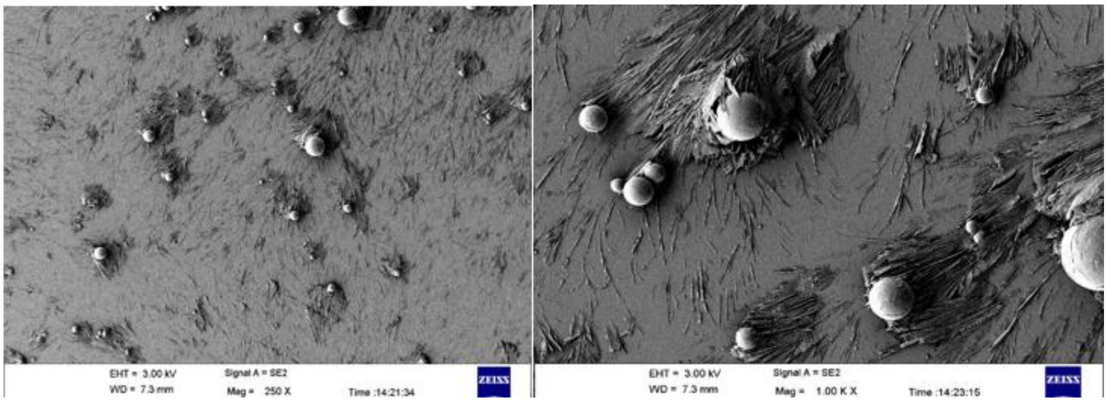


Figure 7: SEM Images of optimized formulation (TNG-3) at different magnifications

In Vitro Diffusion Study

The method was developed at concentrations ranging from 20 ppm to 400 µg per ml. The method is linear with area responses as to the concentration, and the linear plot is observed as 0.999 and r^2 is 1.000. The detection limit (DL) at QL (quantification limit) is 20ppm and the DL is 2 PPM which shows the identification of the drug at the 2 ppm level. The test concentration was prepared by the drug at 1 mg/mL.

No measurable terbinafine permeation through bovine hoof membranes was detected for any nanosponge or marketed formulation within 36 h, remaining below the analytical DL.

Stability Studies

After 2 months, TNG3-O maintained acceptable physicochemical properties with minimal change (pH 5.74→5.71, viscosity 34,300→35,100 mPa·s, spreadability 36.2→36.5 g·cm/s, and drug content 93.74→93.12%). DSC thermograms showed consistent endothermic peaks (213.26 °C → 211.85 °C), confirming no significant interaction or degradation (Figures 8 and 9).

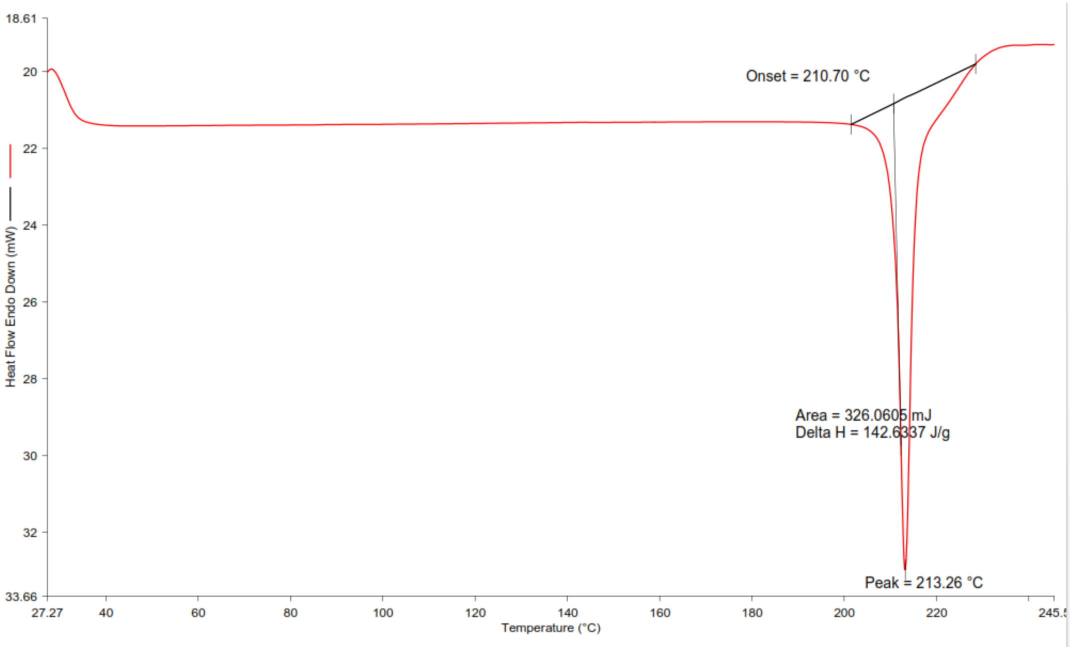


Figure 8: DSC studies of the optimized formulation (TNG3-O) before stability

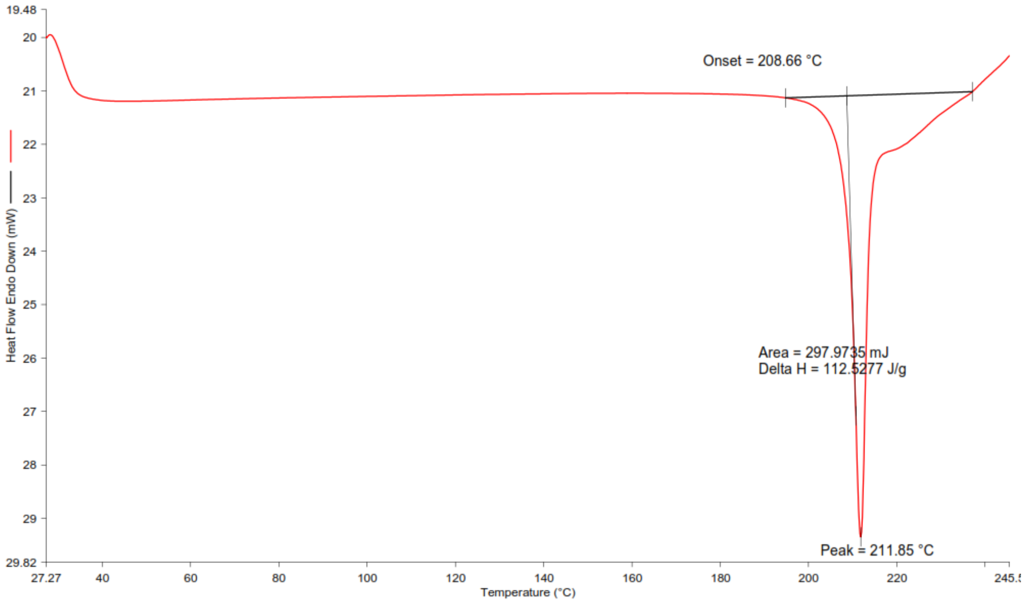


Figure 9: DSC thermograms showed consistent endothermic peaks (213.26 °C → 211.85 °C), confirming no significant interaction or degradation of the optimized formulation (TNG3-O) after stability

Determination of Antimicrobial Activity

TNG3-O showed high antifungal activity, with inhibition zones of 29 ± 0.00 mm for *Candida albicans* and 30 ± 0.00 mm for *Trichophyton rubrum*. This was compared to normal terbinafine, which had inhibition zones of 25 ± 0.34 mm and 23 ± 0.20 mm, respectively. Marked enhancement of the marketed formulation was observed (Table 1, Figure 10).

Table 1: Zone of inhibition (mm) by cup-plate method — TNF formulations

Formulation	***Candida albicans*** **(mm)**	***Trichophyton rubrum*** **(mm)**
Control	-	-
Terbinafine (standard)	25±0.34	23±0.20
TNF-OF (TNG3-O)	29±0.00**	30±0.00**
TNF-MF	25±3.03	24±1.02

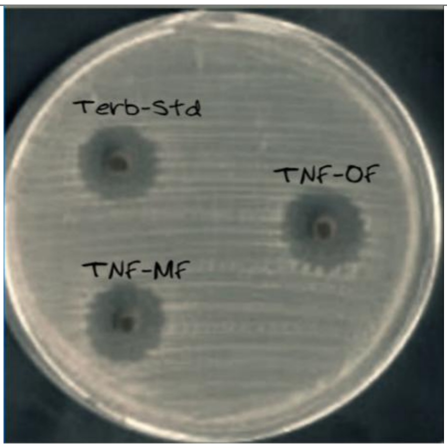


Image 1: Zone of inhibition of TNF-OF (TNG3-O), TNF-MF, and Terbinafine (standard) formulation on *Candida albicans*

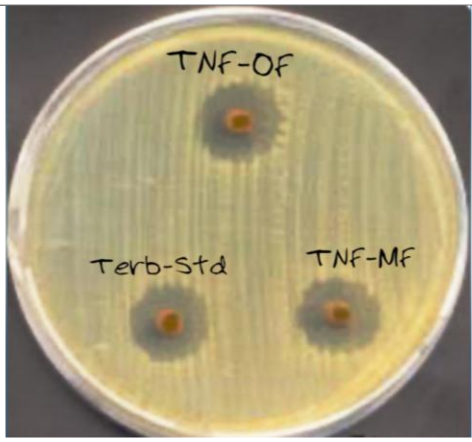


Image 2: Zone of inhibition of TNF-OF (TNG3-O), TNF-MF, and Terbinafine (standard) formulation on *Trichophyton rubrum*

Figure 10: Zone of inhibition of terbinafine (standard), TNF-optimized formulation (TNG3-O), and TNF-marketed formulation against *Candida albicans* and *Trichophyton rubrum* by diffusion (Agar well diffusion) method (mean±SD, n=3)

In Vivo Results

Guinea pig nails with onychomycosis exhibit progressive pathological alterations similar to those observed in human nails, including thickening and fungal infection of the nail bed and plate. Infection rates increased from 57.29% at week 0 to 96.87% at week 6, and both the dorsal and ventral areas were severely ill. Additionally, subungual abscess formation rose from 38.95% to 97.02% over the same period (Table 2).

The optimized terbinafine nanogel formulation (TNG3-O) significantly decreased fungal invasion and subungual abscess formation compared to the untreated controls, demonstrating potent antifungal efficacy. Histopathological analysis at two weeks showed fungal hyphal infiltration. However, 6 weeks after TNG3-O therapy, this had decreased significantly, which means that TNG3-O had worked its way into the tissue and stopped the infection (Table 3, Figures 11 and 12).

Table 2: *In vivo* parameters – post infection period

Post-infection period and location	Infection rate (%)		Total infection rate (%)	Appearance rate of subungual abscesses (%)		Total appearance rate of subungual abscesses (%)
	Dorsal side	Ventral side		Dorsal side	Ventral side	
D^0	0.0(0.00)	15.3(0.541)	57.29	0.0	0.0	38.95
M^0	9.9(0.06)	25.51(0.67)		0.0	25.02	
P^0	49.43(0.93)	38.0.82)		8.4	17.43	
D^2	0.0(0.00)	62.32(3.21)	85.82	0.0	15.43	43.64
M^2	18.35(0.53)	37.92(0.86)		0.0	39.42	
P^2	44.6(0.63)	31.72(0.67)		5.32	15.14	
D^6	43.92(6.25)	93.3(5.34)	96.87	36.57	93	97.02
M^6	53.54(5.43)	49.04(6.23)		34.49	51.02	
P^6	22.76(0.91)	16.28(0.72)		9.32	10.34	

Table 3: *In vivo* parameters - after treatment with TNG3-O, compared to infected but untreated nails (none)

Post-infection period and location	Infection rate (%)		Total infection rate (%)	Appearance rate of subungual abscesses (%)		Total appearance rate of subungual abscesses (%)
	Dorsal side	Ventral side		Dorsal side	Ventral side	
None						
D	0.00(0.00)	66.74(1.03)	66.43	0.00	47.87	57.31
M	27.43(0.43)	39.13(2.04)		22.37	4.01	
P	15.341.23)	6.95(3.18)		7.21	7.95	
TNF- optimization formulations (TNG3-O)						
D	6.75(1.76)	33.97(2.19)	47.34	7.98	40.21	42.81
M	21.35(1.45)	21.98(2.53)		11.09	16.09	
P	15.65(3.19)	11.87(1.56)		6.032	2.01	

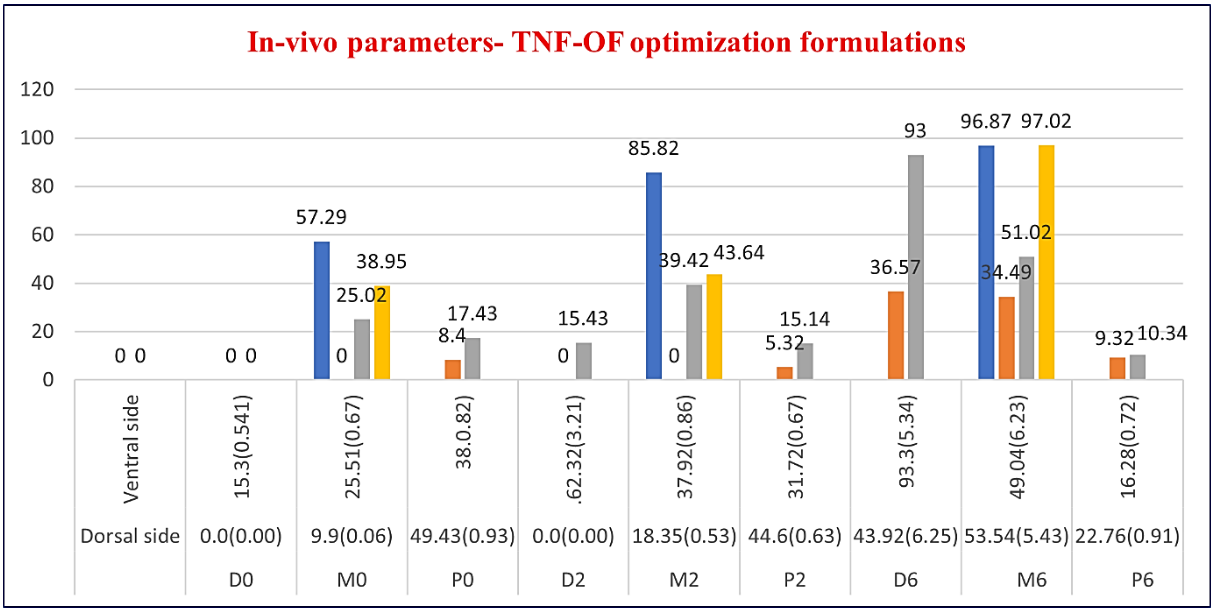


Figure 11: Graphical representation TNF optimization formulations (TNG3-O) with *in vivo* parameters Blue: Untreated Guinea pig, infection rate; light orange: untreated guinea pig, total appearance of subungual abscess; grey: untreated guinea pig-ventral side-subungual abscess; and dark orange: untreated guinea pig-dorsal side-subungual abscess

Figure 12: The infected Guinea pig nails were observed at 6 weeks post-infection for their gross appearance onychomycosis (mean±SD, n=5)

Histopathological images showing fungal invasion at 2 weeks (A) and 6 weeks (B) post-infection for TNF-OF (TNG3-O)-optimized formulations (Figure 13).

Discussion

This study focused on the formulation, optimization, and evaluation of terbinafine-loaded nanosponges incorporated into a nanogel system for enhanced topical delivery and antifungal efficacy against *Candida albicans* and *Trichophyton rubrum*. Using a systematic factorial design approach, the concentrations of the polymer (Eudragit RS100) and stabilizer (PVA) were examined to determine how they affected important formulation characteristics, such as particle size, zeta potential, and drug loading. The optimized nanosponge formulation (TNG3-O) exhibited good physicochemical properties, indicating that terbinafine was successfully trapped and stabilized inside the polymeric matrix. Therefore, TNG-3 was selected as the best formulation for future research (Figure S9).

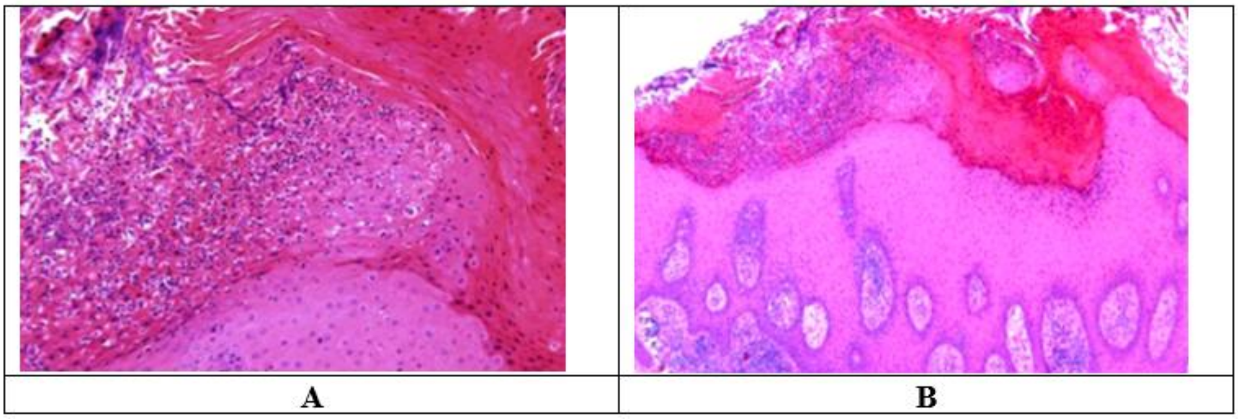


Figure 13: Representative histopathological images of guinea pig nail tissue demonstrating the progression of fungal invasion at 2 weeks (A) and 6 weeks (B) after infection, following application of the optimized TNF-OF (TNG3-O) formulation

The emulsion solvent diffusion technique for producing nanosponges produced empirical and porous nanoparticles, as shown by SEM. This result is consistent with earlier studies on eudragit-based nanosponges characterized by an extensive surface area and controlled drug release properties [20,24]. The average particle size obtained was within the nanometric range suitable for topical delivery, ensuring improved drug deposition at the infection site.

Physicochemical and rheological tests on the nanosponge-loaded nanogels showed that their pH was between 5.26 and 5.74, which is very close to the normal pH of the skin and should not cause any irritation. The optimized formulation (TNG3-O) was easy to apply and spread evenly over the skin. It had a great viscosity (34,300 mPa·s) and spreadability (36.2 g.cm/s) [5]. Drug content uniformity was satisfactory, with minimal variation (91.18–96.32%), confirming homogeneity in drug dispersion within the gel matrix.

In vitro diffusion studies using bovine hoof membranes, which are good models for the absorption of drugs by human nails, showed that the formulation could remain on the nail surface for a long time and act as a drug reservoir within the keratin matrix. There was no detectable flux of terbinafine through the membrane after 36 h. This quality is ideal for the topical treatment of onychomycosis because the antifungal activity depends on how long the drug can stay in the area [19,20].

Short-term stability tests showed that the improved nanogel retained its physicochemical properties with only small changes in pH, viscosity, spreadability, and drug content for two months at both room temperature and cold storage. DSC thermograms showed that there were no drug-excipient interactions or degradation, which provided further proof that the system was thermally and chemically stable [25].

Antifungal studies showed that TNG3-O had much larger zones of inhibition (29–30 mm) than the marketed formulations, and were similar to pure terbinafine (27 mm). This confirmed that TNG3-O was more effective against fungi because it was more soluble, released over time, and better diffused from the nanosponge matrix [16].

The *in vivo* study on guinea pigs with onychomycosis further corroborated these findings. By the sixth week, there was a notable reduction in hyphal invasion and the formation of subungual abscesses, indicating effective fungal eradication, as evidenced by the decline in infection and enhancement of histological profiles after post-TNG3-O administration. Our findings corroborate those of previous studies indicating that nanosponge-mediated drug delivery enhances local drug accumulation and therapeutic outcomes in chronic fungal infections [26].

A promising topical formulation has been developed for the effective management of onychomycosis and other superficial fungal infections. The terbinafine-loaded nanosponge nanogel exhibited enhanced stability, physicochemical compatibility, and increased antifungal efficacy both *in vitro* and *in vivo*.

Conclusion

This study employed a factorial design methodology to produce and enhance Terbinafine-loaded nanosponges within a nanogel system. The optimized formulation (TNG3-O) exhibited superior antifungal efficacy, optimal physicochemical characteristics, and exceptional stability compared with the marketed formulation. Rheological tests showed that viscosity and spreadability were suitable for topical use, and SEM confirmed that the nanosponge structure was uniform and porous. Stability and hydrodynamic stability tests showed that there were no significant physicochemical changes or interactions between the drug and the excipient. Both *in vitro* and *in vivo* tests showed that the nanosponge-based nanogel system prevented fungi from growing and helped drugs stay in the area longer. Despite these promising outcomes, the limitations of the present study should be acknowledged. The stability assessment was limited to a short duration, and long-term stability under various storage conditions remains to be established. Additionally, while *in vitro* diffusion studies suggested prolonged nail retention, quantitative permeation and drug deposition studies have not been performed, limiting the precise evaluation of transungual drug transport. Future investigations should focus on extended stability studies, detailed permeation kinetics, and clinical validation to substantiate the therapeutic potential of this nanosponge-based nanogel system further. Overall, the developed formulation represents a promising, patient-friendly topical approach for the management of onychomycosis and related superficial fungal infections, with scope for further optimization and translational development.

Future Perspective

Future research should focus on the long-term stability of terbinafine-loaded nanosponges under real-time storage conditions and the methods for scaling up their production. Comprehensive clinical evaluations and sophisticated *in vivo* studies are essential to confirm the therapeutic efficacy, safety, and adherence of nanogel formulations in humans. This nanosponge-based delivery technology makes it easier for antifungal and weakly soluble medicines to be absorbed through the skin, released over a longer period, and delivered directly to the skin.

ORCID

Addanki Anusha
https://orcid.org/0000-0003-3655-3486
Annammadevi Govardhini Sayam
https://orcid.org/0000-0002-6670-7071

References

[1] McAuley, W., Jones, S., Traynor, M., Guesné, S., Murdan, S., Brown, M. An investigation of how fungal infection influences drug penetration through onychomycosis patient's nail plates. *European Journal of Pharmaceutics and Biopharmaceutics*, **2016**, 102, 178-184.
[2] Gupta, A.K., Haas-Neill, S., Talukder, M. The safety of oral antifungals for the treatment of onychomycosis. *Expert Opinion on Drug Safety*, **2023**, 22(12), 1169-1178.
[3] Mertin, D., Lippold, B.C. *In-vitro* permeability of the human nail and of a keratin membrane from bovine hooves: Prediction of the penetration rate of antimycotics through the nail plate and their efficacy. *Journal of Pharmacy and Pharmacology*, **1997**, 49(9), 866-872.
[4] Iravani, S., Varma, R.S. Nanosponges for drug delivery and cancer therapy: Recent advances. *Nanomaterials*, **2022**, 12(14), 2440.
[5] Ahmed, M.M., Fatima, F., Anwer, M.K., Ibnouf, E.O., Kalam, M.A., Alshamsan, A., Ansari, M.J. Formulation and *in vitro* evaluation of topical nanosponge-based gel containing butenafine for the treatment of fungal skin infection. *Saudi Pharmaceutical Journal*, **2021**, 29(5), 467-477.
[6] DasPaul, S., Mazumder, R., Bhattacharya, S., Kumar Jha, A. Optimization of polymeric nano drug delivery system using 32 full factorial design. *Current Drug Delivery*, **2013**, 10(4), 394-403.
[7] Amer, R.I., El-Osaily, G.H., Gad, S.S. Design and optimization of topical terbinafine hydrochloride nanosponges: Application of full factorial design: *In*

vitro: and: *in vivo*: evaluation. *Journal of Advanced Pharmaceutical Technology & Research*, **2020**, 11(1), 13-19.

[8] Mahalekshmi, V., Balakrishnan, N., Parthasarathy, V. Recent advancement of nanosponges in pharmaceutical formulation for drug delivery systems. *Journal of Applied Pharmaceutical Science*, **2023**, 13(8), 084-100.

[9] Phagna, M., Badhwar, R., Singh, M., Alhalmi, A., Khan, R., Noman, O.M., Alahdab, A. Development and characterization of terbinafine-loaded nanoemulgel for effective management of dermatophytosis. *Gels*, **2023**, 9(11), 894.

[10] Biswas, G.R., Patra, S., Dutta, P., Khanra, R. *Ex vivo* permeation study of terbinafine hydrochloride from nanostructured lipid carriers-based formulation. *Pharmaceutical and Biomedical Research*, **2024**, 10(3), 229-246.

[11] Jadhav, S., Dighe, P. *In vitro* evaluation, and molecular docking studies of novel pyrazoline derivatives as promising bioactive molecules. *Journal of Pharmaceutical Sciences and Computational Chemistry*, **2025**, 1(3), 190-209.

[12] Kondoros, B.A., Jójárt-Laczkovich, O., Berkesi, O., Szabó-Révész, P., Csóka, I., Ambrus, R., Aigner, Z. Development of solvent-free Co-ground method to produce terbinafine hydrochloride cyclodextrin binary systems; structural and *in vitro* characterizations. *Pharmaceutics*, **2022**, 14(4), 744.

[13] Asif, A.H., Desu, P.K., Alavala, R.R., Rao, G.S.N.K., Sreeharsha, N., Meravanige, G. development, statistical optimization and characterization of fluvastatin loaded solid lipid nanoparticles: a 3^2 factorial design approach. *Pharmaceutics*, **2022**, 14(3), 584.

[14] Nandi, S., Biswas, P. Nanosponge-an emerging nanomaterial in recent advancement of novel drug delivery: An overview and future perspectives. *Indian Journal of Pharmaceutical Sciences*, **2024**, 86(2).

[15] Anwer, M.K., Fatima, F., Ahmed, M.M., Aldawsari, M.F., Alali, A.S., Kalam, M.A., Az, A. Abemaciclib-loaded ethylcellulose based nanosponges for sustained cytotoxicity against MCF-7 and MDA-MB-231 human breast cancer cells lines. *Saudi Pharmaceutical Journal*, **2022**, 30(6), 726-734.

[16] Ankita, K., Asha, D., Baquee, A.A. Formulation and evaluation of transdermal topical gel of ibuprofen. *Journal of Drug Delivery and Therapeutics*, **2020**, 10, 20-25.

[17] Chawalke, P., Zafar, A., Binshaya, A.S., Shmrany, H.A., Hazazi, A., Abalkhail, A., Syed, S.M. Topical miconazole nanogel: *In vitro* characterization, in vivo skin irritation, and enhanced antifungal efficacy. *Drug Development Research*, **2025**, 86(4), e70106.

[18] Yewale, A.S., Sapkal, A.R., Khandare, G.S., Zanwar, M.P., Kalaskar, N.B., Shaikh, F.A., Kale, R. Development and assessment of bio-adhesive formulations containing chlorhexidine gluconate. *Journal of Pharmaceutical Sciences and Computational Chemistry*, **2025**, 1(3), 210-232.

[19] Kappes, S., Faber, T., Nelleßen, L., Yesilkaya, T., Bock, U., Lamprecht, A. Improving transungual permeation study design by increased bovine hoof membrane thickness and subsequent infection. *Pharmaceutics*, **2021**, 13(12), 2098.

[20] Moin, A., Roohi, N.F., Rizvi, S.M.D., Ashraf, S.A., Siddiqui, A.J., Patel, M., Adnan, M. Design and formulation of polymeric nanosponge tablets with enhanced solubility for combination therapy. *RSC Advances*, **2020**, 10(57), 34869-34884.

[21] Montenegro, L., Castelli, F., Sarpietro, M.G. Differential scanning calorimetry analyses of idebenone-loaded solid lipid nanoparticles interactions with a model of bio-membrane: A comparison with *in vitro* skin permeation data. *Pharmaceuticals*, **2018**, 11(4), 138.

[22] Hassan, S.U., Khalid, I., Hussain, L., Imam, M.T., Shahid, I. Topical delivery of terbinafine HCL using nanogels: A new approach to superficial fungal infection treatment. *Gels*, **2023**, 9(11), 841.

[23] Hasegawa, N., Shibuya, K. Development of an animal model of onychomycosis in guinea pigs. *Medical Mycology Journal*, **2020**, 61(4), 55-60.

[24] Sengupta, P., Das, A., Khanam, J., Biswas, A., Mathew, J., Mondal, P.K., Ghosal, K. Evaluating the potential of ethyl cellulose/eudragit-based griseofulvin loaded nanosponge matrix for topical antifungal drug delivery in a sustained release pattern. *International Journal of Biological Macromolecules*, **2024**, 276, 133953.

[25] Siafaka, P.I., Özcan Bülbül, E., Okur, M.E., Karantas, I.D., Üstündağ Okur, N. The application of nanogels as efficient drug delivery platforms for dermal/transdermal delivery. *Gels*, **2023**, 9(9), 753.

[26] Tatsumi, Y., Yokoo, M., Senda, H., Kakehi, K. Therapeutic efficacy of topically applied KP-103 against experimental tinea unguium in guinea pigs in comparison with amorolfine and terbinafine. *Antimicrobial Agents and Chemotherapy*, **2002**, 46(12), 3797-3801.

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