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Fabrication, Characterization and Evaluation of Terbinafine-Loaded Xanthan Gum Nanogels for Enhanced Drug Delivery

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ABSTRACT

This study aimed to fabricate nanogels from nanoemulsion loaded with terbinafine and explored the characteristics and potential applications of FOR2 (Drug-loaded nanoemulsion), and FOR3 (Chitosan-based nanogel prepared from the nanoemulsion). Utilizing scanning electron microscopy (SEM), this study investigated the morphologies of these formulations, revealing that FOR1 displayed a mesh-like structure, while FOR2 and FOR3 exhibited interconnected pores of varying diameters, enhancing their drug loading and release capabilities. The Spreadibility of each formulation was assessed at temperatures of 8°C, 25°C, and 40°C, with FOR1 and FOR2 demonstrating higher Spreadibility, suitable for applications requiring ease of application and rapid absorption. In contrast, FOR3 showed lower, more consistent Spreadibility, indicating its potential for localized or controlled release applications. In vitro drug release studies further differentiated the formulations: FOR2 rapidly released the drug, making it ideal for acute treatment scenarios, whereas FOR3 sustained drug release, a key advantage of nanogels over nanoemulsions, making it suitable for chronic disease management. This sustained release profile of FOR3 could potentially improve therapeutic outcomes and patient compliance by minimizing dosing frequency. Antifungal activity was also evaluated and demonstrated significant and superior antifungal efficacy of the nanogel formulation (FOR3). In conclusions, the findings highlighted the superiority of the nanogels (FOR3) which offered significant advantages for prolonged therapy, accentuating the importance of formulation choice in enhancing treatment efficacy.

Keywords: Nanoemulsion, Nanogel, Terbinafine, Antifungal, Fungal infections.

INTRODUCTION

With the development of advanced nanotechnology in recent decades, nanocarriers have emerged and gained popularity in biomedicine. Because of their ability to encapsulate drugs, nanocarriers are utilised not only as carriers of standard chemotherapeutic agents but also as platforms for theranostics, combinational therapy, and multifunctional diagnostics (Kaur et al., 2017, Kaur et al., 2019, Smoleński et al., 2021). Nanocarriers have been used as an ideal multifunctional drug delivery system (DDS) for a variety of disease therapies, including active targeting enabled by ligand modification of the nanoplatform surface, passive targeting owing to the enhanced permeability and retention (EPR) effect, and site-specific and time-controlled drug delivery strategies mediated by stimuli-responsive materials (Patil et al., 2024, Ranjbar et al., 2023). Hydrogels with a three-dimensional (3D) tunable porous structure and a particle size in the submicrometer range, ranging from 20 to

250 nm, are known as nanogels, a type of systemic drug delivery carrier. They can be distinguished from microgels, which have a particle size ranging from 1 to 350 µm, and in situ-forming hydrogels, which aid in local delivery. Nanogels help encapsulate small molecules, oligonucleotides, and even proteins. They are made of different natural polymers, synthetic polymers, or mixtures of these. Because of their special qualities, nanogels can be used for imaging, diagnostics, and medication delivery (Kothapalli et al., 2024, Szumała and Macierzanka, 2022, Donthi et al., 2022).

The main indication for the antifungal drug terbinafine is the treatment of fungal infections of the skin and nails. It is a member of the allylamine medication class, which suppresses the manufacture of ergosterol, a crucial component of fungal cell membranes, by blocking the enzyme squalene epoxidase (Krishnan-Natesan, 2009). One of the most common uses of terbinafine is in the treatment of fungal nail infections, medically known as onychomycosis. This condition often manifests as thickened, discoloured nails and can be challenging to eradicate. Terbinafine offers a potent solution by penetrating the nail bed and targeting the fungal infection directly, leading to improved nail appearance and overall resolution of the infection. In addition to its efficacy against nail infections, terbinafine is also effective in treating various skin conditions caused by dermatophyte fungi, such as athlete's foot (tinea pedis) and ringworm (tinea corporis) (Krishnan-Natesan, 2009, Newland and Abdel-Rahman, 2009). Its broad-spectrum antifungal activity makes it a versatile option for combating these common fungal infections. Terbinafine is typically available in various formulations, including oral tablets, topical creams, and solutions, allowing for flexibility in treatment depending on the severity and location of the fungal infection. However, like any medication, terbinafine may cause side effects, ranging from mild gastrointestinal disturbances to more serious liver problems, although the latter is rare. Terbinafine stands as a valuable therapeutic option in the management of fungal infections, providing patients with effective relief and the opportunity for improved skin and nail health (Newland and Abdel-Rahman, 2009).

Preparing a nanogel formulation of terbinafine offers several advantages over conventional formulations, making it a compelling choice for the treatment of fungal infections: Nanogels have a nano-sized structure, allowing them to penetrate the skin and nails more effectively than conventional formulations. This enhanced penetration can lead to better delivery of terbinafine to the site of infection, increasing its efficacy. Nanogels can be designed to target specific areas of the body, such as the nail bed or the deeper layers of the skin where fungal infections are located (Jessup et al., 2000). By encapsulating terbinafine within nanogels, it can be delivered directly to the target site, minimizing systemic exposure and reducing the risk of side effects. Nanogels can be engineered to provide sustained release of terbinafine over an extended period. This prolonged release profile ensures that therapeutic levels of the drug are maintained at the site of infection, improving treatment outcomes and reducing the frequency of dosing. Terbinafine is known to have poor aqueous solubility, which can limit its bioavailability and efficacy. By encapsulating it within nanogels, its stability can be enhanced, protecting it from degradation and improving its solubility, thereby increasing its bioavailability and therapeutic effect. Topical formulations such as nanogels are often preferred by patients due to their ease of application and reduced systemic side effects compared to oral medications. This can lead to improved patient

compliance and better treatment outcomes (Balfour and Faulds, 1992, Darkes et al., 2003, Leyden, 1998). In summary, preparing a nanogel formulation of terbinafine offers the potential for enhanced efficacy, targeted delivery, prolonged release, improved stability, and increased patient compliance, making it a promising approach for the treatment of fungal infections affecting the skin and nails. Therefore, this present study was designed to fabricate nanoemulsion based nanogels of terbinafine and evaluate the same for various physicochemical properties as well as for antifungal activities.

MATERIAL AND METHODS

Drugs and chemicals

Natural gums such as Xanthan gum were procured from Merck, India. Chitosan was purchased form Sigma Aldrich, Mumbai, India. All other chemical and reagents were purchased and arranged from reputed and validated vendors only. All the reagents and chemical were of analytical grade.

Preparation of nanoemulsion (NEF)

The previously mentioned procedure was followed to prepare the nanoemulsion (Zhou et al., 2016) with a few required adjustments. The method for preparing the nanoemulsions with different concentrations of components was meticulously executed. Initially, the specified amounts of olive oil, lecithin, and distilled water were measured according to each formulation (NEF-1 to NEF-4). Subsequently, the oil phase (A) was prepared by thoroughly mixing the olive oil with the designated quantity of lecithin until homogenized. Meanwhile, the aqueous phase (B) was created by gradually adding xanthan gum to the distilled water while stirring continuously to prevent clumping. Following this, both phases (A and B) were heated separately on a water bath set at a temperature of 44°C ± 5°C for 35 minutes to ensure optimal dispersion and solubilization of the components. Once heated, the oil phase (A) was slowly added to the aqueous phase (B) while stirring vigorously using a magnetic stirrer or homogenizer at a speed of 4000 rpm for 8 minutes to facilitate emulsification. The mixture was then cooled gradually while stirring at a reduced speed of 1500 rpm for 15 minutes to promote uniform mixing. For formulations containing drug (NEF-2 to NEF-4), the specified amount of drug was incorporated into the oil phase (A) prior to emulsification to ensure even distribution. Finally, the emulsion underwent high-speed/high-shear homogenization at 15000 rpm for 15 minutes to further enhance stability and uniformity. The prepared nanoemulsions were then transferred to suitable containers and stored under specified conditions for stability testing and subsequent use in pharmaceutical applications. The prepared formulas are listed in Table 1 below.

Table 1. Formulation composition table of the nanoemulsions with different concentrations of components

Code for the formulations	**Olive Oil (w/w)**	**Xanthan Gum (w/w)**	**Lecithin (w/w)**	**Drug (w/w)**	**Distilled water Q. S to make 100 g**
NEF-1 (Blank)	18 g	23 g	18 g	-	59 g
NEF-2	23 g	18 g	17 g	250 mg	41.75 g
NEF-3	20 g	21 g	15 g	250 mg	43.75 g

NEF-4	23 g	18 g	13 g	250 mg	45.75 g

Evaluation of nanoemulsions and chitosan gel preparation

Before being incorporated into the gel matrix, multiple nanoemulsion compositions underwent physical examination. The colour changes, consistency, and phase separation of the various formulations were meticulously observed. Samples from each formulation were subjected to storage conditions at 8°C, 25°C, 40°C, and 40°C with 75% relative humidity (RH) for a duration of 28 days. Chitosan was dissolved in distilled water to form the chitosan gel. A precise weight of 2.5 grams of chitosan was accurately measured and then dissolved in 100 millilitres of distilled water containing 1.5% acetic acid, following the method described by Zhou et al., 2016 (Zhou et al., 2016). A high-speed mixer operating at 3500 rpm for a duration of 12 minutes was utilized to prepare the gel. Subsequently, the resulting gel was stored overnight before being incorporated into the emulsion.

Fabrication of the nanoemulsion gel

The fabrication of the nanoemulsion gel began with the preparation of the chitosan gel. Chitosan, accurately weighed to 2.5 grams, was dissolved in 100 millilitres of distilled water containing 1.5% acetic acid using a high-speed mixer operating at 3500 rpm for 12 minutes. This gel was then stored overnight for further use. Meanwhile, several nanoemulsion compositions were physically examined to assess colour changes, consistency, and phase separation. Following the assessment, the chitosan gel was incorporated into the nanoemulsion using a suitable mixing technique for 30 min to ensure homogeneity. The resulting nanoemulsion gel was then subjected to further characterization and stability testing to evaluate its suitability for pharmaceutical applications. Triethanolamine was used to lower the pH (TEA) of the formulations.

Characterizations

Thermodynamic stability and Heat cooling cycle

The optimized formulations underwent thermodynamic stability testing for a period of 28 days, adhering to the guidelines outlined by the International Conference on Harmonisation (ICH). This assessment aimed to evaluate the formulations' resilience under challenging conditions. Following a protocol inspired by a previous study (Burki et al., 2020), with slight modifications, both the manufactured nanoemulsion (NEF) and the chitosan-based nanoemulsion gel (NEFG) formulations were subjected to storage in an incubator set at 40°C for the initial 28 days. Subsequently, both formulations were allowed to return to room temperature. The objective of this test was to observe any physical changes such as turbidity, creaming, or cracking, which could indicate instability or potential issues with the formulations' long-term storage suitability.

Freeze thaw cycle and Centrifugation

For a duration of 28 days, both the nanoemulsion (NEF) and nanoemulsion gel (NEFG) formulations underwent the freeze-thaw cycle test. This involved placing the formulations in a deep freezer at a temperature ranging between 2-4°C. After the freezing period, the formulations were removed from the freezer and allowed to thaw at room temperature. Subsequent to undergoing this rigorous treatment, the formulations were visually inspected to ascertain whether they reverted to their original state. To further assess the formulations' stability, a high-speed centrifuge (Remi, India) was employed. Each sample formulation was transferred to separate Eppendorf tubes and centrifuged at varying speeds of 6000 and

12000 rpm for a duration of 12 minutes. The centrifugation process enabled the examination of the formulations' propensity to separate into distinct oily and aqueous phases, providing insights into their overall stability and emulsion integrity.

The pH, Droplet size, surface charge and PDI of NEF and NEFG

Following the pH analysis, all freshly prepared NEF and NEFG formulations underwent evaluation at specific time intervals: 12 hours, 24 hours, 7 days, and 14 days post-preparation. This comprehensive assessment aimed to monitor any potential changes in pH over time, providing valuable insights into the formulations' stability and suitability. This particular monitoring allowed for early detection of potential stability issues and facilitated adjustments to formulation parameters if necessary, ensuring the maintenance of the desired pH levels throughout the intended shelf-life of the formulations (Burki et al., 2020). The purpose of this test was to determine the polydispersity index, surface charge, and droplet size of the nanoemulsion formulation. To achieve this objective, a Helium-Neon laser and a zeta sizer (Nano ZS 90, Malvern Instruments, UK) were employed, following the previously outlined methodology (Ali et al., 2020). Specifically, 1 ml of nanoemulsion was combined with 9 ml of deionized water, and the resulting mixture was thoroughly stirred for three minutes. This process was repeated three times to ensure consistency, and the obtained results were subsequently averaged to provide a representative measurement (Ali et al., 2020).

Analysis of the drug content

With slight modifications, the analysis of drug content was conducted following the previously described methodology (Burki et al., 2020). To accomplish this, one gram (10 µg/g) of the nanoemulsion gel was accurately weighed and mixed with nine millilitres (w/v) of ethanol. Subsequently, the resulting mixture underwent centrifugation using a Remi centrifuge (India) at 6000 rpm for a duration of two to three minutes. Following centrifugation, the mixture was filtered through a 0.45 micrometer-pore-size nylon filter membrane. Three analyses of the filtrate sample were conducted using a UV-spectrophotometer (Shimadzu, Japan), and the average results were calculated to ensure accuracy and reproducibility.

Viscosity of NEFG and Morphological studies

The viscosities of the NEF (FOR2) and NEFG (FOR3) formulations were evaluated using a viscometer (NDJ, RRS, India) on days 0, 1, 2, 7, 14, and 28. Measurements were conducted at temperatures of 8°C, 25°C, and 40°C to assess the effect of temperature on viscosity over time (Alexander et al., 2013). The primary function of spindle number four is to measure the viscosity of semisolid dosage forms, particularly nanoemulsions (El-Refaie et al., 2015). Beakers were used to hold the 50 g formulations of NEF and NEFG. The viscometer's spindle was carefully positioned in the center of the beakers, ensuring it did not touch the bottom. Subsequently, the spindle's rotation speed was set to 7 rpm, and measurements for both formulations were recorded over a duration of six minutes. For the examination of the morphology and apparent shape of the chitosan-based nanoemulsion gel (NEFG), scanning electron microscopy (SEM) was employed. Metal stubs were coated with NEFG (blank) and drug-loaded samples using double-sided adhesive tape. These formulation-loaded stubs were then placed in a vacuum chamber for drying. Subsequently, the samples were coated with a layer of gold approximately 8–10 nm thick using a sputter

coater for a duration of five minutes prior to inspection. SEM analysis of the stubs was performed at a magnification of 12,000X and an accelerating voltage of 12 KV. The designated areas of the samples were photographed using the aforementioned techniques to capture their microstructural characteristics accurately (El-Refaie et al., 2015, Burki et al., 2020).

FTIR spectroscopy study and Spreadibility studies

FTIR spectroscopy, conducted with a Perkin Elmer instrument, was employed to analyse the drug, chitosan, nanoemulsion, and nanoemulsion gel. The study aimed to determine the degree of compatibility between the polymer and the constituent parts of the formulation, as well as to confirm the presence of functional groups and their corresponding wave numbers. Samples of each mixture and component were applied to the diamond crystal and crushed using the instrument's knob. Spectra for each sample were captured in triplicate within the wave number range of 400–4000 cm^{-1}. Additionally, the Spreadibility of the nanoemulsion gel (NEFG) was assessed using the "Drag & Slip" device, following a previously reported method with slight adjustments (Ali et al., 2020). The device used for assessing Spreadibility consists of a wooden block with a pulley attached to one end. It comprises two identically sized glass slides, one of which is movable while the other is affixed to the block. To evaluate the Spreadibility of a sample, it is placed between the stationary slide and the top mobile slide, and a measured weight is applied. In our evaluation, a fixed (stationary) slide containing 2.0 g of nanoemulsion gel (NEFG) was positioned between the upper slide to test the optimized nanoemulsion gel's Spreadibility. A weight of 50 g was placed on the upper glass slide, and the time taken for the upper slide to travel 8 cm was recorded. The Spreadibility of the test NEFG was calculated using the following formulae.

$S = M \times L \div T$

S stands for Spreadibility in this instance.

The weight on the upper glass slide is denoted by "M"

The glass slides' length is shown by "L" and their travel duration is indicated by "T"

***In vitro* drug release**

The in vitro drug release investigation was conducted following the well-defined methodology outlined in a previous study (Khan et al., 2021). To conduct the experiment, a Franz diffusion cell (IPS Technologies, India) equipped with 6 ml and 3 ml capacities for the donor and receptor compartments, respectively, was employed. The temperature was maintained at 37°C ± 1°C, and the stirring speed was set at 300 rpm before adding the nanoemulsion (NEF) and nanoemulsion gel (NEFG) samples. Given the utilization of artificial membranes for in vitro release studies, a tuffryn membrane (Sortorius, Germany) was securely clamped between the donor and receptor compartments. Each formulation sample (two grams) was carefully placed into the receptor compartments of both cells, and the receptor compartments were subsequently filled with pH 5.5 sodium acetate buffers. Sample collection was conducted at predetermined intervals (0 h, 1 h, 2 h, 4 h, 8 h, and 12 h) using a spinal syringe to withdraw 2 ml from the receptor compartment. Fresh buffer was introduced to maintain the sink state and sodium acetate buffer level in the receptor compartment. Following sample collection, UV-spectrophotometer analysis (at 360 nm) was performed to assess the drug's release behaviour.

Drug release kinetics

Subsequently, the drug release data was fitted into several mathematical models to elucidate the drug's release behavior (Burki et al., 2020).

Antifungal Susceptibility Testing

Biological screening was conducted to determine the formulations' potential as antifungal against fungal strains (Motedayen et al., 2018).

Inoculum Preparation

Mature colonies cultivated on Potato Dextrose Agar (PDA) were subjected to a sterile saline solution (0.85%), supplemented with one drop of tween 20, covering the surface of the colonies. Subsequently, the colonies' surfaces were gently scraped using a sterile swab. The resulting mixture, containing conidia and hyphal fragments, was transferred into a sterile tube and left undisturbed for 5 to 10 minutes at room temperature to allow heavy particles to settle. Following sedimentation, the upper suspension containing predominantly conidia was carefully collected. Conidia concentrations were determined using a hemacytometer, and adjustments were made using RPMI 1640 medium. The RPMI 1640 medium, prepared with glutamine and buffer at pH 7.0 without sodium bicarbonate, was standardized to obtain an inoculum ranging from 1×10^3 to 3×10^3 colony-forming units per millilitre (CFU/mL). This was achieved by dissolving 10.43 grams of RPMI powder and 34.53 grams of MOPS buffer (N-Morpholino Propanesulfonic Acid) in 1 Liter of distilled water with gentle agitation (Motedayen et al., 2018).

Minimum Inhibitory Concentration Test Procedure

The antifungal susceptibility testing followed the standard procedure outlined in the Broth Microdilution CLSI M38 method. Initially, 96-flat-bottomed well microplates (Orange Scientific, E.U) were employed for the assay. Each well was loaded with 100 µL of the respective drug concentration, followed by the addition of 100 µL of the inoculum suspension to each drug-containing well. Growth control and sterilized control wells were also included for each isolate to ensure the validity of the results. Additionally, blank nano-liposomes were incorporated to confirm the absence of any inherent antifungal activity. The microplates were then incubated at 28°C for a period of 4 days. Upon completion of the incubation period, the results were visually assessed, and the minimum inhibitory concentrations (MICs) were recorded. The MIC was defined as the lowest concentration of the drug that inhibited 100% of the fungal growth compared to the growth observed in the control wells. All tests were conducted in duplicate to ensure the reproducibility and accuracy of the results (Kataki, 2010, Kataki et al., 2010, Mukherjee et al., 1995, Motedayen et al., 2018).

Statistical analysis

Using GraphPad Prism, a One Way ANOVA and a student’s T-test were conducted to the data. The data was displayed as mean ± SD after all the variables were averaged three times.

RESULTS & DISCUSSION

Characterizations of formulations (NEF and NEFG)

Physical appraisal and thermodynamic stability

For a duration of 28 days, three formulations were maintained at various temperatures (8 ºC, 25 ºC, 40 ºC, and 40 ºC + 75 relative humidity (RH)). The formulations included blank formulations, chitosan-based nano-emulsion gel, and eucalyptus oil nanoemulsion.

Physical assessments were conducted on these compositions periodically to evaluate their phase separation, consistency, liquefaction, colour change, and cracking. The freshly prepared formulations had a yellowish colour and a smooth, elegant appearance. After centrifugation at 6000 and 12000 rpm, no phase separation was observed. When the initial formulations were evaluated, their pH was found to be 5. 5. The pH of human skin was measured as a reference (Proksch, 2018). Using the student t test, the pH of the formulations was assessed at different intervals after 12 hours, 24 hours, 7 days, 14 days, 1 month, 2 months, and 3 months. Each formulation's pH did not differ noticeably, or $p > 0.05$. In order to avoid skin irritations, the pH of drugs applied topically must be between 5 and 6 (Proksch, 2018, Wagner et al., 2003). Over time, the pH value significantly dropped, which could be due to water diffusing out of its phase or the oil in the formulations creating acidic chemicals (Wagner et al., 2003, Schmid-Wendtner and Korting, 2006). This pH fluctuation was, nevertheless, within the typical range for human skin. According to the characteristics listed above, each formulation was thermodynamically stable.

Droplet size, polydispersity (PDI) and surface charge

The table provided details on the prepared formulations—FOR1 (Blank), FOR2 (Nanoemulsion), and FOR3 (Nanogel) and highlighted important characteristics about each of the formulations, notably in terms of droplet size, zeta potential, and the polydispersity index (PDI).

Size of Droplets: FOR1 as blank exhibits the smallest droplet size of approximately 54.46 nm. This smaller size might be indicative of a simple formulation, possibly containing fewer components that could affect the stability or the aggregation of droplets. FOR2 as nanoemulsion has a slightly larger droplet size (69.67 nm) compared to the blank. This increase might be due to the incorporation of additional components such as oils and surfactants which are typical in nanoemulsions and can influence droplet size by stabilizing larger droplets. FOR3 as nanogel shows the largest droplet size (82.38 nm), which could be attributed to the gel matrix's ability to swell and incorporate more fluid, thus increasing the size of the droplets within the gel structure.

Zeta Potential: FOR1 - Blank and FOR2 – Nanoemulsion have negative zeta potentials of -20.5 mV and -17.5 mV, respectively. Negative values generally indicate stability against aggregation due to electrostatic repulsion between droplets. The difference in values might be due to differences in the surface chemistry influenced by the formulation ingredients. FOR3 – Nanogel, however, exhibits a positive zeta potential of 25.2 mV. This shift to a positive value could be indicative of a different stabilizing agent or a fundamental difference in the composition of the nanogel compared to the other formulations. A positive zeta potential can also confer stability, as similarly charged particles repel each other, preventing aggregation.

PDI Ratio: The PDI values reflect the uniformity in the size distribution of the droplets within each formulation. Lower PDI values (as seen in FOR1 and FOR2) suggest more uniform droplet sizes, which is often desirable for stability and consistency in delivery systems. FOR3's higher PDI value (0.366) suggests a broader size distribution. This could be due to the more complex nature of the nanogel system, where the gel matrix might allow for a more heterogeneous distribution of droplet sizes.

In summary, these characteristics suggest that each formulation offers unique properties suitable for different applications. The smaller, more uniformly sized droplets in the blank and nanoemulsion could be advantageous for applications requiring rapid and efficient delivery, while the larger, positively charged droplets in the nanogel might be better suited for applications where slower release and longer retention are beneficial. The difference in zeta potential among the formulations also hinted at the potential for varied biological interactions, as charge can influence cellular uptake and bio-distribution (Rai et al., 2018, Zhang, 2019, Chakraborty et al., 2020).

Table 2. Zeta potential, PDI, and droplet size of the formulations

Prepared Formulations	**Size of droplets (nm)**	**Zeta potential (Mv)**	**PDI Ratio**
FOR1 - Blank	54.46±1.01	-20.5	0.135
FOR2 – Nanoemulsion (NEF)	69.67±1.11	-17.5	0.124
FOR3 – Nanogel (NEFG)	82.38±1.41	25.2	0.366

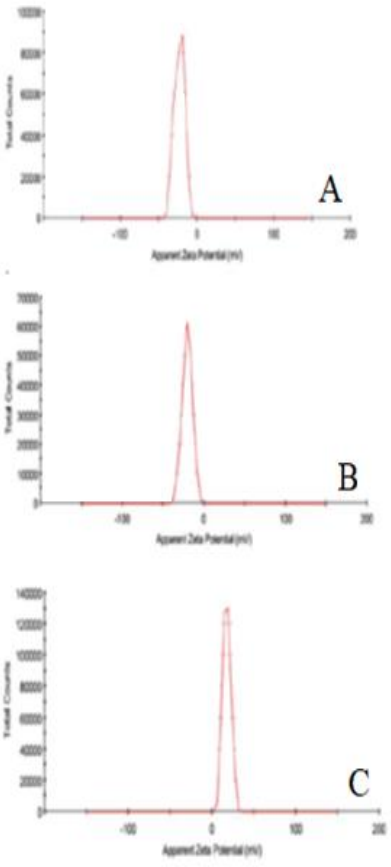

Figure 1. Zeta potential of the three formulations: blank formulation (A), drug-loaded nanoemulsion (B) and Nanogel

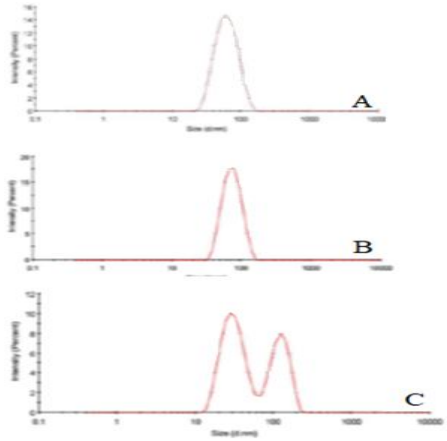

Figure 2. The formulations' particle sizes (A. blank, B. drug-loaded nanoemulsion, and C. nanogel formulation)

FTIR study

The Fourier Transform Infrared (FTIR) spectra of the different samples analysed - including the drug, nanoemulsion containing olive oil, chitosan, and the nanogel formulation -demonstrate no evidence of drug-excipient interaction. The characteristic peaks of each component remain distinct and unaltered across the spectra, indicating that their chemical structures are maintained without any significant modifications or interactions. This suggested that the drug maintains its integrity within the formulation, and the excipients, including the olive oil and lecithin in the nanoemulsion and chitosan in the nanogel, do not chemically interact with the drug. This stability is crucial for ensuring the efficacy and safety of the drug in its intended applications (Pant et al., 2014, Cardenas and Miranda, 2004).

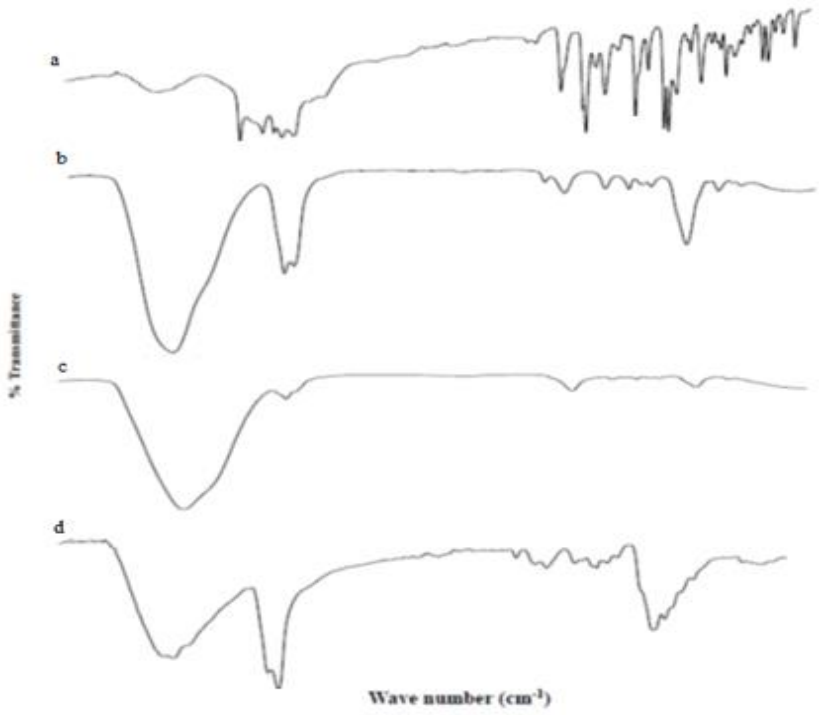

Figure 3. FTIR spectra of a. Drug, b. Nanoemulsion containing olive oil, c. Chitosan and d. Nanogel

Drug content

FOR2 exhibited a drug content of 92.75±1.36%, indicating that the formulation successfully incorporated a high percentage of the drug. This suggests efficient drug loading and stability within the formulation. FOR3 showed a slightly lower drug content of 89.92±1.77% compared to FOR2. While still relatively high, this slight decrease may indicate some variability in drug loading or formulation characteristics. However, it remains within an acceptable range for pharmaceutical formulations. Both formulations (FOR2 and FOR3) demonstrated efficient drug incorporation, with FOR2 having a slightly higher drug content. This suggests that the nanoemulsion and nanoemulsion gel formulations are effective at delivering the desired drug concentration, which is essential for ensuring therapeutic efficacy and consistency in pharmaceutical products (Table 3).

Table 3. Exhibiting the percentage of drug in the formulations for nanoemulsion and nanogel.

Formulation code	Drug Needed (µg)	Drug Found (µg)	% Drug content
FOR1	-	-	-
FOR2	250	92.75±1.36	92.75±1.36
FOR3	250	89.92±1.77	89.92±1.77

Viscosity of formulations

The viscosity measurements for formulations FOR2 (Nanoemulsion) and FOR3 (Nanogel) over various days and at different temperatures (8°C, 25°C, and 40°C) provided insights into their physical stability and potential applications. The viscosity of FOR2 remains relatively stable at 8°C and 25°C throughout the 28 days. However, there's a noticeable decrease in viscosity at 40°C starting from Day 1, which continues to decrease over the 28 days. This decrease at higher temperatures suggests that the nanoemulsion might be susceptible to temperature-induced changes, potentially due to the breakdown of the emulsion structure or the evaporation of volatile components. At lower temperatures (8°C and 25°C), the nanoemulsion exhibits excellent stability with only minor fluctuations in viscosity. This indicates good resistance to phase separation or degradation over the observed period.

The viscosity for FOR3 shows less consistency across temperatures. At 8°C, the viscosity slightly increases over time, suggesting potential thickening or increased cross-linking within the gel. At 25°C and 40°C, the viscosity fluctuates more noticeably, particularly showing a decrease on Day 7 at 40°C before stabilizing. These variations might indicate sensitivity to environmental conditions, which could affect the nanogel's structure and performance. Unlike FOR2, FOR3 exhibits more variation in viscosity, especially at higher temperatures. This could be due to the hydrogel network responding to thermal stress, which might affect its stability and applicability in environments with fluctuating temperatures. FOR2 appeared more suitable for conditions where temperature does not exceed 25°C, maintaining its stability better at these conditions. FOR3, while fluctuating more in viscosity, may require specific storage conditions or usage scenarios where temperature control is feasible, especially to maintain its structural integrity. The stability of FOR2 at lower temperatures suggests its suitability for storage and use in cooler

environments, potentially beneficial for transport and storage. FOR3's behaviour indicated a possible advantage in applications where slight changes in viscosity can be tolerated or where its response to temperature can be used to advantageously modulate the release of active ingredients. The observed data could guide further optimization of these formulations. For FOR2, enhancing thermal stability at higher temperatures could be beneficial, while for FOR3, reducing variability in response to temperature could enhance its applicability (Khan et al., 2021, Alexander et al., 2013, Burki et al., 2020, El-Refaie et al., 2015).

Figure 4. The viscosities of the formulations (FOR2 and FOR3) were shown as centipoise at different times and temperatures.

Morphological studies by SEM

Scanning electron microscopy (SEM) was employed to examine the structures of the drug-loaded nanoemulsion (FOR2), the chitosan-based nanoemulsion gel (FOR3), and the blank nanoemulsion (FOR1), as depicted in Figure 4. The SEM images reveal that the blank nanoemulsion (FOR1) exhibits a mesh-like structure. In contrast, the SEM pictures of both the drug-loaded nanoemulsion (FOR2) and the nanoemulsion gel (FOR3) show interconnected pores with randomly varied diameters. This porous structure is advantageous as it provides ample space for drug incorporation, enhancing both drug movement within the matrix and the rate at which the drug is released from the formulations.

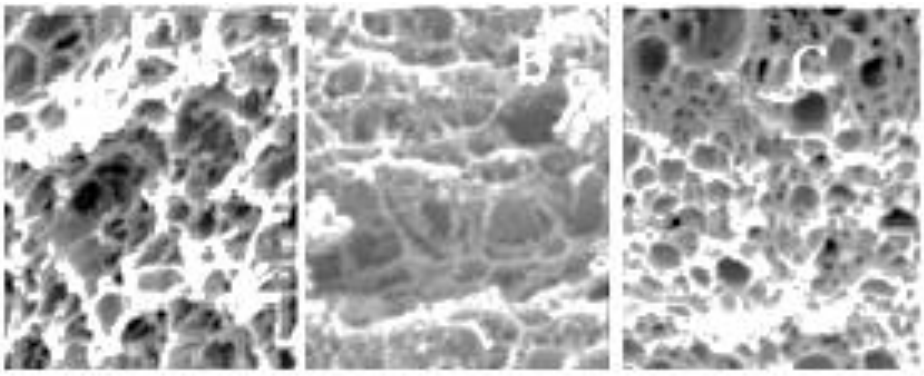

Figure 5. SEM photomicrographs of the formulations A. Blank formulation (FOR1), B. Nanoemulsion formulation (FOR2) and C. Nanogel formulation (FOR3)

Spreadibility

The Spreadibility data for the formulations FOR1 (Blank nanoemulsion), FOR2 (Drug-loaded nanoemulsion), and FOR3 (Chitosan-based nanoemulsion gel) were analysed across various temperatures (8°C, 25°C, and 40°C), revealing key insights into their performance characteristics and suitability for potential applications. In terms of temperature influence, both FOR1 and FOR2 demonstrated an increase in Spreadibility as the temperature rose from 8°C to 40°C. This behaviour is typical as higher temperatures generally reduce the viscosity of formulations, thereby enhancing their ease of spread. However, FOR3 also exhibited an increase in Spreadibility with rising temperatures but maintained significantly lower values at all temperatures compared to FOR1 and FOR2. This suggests that FOR3, being a gel-based formulation, inherently possesses a higher resistance to flow due to its thicker or more viscous composition. Comparatively, FOR1 and FOR2 showed very similar Spreadibility metrics at all studied temperatures, significantly higher than those of FOR3. This similarity indicates that the drug loading in FOR2 did not significantly alter its physical properties affecting Spreadibility compared to the blank nanoemulsion (FOR1). The lower Spreadibility of FOR3 is attributed to its gel matrix, which likely provides a more structured and less fluid consistency favourable for applications requiring slower release or more localized application where reduced spread is beneficial. The implications for application are clear. The higher Spreadibility of FOR1 and FOR2 at elevated temperatures suggests that these formulations are well-suited for applications where easy

application and quick absorption are required, such as topical treatments in warmer environments. Conversely, FOR3, with its lower Spreadibility, might be better suited for applications where a thicker, more controlled release formulation is beneficial, such as in transdermal patches or localized therapy. The stability in Spreadibility of FOR2, despite the drug loading, suggests that effective formulation techniques were employed to incorporate the drug without adversely affecting the physical properties, ensuring consistent performance in drug delivery applications. The consistent increase in Spreadibility with temperature across all formulations indicates predictable behaviour, advantageous for managing performance under varying environmental conditions (Burki et al., 2020, Khan et al., 2021).

Table 4. For the formulations (FOR1, FOR2, and FOR3) at the different temperatures under study, the Spreadibility presented as mean ± SD.

	Spreadibility		
Formulation codes	**8 ^{0}C**	**25 ^{0}C**	**40 ^{0}C**
FOR1	17.68±1.23	21.65±1.11	27.86±1.03
FOR2	17.56±1.43	21.75±1.05	27.98±1.21
FOR3	13.56±1.22	15.86±1.05	17.58±1.09

***In vitro* drug release**

The in vitro drug release data for the formulations FOR2 (Drug-loaded nanoemulsion) and FOR3 (Chitosan-based nanoemulsion gel) provided insights into their release kinetics and potential efficacy as drug delivery systems. The data was expressed as the percentage of drug released over time, revealing distinct behaviours for each formulation. Initially, FOR2 exhibited a rapid drug release, with approximately 54.57% of the drug released within the first hour. This quick release profile suggested that FOR2 facilitated faster dispersion and dissolution of the drug, likely due to its nanoemulsion structure. In contrast, FOR3 showed a somewhat slower initial release at 50.58%, which could be attributed to its gel matrix that may restrict immediate drug release. As time progressed, FOR2 continued to release the drug steadily, achieving about 89.07% release by the 12th hour. This indicated a gradual decrease in the release rate following the initial burst. FOR3, on the other hand, displayed a more controlled release pattern, reaching 79.39% by the 12th hour. The more gradual release rate of FOR3 was likely influenced by the gel matrix's role in modulating the drug release. The faster release rate of FOR2 was potentially ideal for applications requiring rapid onset of action, such as acute pain management or conditions where quick drug absorption is crucial. FOR3, with its slower and more controlled release, seemed better suited for applications where prolonged drug release is beneficial, such as in chronic therapy management. This could reduce dosing frequency and potentially improve patient compliance. Overall, the distinct drug release profiles of FOR2 and FOR3 highlighted their unique capabilities and potential applications in different therapeutic contexts. FOR2 was suited for acute treatment scenarios requiring fast drug absorption, while FOR3 was aligned

with long-term treatment strategies that benefit from a consistent, controlled release (Burki et al., 2020, Khan et al., 2021).

Table 5. In Vitro drug release study presented the % drug release data for the formulations (FOR2 and FOR3).

	Formulations	
Time (Hr)	**FOR2**	**FOR3**
0	0	0
1	54.57±2.03	50.58±1.07
2	64.81±2.01	57.49±1.18
4	73.81±2.09	68.46±1.49
6	79.39±2.11	73.72±1.47
8	82.13±2.01	76.19±1.26
12	89.07±2.09	79.39±1.19

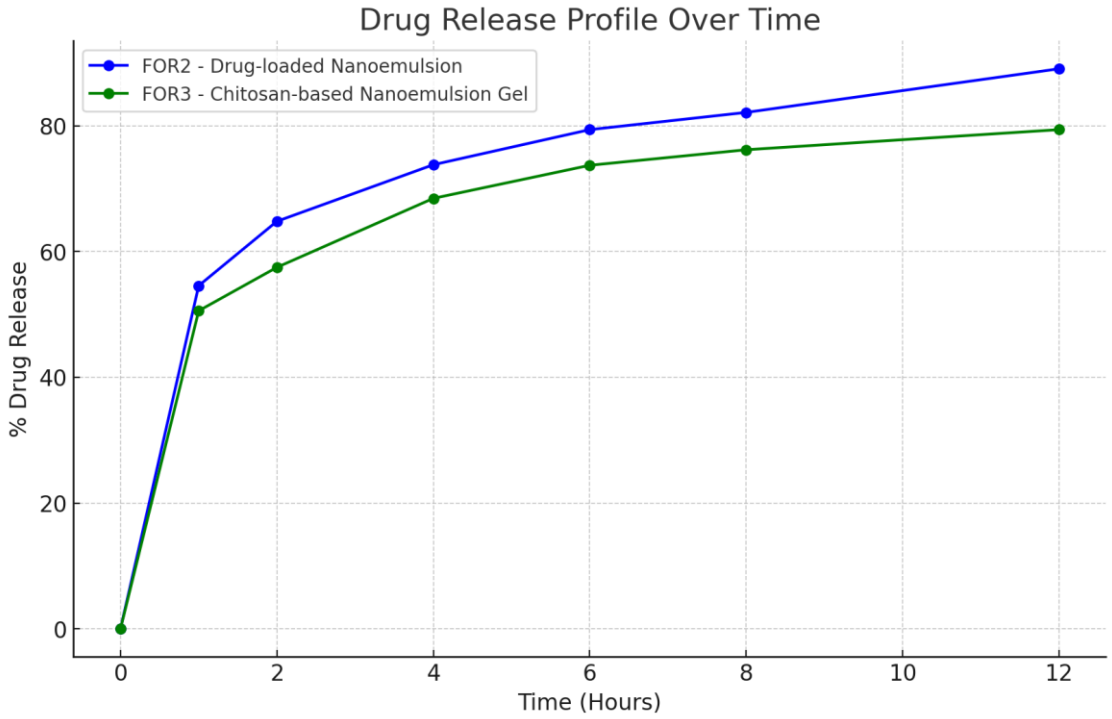

Figure 6. Depicting the percentage drug release for the formulations (FOR2 and FOR3).

Antifungal Susceptibility Testing

The MIC (Minimum Inhibitory Concentration) values for Terbinafine and Nanoemulsion Gel (NEFG) against *T. rubrum* and *M. canis* fungal strains were determined. For *T. rubrum*, the MIC range of Terbinafine was found to be 0.0614 - 1 µg/mL, with MIC50 and MIC90 values of 0.49 µg/mL and 1 µg/mL, respectively. On the other hand, NEFG exhibited a lower MIC range of 0.0145 - 0.26 µg/mL, with MIC50 and MIC90 values of 0.0514 µg/mL and 0.114 µg/mL, respectively. Similarly, for *M. canis*, Terbinafine displayed a MIC range of 0.0302 - 0.56 µg/mL, with MIC50 and MIC90 values of 0.114 µg/mL and 0.49 µg/mL, respectively. Contrastingly, NEFG showed a narrower MIC range of 0.0067 - 0.114 µg/mL, with MIC50 and MIC90 values of 0.0202 µg/mL and 0.115 µg/mL, respectively. These results indicate that the Nanoemulsion Gel (NEFG) formulation possesses stronger

antifungal activity compared to Terbinafine alone against both *T. rubrum* and *M. canis* strains. The lower MIC values for NEFG suggest that it requires lower concentrations to inhibit fungal growth effectively. This enhanced efficacy could be attributed to the synergistic effect of the nanoemulsion formulation, which may facilitate better penetration of the antifungal agent into the fungal cells or provide sustained release of the drug, leading to prolonged antifungal activity. Further studies are warranted to elucidate the mechanisms underlying the improved antifungal efficacy of NEFG and to assess its potential for clinical applications in treating fungal infections.

Table 6. Antifungal activity of the nanoemulsion and nanogel formulations compared to Terbinafine alone against both *T. rubrum* and *M. canis* strains

Species/Antifungal Agent	**MIC Range (µg/mL)**	**MIC50 (µg/mL)**	**MIC90 (µg/mL)**
T. rubrum (n = 60)			
Terbinafine	0.0614 - 1	0.49	1
NEFG	0.0145 - 0.26	0.0514	0.114
M. canis (n = 60)			
Terbinafine	0.0302 - 0.56	0.114	0.49
NEFG	0.0067 - 0.114	0.0202	0.115

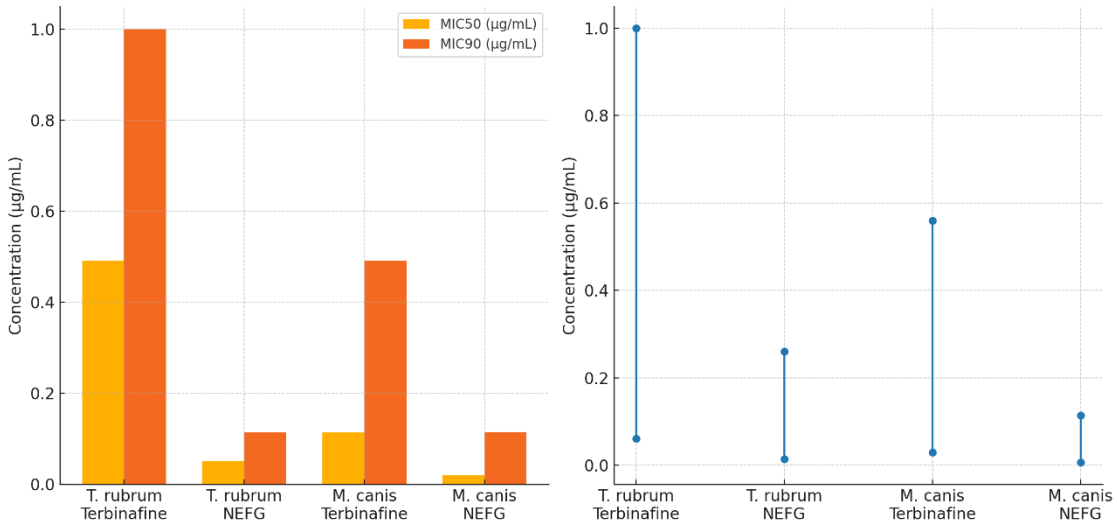

Figure 7. Antifungal activity of the nanoemulsion and nanogel formulations compared to Terbinafine alone against both *T. rubrum* and *M. canis* strains

CONCLUSION

The present study successfully fabricated and evaluated nanoemulsion (FOR2) and nanogel (FOR3) formulations of terbinafine hydrochloride. The comprehensive study of the formulations FOR1, FOR2, and FOR3, encompassing their morphological, release, and physical properties, offered valuable insights into their potential applications and benefits. Scanning electron microscopy (SEM) analysis showed that FOR1, a blank nanoemulsion, exhibited a mesh-like structure, while FOR2 and FOR3, which are drug-loaded formulations, demonstrated interconnected pores with varying diameters. These structural characteristics are crucial for enhanced drug loading and release capabilities. Spreadibility tests indicated that FOR1 and FOR2 maintained higher Spreadibility across various

temperatures, suggesting their suitability for applications requiring easy application and rapid absorption. In contrast, FOR3, a chitosan-based nanogel, showed lower and more stable Spreadibility suitable for localized or controlled release applications. Drug release studies revealed that FOR2 had a quicker release profile than FOR3, which is aligned with scenarios needing immediate drug action, such as acute treatments. However, FOR3, designed as a nanogel, excelled in sustaining the release of the drug, an inherent advantage of nanogels over nanoemulsions. This controlled release profile made FOR3 particularly beneficial for chronic disease management, where sustained delivery can enhance therapeutic outcomes and patient adherence by reducing the frequency of dosing. The nanogel (FOR3) formulation also demonstrated superior antifungal activity. In conclusions, FOR3 as the nanogel formulation offered significant benefits for prolonged therapy, emphasizing the strategic use of nanogel technology to optimize treatment efficacy and patient compliance.

DECLARATION OF INTEREST

None

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Nil

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