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Formulation and development of griseofulvin-loaded glycerosomal nanogel for the management of onychomycosis

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The study focused on the development and evaluation of Griseofulvin-loaded glycerosomal nanogels for the treatment of Onychomycosis using the lipid thin-film hydration technique. This method was employed to prepare several formulation batches (F1-F6) with the aim of optimizing the drug delivery system by varying the concentration of glycerol. Among these formulations, some batches were excluded based on preliminary evaluation parameters, including particle size and entrapment efficiency. The nanogels were formulated using lecithin as a phospholipid to enhance drug encapsulation efficiency. Their physicochemical characteristics, such as particle size and morphology, were evaluated using transmission electron microscopy (TEM). The optimized formulations were further analyzed for particle size distribution and *in-vitro* drug release in phosphate buffer solution (pH 7.4). In addition, stability studies were carried out under different storage conditions to evaluate the effect of temperature on formulation integrity and stability. The primary objective of this research was to improve drug penetration through the nail bed and reduce the frequency of drug administration, thereby enhancing patient compliance. The findings suggest that the lecithin-based lipid thin-film hydration technique is a promising strategy for the effective delivery of Griseofulvin in the management of Onychomycosis.

Keywords: Onychomycosis, Nanogels, Griseofulvin, Encapsulation, Glycerosomes

INTRODUCTION

Fungal infections are among the most common skin disorders caused by the exposure of fingernails and toenails to contaminated environments. Fungi are naturally present on human skin, indoor surfaces, outdoor soil, and are also commonly found in diabetic patients[1-3]. These infections are typically characterized by red rashes, irritation, and severe itching that may recur frequently[4,5]. Various dosage forms, including liquid, semi-solid, and solid preparations, are prescribed for treatment; however, most conventional therapies require long-term oral administration for approximately 3-9 months, which may increase the risk of adverse effects. Therefore, topical drug delivery is considered a more suitable approach for reducing treatment duration and minimizing systemic side effects, and it remains one of the most preferred methods for the treatment of fungal infections[6]. Ringworm, also known as tinea, is a fungal infection caused by dermatophytes rather than heat and commonly affects the trunk and limbs, while infections occurring on other body parts are referred to as athlete's foot or jock itch. It is characterized by a ring-shaped rash with slightly raised borders and comparatively clear skin in the center, often accompanied by itching and gradual spreading of lesions. Ringworm is highly contagious but can usually be treated effectively with topical antifungal agents. Another common fungal infection is cutaneous candidiasis, which is caused by the overgrowth of Candida species[7-13].

Although Candida normally exists on and inside the human body, excessive growth under warm, moist, and poorly

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ventilated conditions can lead to infection, particularly in areas such as beneath the breasts, diaper-covered regions, and folds of the buttocks, resulting in redness and irritation. Onychomycosis is a fungal infection of the nails that primarily affects toenails, although fingernails may also be involved[14-16]. The increasing prevalence of fungal infections has encouraged the development of safer and more effective treatment approaches. Recent advances in nanotechnology have provided promising opportunities for improving antifungal therapy through enhanced drug delivery systems. Nanotechnology-based formulations offer several advantages over conventional dosage forms, including reduced toxicity, improved biodistribution, enhanced drug permeation, and better patient compliance[17-18]. Consequently, there is growing interest in the development of topical nano-based drug delivery systems[19,20]. Among these systems, nanogels have attracted considerable attention because of their excellent drug transport properties, biodegradability, and enhanced permeation capability. Pharmaceutical industries are increasingly focusing on novel drug delivery approaches to improve therapeutic efficacy and patient convenience. Griseofulvin, a well-known antifungal drug, is widely used in the treatment of fungal infections affecting the scalp, skin, fingernails, toenails, feet, groin, and thighs. Therefore, griseofulvin was selected as the therapeutic agent for the present investigation involving the development of a topical nanogel formulation. The main objective of this research was to formulate and evaluate an antifungal nanogel with improved *in vitro* drug release and reduced dosing frequency. Griseofulvin may be used alone or in combination with other topical antifungal agents, and its antifungal activity is mainly attributed to the inhibition of fungal cell mitosis and nucleic acid synthesis[21-23].

Materials and Methods

Materials

The chemicals and drug used in the present study, namely Griseofulvin, were purchased from Life Sciences Private Limited. Lecithin and hydrochloric acid were procured from Nice Laboratory Reagents, Kochi, while cholesterol was obtained from Yarrow Chem Products, Mumbai. Stearyl amine was purchased from Ottokemi, Mumbai, and Sephadex G-50 was obtained from Sigma-Aldrich. In addition, propylene glycol was procured from Central Drug House, New Delhi. All the chemicals and reagents used throughout the study were of high analytical grade.

Preformulation study

UV spectrophotometric analysis

A stock solution of Griseofulvin (100 µg/mL) was prepared in phosphate buffer (pH 7.4). The solution was scanned over the wavelength range of 200-400 nm using a UV-Visible double-beam spectrophotometer (Systronics) to determine the wavelength of maximum absorbance (λmax)[24].

Fourier Transform Infrared (FTIR) spectroscopy

FTIR analysis of pure Griseofulvin was performed using an FTIR spectrophotometer (Bruker, Germany) within the range of 4000-400 cm^{-1} to identify characteristic functional groups and confirm drug identity[25].

Determination of melting point

The melting point of Griseofulvin was determined using a digital melting point apparatus and compared with reported values.

Solubility studies

The solubility of Griseofulvin was determined in purified water, phosphate buffer pH 7.4, glycerol, propylene glycol, and methanol using the shake-flask method. Excess drug was added to each solvent and shaken at room temperature until equilibrium was achieved. The solutions were filtered and analyzed spectrophotometrically.

Partition coefficient determination

The partition coefficient of Griseofulvin was determined using the n-octanol/water system by the shake-flask method. Drug concentrations in both phases were quantified spectrophotometrically after equilibrium[26].

Drug-excipient compatibility study

Compatibility studies were carried out using FTIR spectroscopy. Griseofulvin was mixed individually with each excipient in a 1:1 ratio and stored in sealed glass vials at 40 ± 2°C and 75 ± 5% relative humidity for 30 days. After storage, FTIR spectra of the mixtures were recorded and compared with those of the pure drug to identify any potential interactions[27-28].

Method of preparation of Glycerosomes

The Glycerosomal Nanogel was prepared using the lipid thin-film hydration method. Briefly, cholesterol, lecithin, and stearylamine were co-dissolved in a small quantity of chloroform in a separate beaker[29-30.] The organic solutions were then combined, and the solvent was evaporated using a rotary evaporator under reduced pressure until a completely dry thin film was formed. The dried lipid film was subsequently hydrated with different concentrations of glycerol solution prepared in phosphate buffer (pH 7.4) and homogenized using an ultrasonic device to obtain glycerosomal nanogels of nanometric size. The unentrapped free drug was separated by passing the formulation through a Sephadex G-50 column[31-33]. The compositions of all six formulations are presented in Table 1.

Table 1. Composition of different formulations of Glycerosomes

Ingredients	F1	F2	F3	F4	F5	F6
Griseofulvin (% w/v)	1	1	1	1	1	1
Phosphotidylcholine (%w/v)	-	-	-	5	5	5
Phosphatidylserine	5	5	5	-	-	-
Cholesterol (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5
Stearylamine (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5
Glycerol (% v/v)	20	20	20	30	30	30

Formulation design and optimization

Six formulations (F1-F6) were prepared by varying the type of phospholipid (phosphatidylserine or phosphatidylcholine) and glycerol concentration (20% and 30% v/v), while maintaining constant concentrations of Griseofulvin, cholesterol, and stearylamine.

The objective of formulation optimization was to evaluate the influence of phospholipid type and glycerol concentration on the physicochemical characteristics of glycerosomes[34].

Optimization criteria

All formulations were evaluated based on:

- Vesicle size
- Entrapment efficiency
- Drug release profile
- Physical stability

The optimized formulation was selected on the basis of: Minimum vesicle size, low PDI indicating uniform size distribution, high entrapment efficiency and sustained drug release behavior.

Characterization of Glycerosomes

Particle size analysis by Malvern Mastersizer

The particle size of the prepared glycerosomes was evaluated using the Malvern Mastersizer[35].

Surface and shape analysis by TEM

The surface morphology and shape characteristics of the glycerosomes were analyzed using a Transmission Electron Microscope (TEM) (Model H-7500 Hitachi, Japan)[36].

Drug entrapment efficiency (%)

For the determination of drug entrapment efficiency, 0.2 mL of undiluted griseofulvin suspension was carefully applied dropwise onto the center of the gel bed. The void volume containing the nanogel was separated by centrifugation at 3000 rpm for 3 minutes. After collecting the elutes, 0.2 mL of saline solution was added to each column, and the centrifugation process was repeated. The amount of drug entrapped within the nanogel vesicles was determined by lysing the vesicles with Triton X-100 (0.5% v/v) and measuring the drug content at 291 nm using a UV-visible spectrophotometer[37-39]. The percentage entrapment efficiency was calculated using the following equation:
Entrapment Efficiency = (Observed drug content / Initial drug content) x 100

In vitro drug release studies

In vitro drug release studies were performed using a Franz diffusion cell equipped with an egg membrane as the diffusion barrier. The receptor compartment was filled with phosphate buffer (pH 7.4) and maintained at 37 ± 0.5°C under continuous magnetic stirring at 100 rpm. The glycerosomal formulation containing a drug equivalent to 2 mg of Griseofulvin was placed in the donor compartment over the membrane. At predetermined intervals, 5 mL samples were withdrawn from the receptor compartment and replaced immediately with an equal volume of fresh phosphate buffer to maintain sink conditions. The samples were analyzed spectrophotometrically at 291 nm, and the cumulative percentage drug release was calculated[40].

Release kinetic analysis

To elucidate the mechanism of drug release from the glycerosomal formulation, the *in vitro* release data were fitted to various mathematical kinetic models, including:

- Zero-order model
- First-order model
- Higuchi model
- Korsmeyer-Peppas model

Stability studies

Stability studies were carried out on the optimized formulation by storing it in tightly closed containers at a temperature of 4 ± 1°C. The samples were evaluated for drug content at predetermined time intervals of 15, 30, 45, 60, and 90 days. The initial drug content of each formulation was considered as 100% for the stability assessment[41-42].

Characterization of nanogel

Homogeneity

The prepared nanogel was visually evaluated for its color, clarity, homogeneity, and overall appearance[43-44.]

pH stability study

For the determination of pH, 1 g of the gel was dispersed in 100 mL of distilled water and allowed to stand for 2 hours. The pH of the formulation was measured three times, and the average value was calculated along with the standard deviation. The pH of the gel was maintained as close as possible to the natural pH of the skin in order to minimize the risk of irritation[45-46].

Viscosity study

The viscosity of the prepared nanogel was measured using a Brookfield viscometer. The formulation was rotated at 2.5 rpm using spindle number C75-1, and the corresponding dial readings were recorded for viscosity determination[47-48].

Spreadability study

The spreadability of the formulation plays an important role in its therapeutic effectiveness. Spreadability was determined by measuring the spreading diameter of 1 g of gel placed between two horizontal glass plates (20 cm × 20 cm) after 1 minute. A standard weight of 125 g was placed on the upper plate to facilitate uniform spreading of the gel. The diameter of the spread gel circle was measured in centimeters, and the final result was expressed as the average of three determinations[49].

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Extrudability study

After drying, the gel formulations were filled into collapsible tubes. The extrudability of the formulations was evaluated by determining the weight in grams required to extrude a 0.5 cm ribbon of gel within 10 seconds[50].

Consistency

The consistency of the prepared gels was evaluated by dropping a cone attached to a holding rod from a fixed height of 10 cm into the center of a glass cup containing the gel. The depth of penetration of the cone was measured from the surface of the gel to the tip of the cone after 10 seconds. The penetration distance was recorded as an indicator of gel consistency[50].

Results and Discussion

Preformulation studies

The drug was identified and characterized using various analytical techniques, including the melting point method, UV spectroscopy, and FTIR spectroscopy. All evaluated parameters were found to be within the acceptable limits and complied with the standards specified in official compendia. The UV spectroscopic analysis of the drug exhibited a maximum absorbance (λmax) at 291 nm. Additionally, the melting point of the drug was observed to be approximately 220°C, which confirmed the purity and identity of the drug sample. The results obtained from these characterization studies indicated that the drug possessed suitable physicochemical properties for further formulation development.

Partition coefficient

The partition coefficient of the drug was found to be 2.16, which falls within the acceptable range when compared with the standard reference drug. This result indicates that the drug possesses suitable lipophilic characteristics, which may contribute to its effective permeation and formulation performance.

Solubility of drug

The drug was found to be highly soluble in N, N-dimethylformamide, while it exhibited slight solubility in ethanol (C_2H_5OH), carbon tetrachloride (CCl_4), and methanol (CH_3OH). However, the drug was found to be practically insoluble in water. These solubility characteristics indicate the lipophilic nature of the drug, which is an important factor to consider during formulation development.

Drug excipient compatibility study

The results of the physical and chemical incompatibility studies revealed that the active pharmaceutical ingredient (API) was compatible with all the excipients used in the formulation. No significant interactions or incompatibilities were observed, indicating the suitability of the selected excipients for the development of the formulation.

Characterizatiion of glycerosomes

Particle size analysis by Malvern Mastersizer

Particle size analysis was carried out using a Malvern Mastersizer, and the average particle size of the formulation was found to be 62.76 nm. The obtained results indicated a uniform particle size distribution, which may contribute to the stability and performance of the formulation in Fig. 1.

Surface analysis and shape by TEM

The surface morphology of the glycerosomes was evaluated using TEM. The obtained images revealed that the glycerosomes were spherical in shape, smooth in surface texture, and vesicular in structure (Fig. 2).

Entrapment efficiency

In vitro drug release study

The entrapment efficiency of griseofulvin-loaded glycerosomes was determined to evaluate the ability of the vesicular system to encapsulate and retain the drug within the lipid bilayer. The results presented in Table 2 and Fig. 3 showed that the entrapment efficiency varied significantly among the different formulation batches (F1–F6), ranging from 65.49 ± 1.18% to 85.45 ± 0.32%.

Among all the formulations, F4 exhibited the highest entrapment efficiency (85.45 ± 0.32%), indicating its superior drug-loading capacity. The increase in entrapment efficiency from F1 to F4 may be attributed to the optimum concentration of glycerol, which enhanced vesicle flexibility and improved drug incorporation within the glycerosomal structure. However, a further increase in

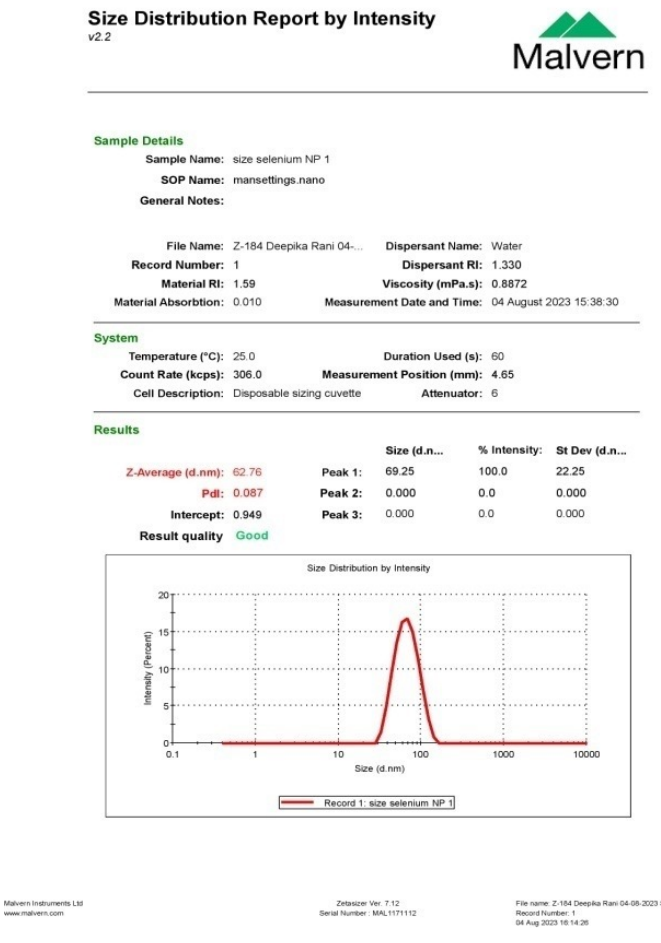

Figure 1. Particle size analysis by Malvern Mastersizer

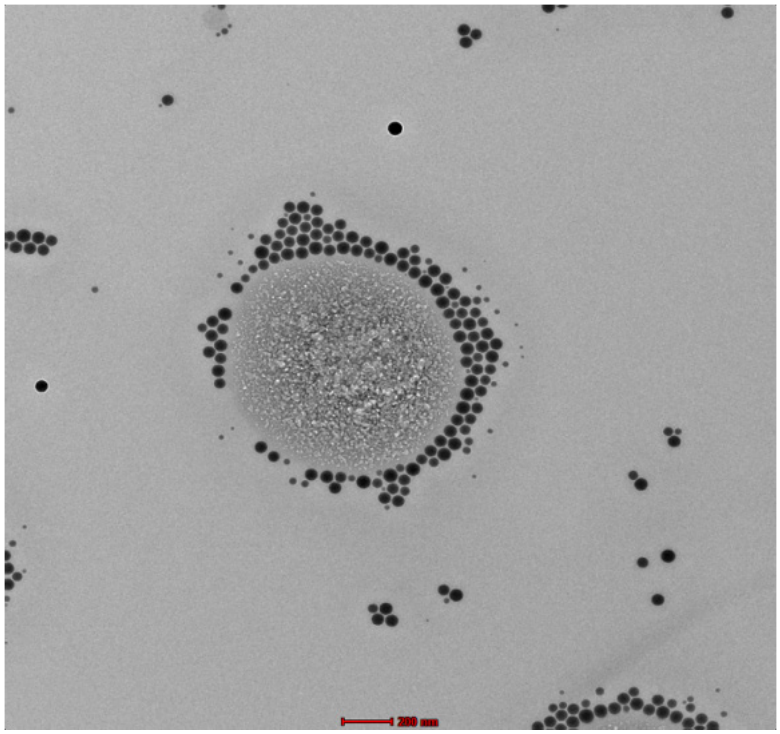

Figure 2. TEM photograph of griseofulvin loaded glycerosomes

Table 2. Entrapment efficiency of (F1-F6) glycerosomes

Batch code	Entrapment efficiency (%) ± S.D.
F1	65.49 ± 1.18
F2	73.39 ± 1.06
F3	78.62 ± 1.04
F4	85.45 ± 0.32
F5	80.91 ± 0.46
F6	71.49 ± 0.18

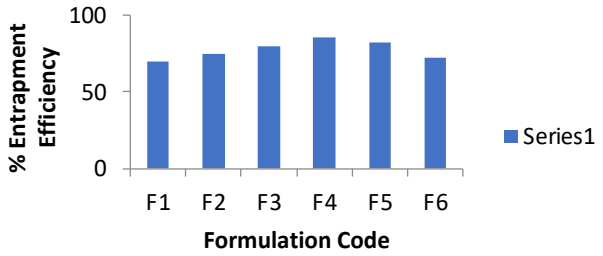

Figure 3. Comparative graph of entrapment efficiency of different batches

glycerol concentration in formulations F5 and F6 resulted in a decline in entrapment efficiency to 80.91 ± 0.46% and 71.49 ± 0.18%, respectively. This reduction may be due to excessive glycerol causing increased membrane fluidity and drug leakage from the vesicles.

The results indicate that glycerol concentration plays a crucial role in determining the drug entrapment capability of glycerosomes. Based on the obtained data, F4 was selected as the optimized formulation due to its highest entrapment efficiency and potential for enhanced drug delivery.

Table 2 summarizes the entrapment efficiency values of all formulation batches, while Fig. 3 provides a comparative graphical representation of the entrapment efficiency of different glycerosomal formulations.

In vitro drug release study

The *in vitro* drug release study was performed to evaluate the release behavior of Griseofulvin from different glycerosomal formulations (F1–F6). The cumulative percentage drug release data are presented in Table 3, while the comparative release profiles are illustrated in Fig. 4.

The results demonstrated a gradual and sustained release of Griseofulvin from all glycerosomal formulations over a period of 16 hours. An initial burst release was observed during the first hour, followed by a controlled and prolonged release phase. The initial release may be attributed to the drug adsorbed on or near the surface of the glycerosomal vesicles, whereas the subsequent sustained release was due to the diffusion of the entrapped drug through the lipid bilayer.

Among all formulations, F4 exhibited the highest cumulative drug release of 80.01 ± 0.32% at the end of 16 hours, indicating its superior release characteristics. The enhanced release from F4 may be attributed to the optimum glycerol concentration, which improved vesicle flexibility and facilitated efficient drug diffusion. Formulations F3 and F5 also showed appreciable drug release values of 75.91 ± 0.65% and 78.19 ± 0.73%, respectively. In contrast, F1 exhibited the lowest cumulative drug release (71.98 ± 0.32%) among all formulations.

The release profiles suggest that glycerol concentration significantly influenced the drug release behavior of the glycerosomes. An optimum concentration of glycerol enhanced drug release, while either lower or higher concentrations resulted in comparatively reduced release rates. Based on the *in vitro* drug release data, F4 was considered the optimized formulation, as it showed the highest and most sustained drug release profile along with superior entrapment efficiency.

Table 3 summarizes the cumulative percentage drug release of all formulations at different time intervals, whereas Fig. 4 presents the comparative *in vitro* drug release profiles of glycerosomal formulations (F1–F6), highlighting the superior performance of formulation F4.

Drug release kinetics

To understand the mechanism of drug release from the optimized glycerosomal formulation (F4), the *in vitro* drug release data were fitted into various kinetic models, namely Zero-Order, First-Order, Higuchi, and Korsmeyer–Peppas models. The regression coefficient (R^2) values obtained from these models are presented in Table 4.

The results indicated that the Higuchi model showed the highest regression coefficient (R^2 = 0.983) among all the kinetic models evaluated. This suggests that the release of Griseofulvin from the optimized glycerosomal formulation primarily follows the Higuchi diffusion model, where drug release occurs through diffusion from the vesicular matrix. The Korsmeyer–Peppas model also exhibited a relatively high correlation coefficient (R^2 = 0.933), indicating that diffusion plays a significant role in the drug release process. The Zero-Order model showed a moderate fit (R^2 = 0.874), suggesting that the release was not completely concentration-independent. In contrast, the First-Order model exhibited the lowest regression coefficient (R^2 = 0.486), indicating poor conformity of the release data to first-order kinetics.

Overall, the kinetic analysis revealed that the optimized formulation F4 predominantly follows the Higuchi model, suggesting a diffusion-controlled release mechanism. The sustained drug release behavior observed in the formulation may be attributed to the gradual diffusion of Griseofulvin through the glycerosomal lipid matrix, thereby supporting prolonged drug availability and improved therapeutic performance.

Table 4 confirming that the Higuchi model best describes

Table 3. *In vitro* drug release data of glycerosomes

Time (hrs)	F1	F2	F3	F4	F5	F6
0.5	8.91±0.54	10.19±0.23	12.41±0.98	13.07±0.21	12.91±0.63	9.29±0.78
1	20.03±0.61	22.03±0.34	23.19±0.46	25.59±0.27	23.85±0.73	21.26±1.34
2	31.98±0.32	30.84±0.54	33.74±0.34	32.78±0.56	31.01±0.84	29.06±0.56
4	38.95±0.61	41.91±0.74	43.72±0.26	45.31±1.13	43.92±0.42	41.97±0.52
6	45.74±0.63	52.46±1.52	55.63±0.67	58.85±0.53	55.27±0.34	55.03±0.32
8	49.12±0.82	54.78±0.67	58.12±0.29	66.76±0.42	60.76±0.53	59.12±0.74
10	61.9±0.64	63.51±0.73	65.07±1.23	69.99±0.51	68.01±1.54	65.12±0.62
12	67.08±0.73	69.29±0.14	71.65±0.54	73.28±0.63	71.31±0.35	68.78±0.15
14	72.49±0.91	73.11±0.72	75.05±0.24	79.32±0.47	77.18±0.51	75.28±0.42
16	71.98±0.32	73.86±0.63	75.91±0.65	80.01±0.32	78.19±0.73	76.45±0.92

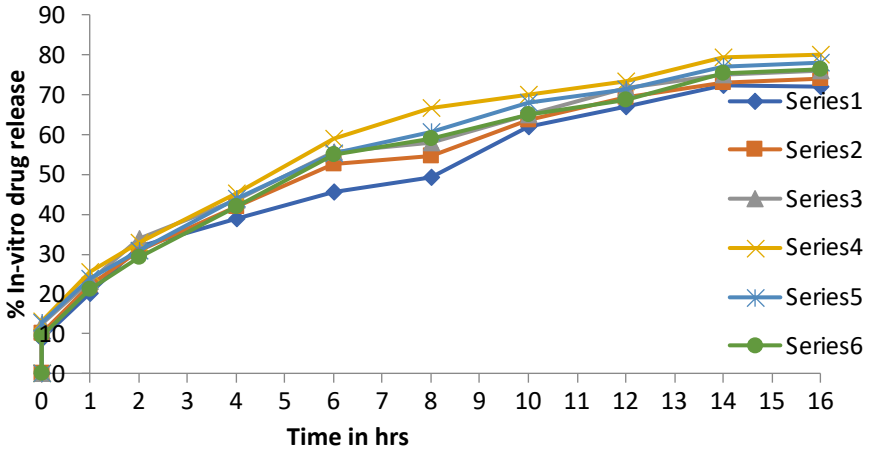

Figure 4. *In vitro* drug release profiles of glycerosomes

Table 4. Derived from several kinetics models as regression co-efficient (R2)

Batch code	Zero order	Higuchi	Korsmeyer peppas	First order
F4	0.874	0.983	0.933	0.486

the drug release behavior of formulation F4.summarizes the regression coefficient (R^2) values obtained from different kinetic models,

Spreadability

Rgression co-efficient values of optimized formulation (F4)

Spreadability refers to the ease with which a gel spreads over the skin or affected area after application. To evaluate the spreadability of the formulation, 0.5 g of gel was placed on a glass plate marked with a pre-defined circle of 1 cm diameter. Another glass plate was carefully placed over it, and a weight of 500 g was allowed to rest on the upper plate for 5 minutes. After the application of weight, the diameter of the spread gel increased to 6.70 ± 0.07 cm. The obtained results indicated that the prepared gel formulation possessed good spreadability. Spreadability is an important parameter in topical formulations, as it directly influences patient compliance and ease of application. A gel with good spreadability can be applied more comfortably and uniformly over irritated or diseased skin, thereby improving patient convenience and therapeutic effectiveness.

Characterization of drug loaded glycerosomal nanogel

Homogeneity

The prepared Griseofulvin-loaded glycerosomal nanogel exhibited excellent homogeneity and was found to be free from lumps or aggregates. The formulation showed a smooth and uniform appearance, indicating proper dispersion of all components within the gel system.

Measurement of pH

The apparent pH of the gel formulation was measured in triplicate using a digital pH meter at 25°C. The pH of the optimized formulation (F4) was found to be 6.3 ± 0.04. This pH value falls within the acceptable range for

topical applications and is compatible with the natural pH of the skin. Therefore, the prepared Griseofulvin-loaded glycerosomal nanogel is expected to minimize the risk of skin irritation and provide better patient acceptability.

Viscosity

The viscosity of the prepared formulations was measured using a Brookfield viscometer. The optimized formulation (F4) showed a viscosity of 501 ± 0.57 cps. This viscosity value indicates that the gel possessed appropriate flow properties and consistency, making it suitable for topical application and easy spreadability over the skin surface.

Spreadability study

The spreadability of the gel formulation was evaluated by measuring the spreading diameter of 1 g of gel placed between two horizontal glass plates (20 cm) after one minute. The spreadability of the optimized formulation (F4) was found to be 6.5 g·cm/sec, indicating that the Griseofulvin-loaded glycerosomal nanogel possessed good spreading characteristics. This property is important for topical formulations, as it ensures easy and uniform application over the affected area, thereby enhancing patient convenience and compliance.

Extrudability study

Extrudability studies were carried out to evaluate the ease of gel extrusion from the collapsible tube. The optimized formulation (F4) showed satisfactory extrudability, as a 0.5 cm ribbon of gel was smoothly extruded from the tube within 10 seconds. The results indicated that the formulation possessed suitable consistency and could be conveniently dispensed for topical application.

Consistency

The consistency of the gel indicates its ability to be expelled from the tube in a uniform and appropriate amount upon application of pressure. The consistency of the formulation was evaluated by measuring the distance traveled by the cone, which was found to be 5 mm. This result suggests that the gel possessed suitable consistency for convenient handling and topical application.

Stability study

hort-term accelerated stability studies were carried out for the optimized formulation according to the procedure described previously. During the study, the formulation was evaluated for physical appearance, pH, cumulative percentage drug release, and drug content under different storage conditions. The results indicated that the formulation remained physically stable throughout the study period, with no noticeable changes in color, phase separation, or consistency. In addition, the drug release profile and drug content did not show any major variations under refrigerated storage conditions, suggesting good formulation stability.

However, a comparatively greater reduction in drug content was observed when the formulation was stored at room temperature, indicating that elevated temperature conditions may affect the integrity of the glycerosomal vesicles and lead to gradual drug degradation. This finding highlights the temperature-sensitive nature of the nanogel formulation and suggests that refrigerated storage conditions are more appropriate for maintaining its physicochemical stability and therapeutic effectiveness over an extended period.

The stability behavior of the formulation may be influenced by factors such as vesicle aggregation, lipid oxidation, and possible leakage of the entrapped drug during storage. Therefore, careful selection of storage conditions is essential to preserve the quality and performance of the formulation. From a practical perspective, the observed stability profile indicates that the optimized Griseofulvin-loaded glycerosomal nanogel has the potential to remain stable during storage and handling when maintained under suitable environmental conditions.

Effect of aging on residual drug content

At the end of the three-month stability study, the residual drug content of the optimized formulation was determined by chemical analysis. The formulation retained 97.12 ± 0.79% of the drug when stored under refrigerated conditions (4 ± 1°C), whereas the residual drug content decreased to 92.14 ± 0.88% when stored at room temperature, as shown in Tables 5, 6 and Fig. 5.

The higher drug retention observed under refrigerated conditions indicates that lower temperatures help preserve the structural integrity of the glycerosomal vesicles and minimize drug degradation during storage. In contrast, storage at room temperature resulted in a comparatively greater reduction in drug content, which may be attributed to physicochemical changes such as lipid degradation, vesicle aggregation, or drug leakage. Furthermore, a slight yellow discoloration was observed after 60 days of storage at room temperature, indicating possible formulation instability under elevated storage conditions.

Despite the reduction in drug content and minor color change at room temperature, the formulation remained physically stable without any noticeable changes in consistency or phase separation throughout the study period. These findings demonstrate that the developed Griseofulvin-loaded glycerosomal nanogel possesses satisfactory stability characteristics, particularly under refrigerated conditions.

Overall, the stability study confirms that the optimized formulation can maintain its quality and drug content during storage and has the potential for effective topical application in the treatment of onychomycosis. However,

Table 5. Effect of aging on residual drug content at 4±1°C

Sr. No.	Days	Physical change	Mean % residual drug content (at 4 ± 1°C)
1	0	No change	100
2	15	No change	99.29±0.08
3	30	No change	99.04±0.23
4	45	No change	98.38±0.45
5	60	No change	97.9±0.67
6	90	No change	97.12±0.79

Table 6. Effect of aging on residual drug content at room temperature

Sr. No.	Days	Physical change	Mean % residual drug content (at room temp.)
1	0	No change	100
2	15	No change	98.04±0.69
3	30	No change	97.52±0.91
4	45	No change	95.24±0.45
5	60	Color changes to slight yellow	95.12±0.70
6	90	Color changes to slight yellow	92.14±0.88

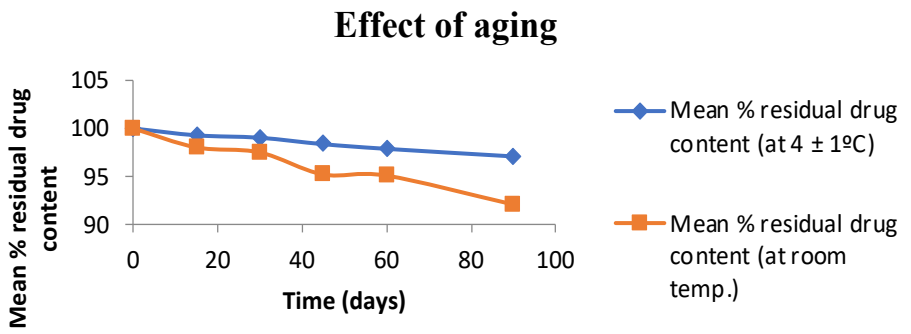

Figure 5. Effect of aging on percent residual drug content at 4±1°C and at room temperature

refrigerated storage is recommended to ensure maximum stability and therapeutic efficacy.

Conclusions

The present study was undertaken to develop and evaluate a griseofulvin-loaded glycerosomal nanogel for the topical treatment of onychomycosis. The formulation was designed using the lipid thin-film hydration technique with the objective of enhancing drug penetration through the nail plate, providing sustained drug release, and improving patient compliance.

Preformulation studies confirmed the suitability of Griseofulvin for formulation development. The drug exhibited a maximum absorbance (λmax) at 291 nm and a melting point of approximately 220°C. The partition coefficient value of 2.16 indicated favorable lipophilic characteristics, while FTIR compatibility studies confirmed the absence of significant interactions between the drug and selected excipients.

Six glycerosomal formulations (F1–F6) were prepared by varying the phospholipid type and glycerol concentration. Particle size analysis revealed the formation of nanosized vesicles with an average particle size of 62.76 nm. Transmission electron microscopy showed that the prepared glycerosomes were spherical in shape with smooth surfaces and a vesicular structure.

Among all formulations, F4 was identified as the optimized formulation due to its superior performance. It exhibited the highest entrapment efficiency of 85.45 ± 0.32% and the highest cumulative drug release of 80.01 ± 0.32% after 16 hours. Drug release kinetic analysis demonstrated that the release profile best fitted the Higuchi model (R^2 = 0.983), indicating a diffusion-controlled release mechanism.

The optimized glycerosomal formulation was successfully incorporated into a nanogel system and evaluated for its physicochemical properties. The nanogel showed excellent homogeneity, a skin-compatible pH of 6.3 ± 0.04, viscosity of 501 ± 0.57 cps, good spreadability, satisfactory extrudability, and appropriate consistency,

making it suitable for topical application.

Stability studies conducted for 90 days demonstrated that the optimized formulation remained stable under refrigerated conditions. The residual drug content was found to be 97.12 ± 0.79% at 4 ± 1°C and 92.14 ± 0.88% at room temperature after three months. Although slight discoloration was observed under room-temperature storage, no significant changes in consistency or phase separation were detected.

In conclusion, the developed griseofulvin-loaded glycerosomal nanogel exhibited desirable physicochemical properties, high drug entrapment efficiency, sustained drug release, and satisfactory stability. The optimized formulation has the potential to improve topical delivery of Griseofulvin and may offer an effective alternative for the management of onychomycosis. Further *in vivo* and clinical investigations are recommended to establish its therapeutic efficacy, safety, and long-term clinical applicability.

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Conflict of interest

All the authors declare that they have no conflicts of interest.

References

1. **Gupta, A.K., Sibbald, R.G., Andriessen, A., Belley, R., Boroditsky, A., Botros, M. et al. (2015).** Toenail onychomycosis - A Canadian approach with a new transungual treatment: Development of a clinical pathway. Journal of Cutaneous Medicine and Surgery. 19(5): 440-449.
2. **Joyce, A., Gupta, A.K., Koenig, L., Wolcott, R., Carviel, J. (2019).** Fungal diversity and onychomycosis: An analysis of 8,816 toenail samples using quantitative PCR and next-generation sequencing. Journal of the American Podiatric Medical Association. 109(1): 57-63.
3. **Thomas, J., Jacobson, G.A., Narkowicz, C.K., Peterson, G.M., Burnet, H., Sharpe, C. (2010).** Toenail onychomycosis: An important global disease burden. Journal of Clinical Pharmacy and Therapeutics. 35(5): 497-519.
4. **Youssef, A.B., Kallel, A., Azaiz, Z., Jemel, S., Bada, N., Chouchen, A. et al. (2018).** Onychomycosis: Which fungal species are involved? Experience of the Laboratory of Parasitology-Mycology of the Rabta Hospital of Tunis. Journal of Medical Mycology. 28(4): 651-654.
5. **Fike, J.M., Kollipara, R., Alkul, S., Stetson, C.L. (2018).** Case report of onychomycosis and tinea corporis due to Microsporum gypseum. Journal of Cutaneous Medicine and Surgery. 22(1): 94-96.
6. **Shah, P.P., Desai, P.R., Patel, A.R., Singh, M.S. (2012).** Skin permeating nanogel for cutaneous co-delivery of the two anti-inflammatory drugs. Biomaterials. 33(Suppl 5): 1607-1617.
7. **Findley, K., Oh, J., Yang, J. et al. (2013).** Topographic diversity of fungal and bacterial communities in human skin. Nature. 498: 367-370.
8. **Kashem, S.W., Kaplan, D.H. (2016).** Skin immunity to Candida albicans. Trends in Immunology. 37: 440-450.
9. **Havlickova, B., Czaika, V.A., Friedrich, M. (2008).** Epidemiological trends in skin mycoses worldwide. Mycoses. 51: 2-15.
10. **Sudbery, P., Gow, N., Berman, J. (2004).** The distinct morphogenic states of Candida albicans. Trends in Microbiology. 12: 317-324.
11. **Bineshian, F., Yadegari, M.H., Sharifi, Z. et al. (2015).** Identification of Candida species using MP65 gene and evaluation of the Candida albicans MP65 gene expression in BALB/C mice. Jundishapur Journal of Microbiology. 8: e18984.
12. **Coppola, R., Zanframundo, S., Rinati, M.V. et al. (2015).** Rhodotorula mucilaginosa skin infection in a patient treated with sorafenib. Journal of the European Academy of Dermatology and Venereology. 29: 1028-1029.
13. **Vlahovic, T.C. (2016).** Onychomycosis: Evaluation, treatment options, managing recurrence, and patient outcomes. Clinics in Podiatric Medicine and Surgery. 33(3): 305-318.
14. **Hoy, N.Y., Leung, A.K., Metelitsa, A.I., Adams, S. (2012).** New concepts in median nail dystrophy, onychomycosis, and hand, foot, and mouth disease nail pathology. ISRN Dermatology. 2012: 680163.
15. **Vlahovic, T.C. (2016).** Onychomycosis: Evaluation, treatment options, managing recurrence, and patient outcomes. Clinics in Podiatric Medicine and Surgery. 33(3): 305-318.
16. **Queller, J.N., Bhatia, N. (2015).** The dermatologist's approach to onychomycosis. Journal of Fungi. 1(2): 173-184.
17. **Patra, J.K., Baek, K.H. (2014).** Green nanobiotechnology: Factors affecting synthesis and characterization techniques. Journal of Nanomaterials. 2014: 219.
18. **Joseph, R.R., Venkatraman, S.S. (2017).** Drug delivery to the eye: What benefits do nanocarriers offer? Nanomedicine. 12: 683-702.
19. **Mirza, A.Z., Siddiqui, F.A. (2014).** Nanomedicine and drug delivery: A mini review. International Nano Letters. 4: 94.
20. **Rudramurthy, G.R., Swamy, M.K., Sinniah, U.R., Ghasemzadeh, A. (2016).** Nanoparticles: Alternatives against drug-resistant pathogenic microbes. Molecules. 21: 836.
21. **Kumar, S., Talegaonker, S., Negi, L.M., Khan, Z.I. (2012).** Design and development of ciclopirox topical nanoemulsion gel for the treatment of subungal onychomycosis. Indian Journal of Pharmaceutical Education and Research. 46(Suppl 4): 303-310.
22. **Panonnummal, R., Jayakumar, R., Sabitha, M. (2017).** Comparative anti-psoriatic efficacy studies of clobetasol loaded chitin nanogel and marketed cream. European Journal of Pharmaceutical Sciences. 96: 193-206.
23. **Kamble, A., Badde, S., Kumar, A., Pokharkar, V.B.**

(2010). Development and characterization of liposomal drug delivery system for nimesulide. International Journal of Pharmacy and Pharmaceutical Sciences. 2(Suppl 4): 87-89.

24. **Abu, T.M.S., Ajit, B.T., Rabin, N.G., Micheal, G.F., Sunanda, A.R., Kenneth, R.M. (1999).** Selection of solid dosage form composition through drug excipient compatibility testing. Journal of Pharmaceutical Sciences. 88(7): 696-704.
25. **Adhikari, B., Cherukuri, S., Reddy, S.C., Chinthaginjala, H., Bhatta, H., Naidu, R., Inturi. (2016).** Recent advances in nanogels in drug delivery system. World Journal of Pharmacy and Pharmaceutical Sciences. 5(9): 505-530.
26. **Agarwal, M., Nagar, D.P., Srivastava, N., Agarwal, M.K. (2015).** Chitosan nanoparticles-based drug delivery: An update. International Journal of Advanced Multidisciplinary Research. 2(4): 1-13.
27. **Sangster, J. (1989).** Octanol-water partition coefficients of simple organic compounds. Journal of Physical and Chemical Reference Data. 18(3): 1111-1229.
28. **Angstwurm, M.W., Engelmann, L., Zimmermann, T., Lehmann, C., Spes, C.H., Abel, P. et al. (2007).** Selenium in Intensive Care (SIC): Results of a prospective randomized, placebo-controlled, multiple-center study in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock. Critical Care Medicine. 35(1): 118-126.
29. **Shirsand, S.B., Para, M.S., Nagendrakumar, D., Kanani, K.M., Keerthy, D. (2012).** Formulation and evaluation of ketoconazole niosomal gel drug delivery system. International Journal of Pharmaceutical Investigation. 2(4): 201-207.
30. **Thomas, L., Viswanad, V. (2012).** Formulation and optimization of clotrimazole-loaded proniosomal gel using 32 factorial designs. Scientia Pharmaceutica. 80(3): 731-748.
31. **Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S.W., Zarghami, N., Hanifehpour, Y., Samiei, M., Kouchi, M., Nejati-Koshki, K. (2013).** Liposomes: Classification, preparation and applications. Nanoscale Research Letters. 8(102): 1-9.
32. **Albuquerque, P., Coelho, L.C., Teixeira, J.A., Carneiro-da-Cunha, M.G. (2016).** Approaches in biotechnological applications of natural polymers. AIMS Molecular Science. 3(3): 386-425.
33. **Annakula, D., Errabelli, M.R., Jukanti, R., Bandari, S., Veerareddy, P.R. (2010).** Provesicular drug delivery systems: An overview and appraisal. Archives of Applied Science Research. 2(4): 135-146.
34. **Bhowmik, D., Gopinath, H., Kumar, B.P., Duraivel, S., Kumar, K.P.S. (2012).** Recent advances in novel topical drug delivery systems. The Pharma Innovation. 1(9): 12-31.
35. **Hashim, F., El-Ridy, M., Nasr, M., Abdallah, Y. (2010).** Preparation and characterization of niosomes containing ribavirin for liver targeting. Drug Delivery. 17(5): 282-287.
36. **Blanco, E., Shen, H., Ferrari, M. (2015).** Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature Biotechnology. 33: 941-951.
37. **Raja, C.H.N.V., Kiran Kumar, G., Kotapatianusha. (2013).** Fabrication and evaluation of ciprofloxacin loaded nanosponges for sustained release. International Journal of Research in Pharmaceutical and Nano Sciences. 2(1): 1-9.
38. **Shunmugam, R., Balusamy, S.R., Kumar, V., Menon, S., Lakshmi, T., Perumalsamy, H. (2021).** Biosynthesis of gold nanoparticles using marine microbe (Vibrio alginolyticus) and its anticancer and antioxidant analysis. Journal of King Saud University-Science. 33(1): 101260.
39. **Silva, L.D., Fu, J.Y., Htar, T.T., Muniyandy, S., Kasbollah, A., Wan Kamal, W.H.B., Chuah, L.H. (2019).** Characterization, optimization, and *in vitro* evaluation of Technetium-99m-labeled niosomes. International Journal of Nanomedicine. 14: 1101-1117.
40. **Shaikh, S., Fatima, J., Shakil, S., Rizvi, S.M.D., Kamal, M.A. (2015).** Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi Journal of Biological Sciences. 22(1): 90-101.
41. **Dash, S., Murthy, P.N., Nath, L., Chowdhury, P. (2010).** Kinetic modeling of drug release from controlled drug delivery systems. Acta Poloniae Pharmaceutica-Drug Research. 67(3): 217-223.
42. **Dhandapani, N.V., Thapa, A., Sandip, G., Shrestha, A., Shrestha, N., Bhattarai, R.S. (2013).** Liposomes: A novel drug delivery system: Comprehensive review. International Journal of Research in Pharmaceutical Sciences. 4(2): 187-193.
43. **Bermudez, N.M., Rodríguez-Tamez, G., Perez, S. and Tosti, A. (2023).** Onychomycosis: Old and new. Journal of Fungi. 9(5): 559.
44. **Elewski, B.E. (1998).** Onychomycosis: Pathogenesis, diagnosis, and management. Clinical Microbiology Reviews. 11(3): 415-429.
45. **Weinberg, J.M., Koestenblatt, E.K., Tutrone, W.D., Tishler, H.R. and Najarian, L. (2003).** Comparison of diagnostic methods in the evaluation of onychomycosis. Journal of the American Academy of Dermatology. 49(2): 193-197.
46. **Jeelani, S., Ahmed, Q.M., Lanker, A.M., Hassan, I., Jeelani, N. and Fazili, T. (2015).** Histopathological examination of nail clippings using PAS staining (HPE-PAS): Gold standard in diagnosis of onychomycosis. Mycoses. 58(1): 27-32.
47. **Lee, N.Y. and Lee, D.Y. (2015).** Comparison of diagnostic methods for onychomycosis, and proposal of a diagnostic algorithm. Clinical and Experimental Dermatology. 40(5): 479-484.
48. **Begari, V., Pathakumari, P. and Takalkar, A.A. (2019).** Comparative evaluation of KOH mount, fungal culture and PAS staining in onychomycosis. International Journal of Research in Dermatology. 5(3): 554-558.
49. **Piraccini, B.M. and Alessandrini, A. (2015).** Onychomycosis: A review. Journal of Fungi, 1(1): 30-43.
50. **Lipner, S.R. and Scher, R.K. (2019).** Onychomycosis: Clinical overview and diagnosis. Journal of the American Academy of Dermatology. 80(4): 835-851.