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Research

Characterization of Griseofulvin Niosomes via Thin Film Hydration Technique

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	Abstract
Published on: 29 Oct 2025	Griseofulvin, a potent antifungal drug, suffers from poor solubility and low oral bioavailability, necessitating advanced delivery approaches. In this study, eighteen niosomal formulations were prepared using the thin-film hydration method by varying surfactant type, surfactant-to-cholesterol ratios, and Stearylamine levels. Tween-60-based formulations showed superior performance, with Formulation F18 (Tween-60:Cholesterol 2:1, 0.75% Stearylamine) emerging as the optimized system. F18 achieved the highest entrapment efficiency (72.73%), smallest vesicle size (3.38 μm), and near-complete sustained release (94.86% in 24 hrs). Release kinetics followed the Korsmeyer–Peppas model, confirming a diffusion-controlled, non-Fickian mechanism. Stability studies demonstrated that F18 remained physically and functionally stable for three months at both refrigerated and room temperature conditions. Overall, niosomal encapsulation—particularly the optimized F18—offers a promising strategy to enhance the solubility, stability, and controlled delivery of Griseofulvin, potentially improving its therapeutic efficacy.
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	Keywords: Griseofulvin, Niosomes, Entrapment Efficiency, Sustained Release, Optimization, Stability, Tween-60, Stearylamine.

INTRODUCTION

Niosomes are non-ionic surfactant-based vesicular systems that serve as efficient drug delivery carriers, offering better stability, lower cost, and tunable properties compared to liposomes. Composed of surfactants, cholesterol, and stabilizers, they can encapsulate both hydrophilic and hydrophobic drugs, improving bioavailability, controlled

release, and targeted delivery. Their adjustable size, charge, and permeability make them suitable for various routes of administration with reduced immunogenicity and improved shelf life. The thin film hydration method is a simple and scalable technique for niosome preparation. A mixture of surfactants and cholesterol is dissolved in an organic solvent, which is evaporated to form a thin film. Upon hydration with an aqueous solution, the film forms multilamellar vesicles that can be sonicated or extruded for uniform size. Factors such as surfactant type, cholesterol ratio, hydration conditions, and processing steps critically influence vesicle properties, requiring careful optimization to achieve stable and effective niosomal formulations⁽¹⁾.

Grisofulvin is selected as the model drug for preparing niosomes due to its poor aqueous solubility, classifying it as a BCS Class II compound (low solubility, high permeability). This characteristic makes Grisofulvin a particularly suitable candidate for encapsulation within niosomes, which can enhance its solubility and dissolution performance. Additionally, Grisofulvin exhibits low bioavailability because conventional formulations suffer from erratic absorption. By employing niosomes, the solubility and drug release characteristics of Grisofulvin can be improved, which is anticipated to translate into enhanced overall bioavailability. The aim of this study is to characterize Grisofulvin-loaded niosomes prepared via the thin-film hydration technique, optimizing their formulation for enhanced drug delivery, stability, and therapeutic efficacy.

MATERIALS AND METHODS

Griseofulvin - Benevolent gift from Glaxo Smith Kline Pharmaceuticals Ltd., Mumbai, Span 20, 60 and Tween 20, 60 from S.D. Fine Chem Ltd, Boisar.

FTIR Interaction Study:

To assess drug-polymer interactions, IR spectra were recorded for pure griseofulvin and drug-polymer physical mixtures (1:1) using a Shimadzu IR-470. Samples were prepared as KBr discs pressed at 6 ton/m². Spectra were collected from 400 to 4000 cm⁻¹ (PerkinElmer FTIR). The mixture spectrum was compared with those of the pure drug and polymers to detect any peak appearance or disappearance⁽²⁾.

Formulation of Griseofulvin niosomes:

Griseofulvin-loaded niosomes were prepared by the thin film hydration technique. Accurately weighed amounts of cholesterol and surfactant were dissolved in a chloroform:methanol mixture using a 100 ml volumetric flask. To this solvent mixture, the required quantity of drug, stearylamine and dicetyl phosphate was added. The solvent system was then evaporated using a rotary evaporator at 60 °C and a rotation speed of 150 rpm to obtain a thin lipid film on the wall of the flask. Complete removal of solvent was ensured by applying vacuum. The resulting dry lipid film was hydrated with 5 ml of PBS (pH 7.4) at a temperature of 60 ± 2 °C for 2 hours, allowing the formation of niosomes. Finally, all the prepared batches were subjected to sonication using a probe sonicator for 2 minutes to obtain the desired niosomal dispersion. The untrapped drug from niosomal formulation was separated by centrifugation method at 15,000 rpm for 30 min using cooling centrifuge at 5°C. The supernatant was separated and the pellet was resuspended in PBS pH 7.4 to obtain a niosomal suspension free from untrapped drug.

F. Code	Surfactant Type	Surfactant: Cholesterol	Stearylamine	F. Code	Surfactant Type	Surfactant: Cholesterol	Stearylamine
F1	Span-20	1:1	-	F10	Tween-20	1:1	-
F2	Span-20	1:2	-	F11	Tween-20	1:2	-
F3	Span-20	2:1	-	F12	Tween-20	2:1	-
F4	Span-60	1:1	-	F13	Tween-60	1:1	-
F5	Span-60	1:2	-	F14	Tween-60	1:2	-
F6	Span-60	2:1	-	F15	Tween-60	2:1	-
F7	Span-60	2:1	0.25	F16	Tween-60	2:1	0.25
F8	Span-60	2:1	0.50	F17	Tween-60	2:1	0.50
F9	Span-60	2:1	0.75	F18	Tween-60	2:1	0.75

Table 1: Formulation of Griseofulvin-loaded niosomes

EVALUATION OF GRISEOFULVIN-LOADED NIOSOMES

Encapsulation efficiency⁽³⁾: For the determination of entrapment efficiency, the untrapped drug in the niosomal formulation was separated using the centrifugation method. A 1 ml aliquot of the separated drug loaded niosomal suspension was then disrupted with 0.1 ml of 0.1% Triton X-100 prepared in distilled water and kept for 5 minutes. The resulting solution was subjected to centrifugation at 3,000 rpm for 5 minutes. The supernatant obtained was carefully decanted and suitably diluted with PBS, pH 7.4. The amount of drug present was finally estimated spectrophotometrically at λ_{max} 291 nm using PBS containing 0.1% Triton X-100 as the blank.

$$\text{Percent Entrapment} = \frac{\text{Amount of Drug Entrapped}}{\text{Total Amount of Drug Added}} \times 100$$

Zeta potential and Particle Size Analysis⁽⁴⁾: The zeta potential and vesicles size of optimized niosomal formulation was measured using Malvern zeta potential analyser.

Scanning electron microscopy⁽⁵⁾: The optimized formulation was morphologically characterized by scanning electron microscopy (SEM). The sample for SEM analysis was mounted in the specimen stub using an adhesive small sample was mounted directly in scotch double adhesive tape. The sample was analysed in Hitachi scanning electron microscope operated at 15 kv and photograph was taken.

In vitro release study for niosomal preparation⁽⁶⁾: The niosomal formulation was taken in a dialysis membrane of 5 cm length and suitably suspended in a beaker containing 100 ml diffusion medium of PBS pH 7.4. The temperature of medium was maintained at $37 \pm 0.5^\circ\text{C}$. The medium was stirred by means of magnetic stirrer at a constant speed. 1 ml of sample was withdrawn at every 1 hour and replaced with 1 ml of fresh buffer, so that the volume of diffusion medium was maintained constant at 100 ml. The withdrawn samples were diluted up to 10 ml using PBS pH 7.4. The samples were measured spectrophotometrically at 291 nm.

Drug Release Kinetic Study⁽⁷⁾: The prepared formulations were evaluated to determine the order of drug release from the niosomes using Zero order Vs First order and mechanism of drug release using Higuchi Vs Hixson-Crowell models. Finally the drug release mechanism was confirmed using Korsmeyer-Peppas model.

Stability study⁽⁸⁾: The optimized niosomal formulation was examined for stability study. The formulations were taken in a 20 ml sealed glass vial and stored in three different environments such as room temperature, 4°C , and $45^\circ\text{C}/75\% \text{ RH}$ for a period of three months. Samples from each batch were withdrawn at the interval of one month and evaluated for entrapment efficiency and *in vitro* drug release.

RESULTS AND DISCUSSION

FTIR Interaction Study

Compatibility Study:

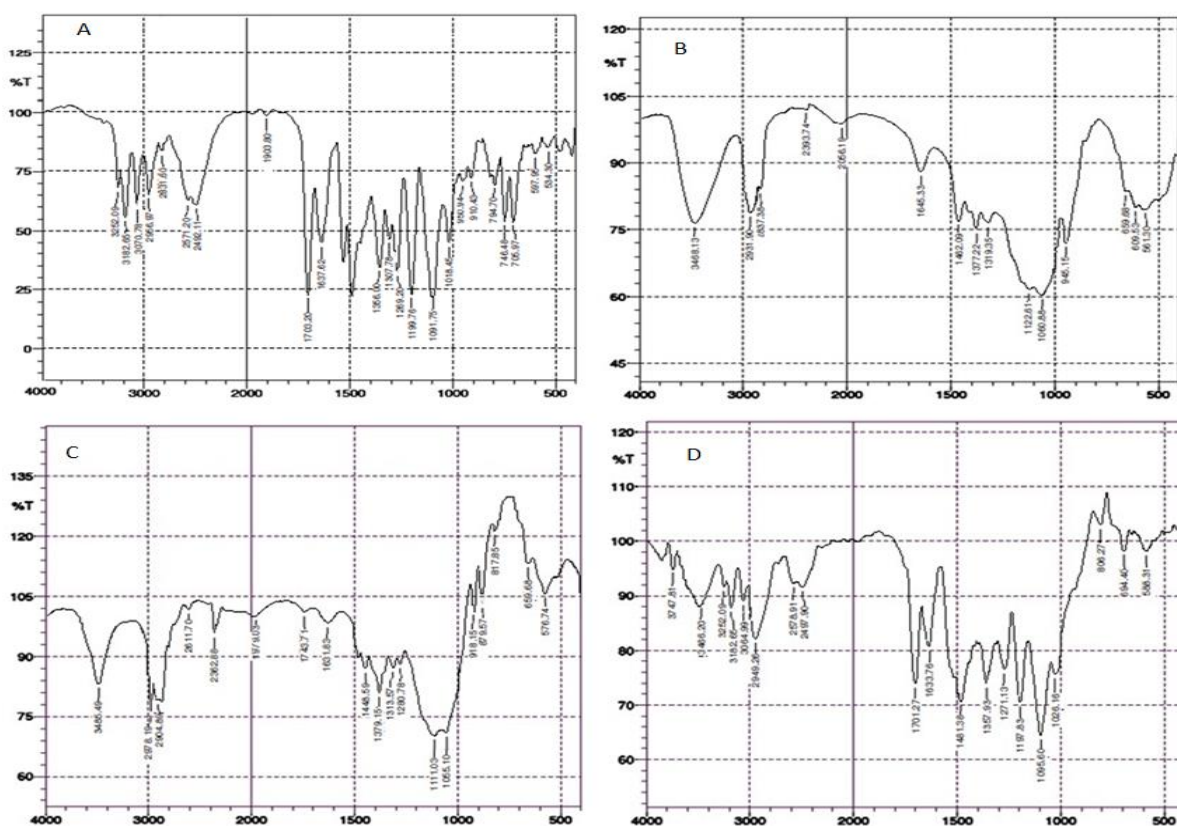


Figure 1: IR spectrum of A. Griseofulvin; B. Griseofulvin + Tween 60; C. Griseofulvin + Stearylamine; D. Griseofulvin + Tween 60 + Stearylamine

FTIR analysis shows that mixing griseofulvin with Tween 60 and stearylamine produces no new bands or large systematic shifts ($>10\text{--}15\text{ cm}^{-1}$) and all key functional-group bands of Griseofulvin (aromatic C=O $\sim 1701\text{--}1703\text{ cm}^{-1}$, aromatic C=C $\sim 1633\text{--}1637\text{ cm}^{-1}$, aromatic C-H $\sim 3064\text{--}3070\text{ cm}^{-1}$) are retained with only minor shifts ($\sim 2\text{--}7\text{ cm}^{-1}$) due to normal solid-state variations; the surfactant/amine markers (broad O-H $\sim 3466\text{--}3468\text{ cm}^{-1}$ and aliphatic C-H $\sim 2949\text{--}2978\text{ cm}^{-1}$) are preserved without significant displacement. The broad $3460\text{--}3200\text{ cm}^{-1}$ region remains present, slightly broadened due to weak, non-covalent hydrogen-bonding interactions rather than covalent bonding, and does not align with any diagnostic shifts of the Griseofulvin carbonyl or other major bands, indicating no chemical reaction. Together, these observations support solid-state compatibility and preservation of the drug's structural integrity upon mixing, favorable for subsequent formulation (e.g., niosomes), with only expected physical mixing effects and weak intermolecular associations rather than chemical incompatibility.

Encapsulation efficiency

F. Code	% Encapsulation efficiency (n=3)	Mean vesicle size (μm) (n=3)	Zeta Potential (mV)
F1	31.56 $\pm$ 1.37	4.86 $\pm$ 0.26	30.4
F2	33.28 $\pm$ 2.21	4.53 $\pm$ 0.18	31.7
F3	35.73 $\pm$ 2.43	4.41 $\pm$ 0.22	31.9
F4	54.82 $\pm$ 1.68	4.46 $\pm$ 0.15	33.5
F5	56.38 $\pm$ 1.44	4.25 $\pm$ 0.18	33.8
F6	56.96 $\pm$ 2.53	4.11 $\pm$ 0.15	34.1
F7	58.48 $\pm$ 1.88	4.28 $\pm$ 0.22	34.2
F8	58.96 $\pm$ 2.11	4.15 $\pm$ 0.14	34.8
F9	59.55 $\pm$ 1.75	4.07 $\pm$ 0.17	35.6

F10	44.28±2.16	4.38±0.16	31.4
F11	46.83±1.76	4.31±0.21	32.7
F12	47.29±1.88	4.24±0.14	33.2
F13	66.27±1.47	3.94±0.11	33.8
F14	68.53±2.18	3.76±0.24	34.2
F15	68.98±1.67	3.55±0.16	34.9
F16	70.16±1.84	3.69±0.21	35.1
F17	70.48±2.18	3.43±0.26	36.6
F18	72.73±1.32	3.38±0.15	36.8

Table 2: Encapsulation efficiency, Mean vesicle size and Zeta Potential values of prepared formulations.

Encapsulation efficiency (EE) in this study is governed chiefly by surfactant chain length and formulation design. Span-20 formulations (F1–F3) with shorter C12 chains and lower phase-transition temperature yield the lowest EE (31.56%–35.73%) due to hydrophilicity (HLB ~8.6) and weaker, less packed bilayers, with only a slight EE rise from 1:1 to 2:1 surfactant:cholesterol. Replacing Span-20 with Span-60 (F4–F6, C18 chains) markedly improves EE to 54.82%–56.96% because the longer saturated chains promote tighter packing, higher rigidity, and reduced permeability; a 2:1 ratio further enhances drug retention. Adding Stearylamine to the optimal 2:1 Span-60 system (F7–F9) yields a gradual EE increase from 58.48% to 59.55% as positive surface charge improves vesicle stability and prevents aggregation. Tween-series show that Tween-20 (F10–F12, high HLB ~16.7) gives moderate EE (44.28%–47.29%) due to membrane fluidity, whereas switching to Tween-60 (F13–F15, C18 chains, HLB ~14.9) substantially boosts EE to 66.27%–68.98% by forming a more rigid bilayer. The peak EE occurs with Tween-60 and Stearylamine (F16–F18), reaching up to 72.73% (notably F18), where C18 chains provide rigidity and Stearylamine confers positive charge, enhancing colloidal stability and minimizing leakage. Overall, C18-chain surfactants (Span-60, Tween-60) outperform C12 analogs, and combining a long-chain surfactant with a charge-inducing agent is the most effective strategy for maximizing Griseofulvin loading in niosomes, with Tween-60/Stearylamine (F18) being the best performer.

Zeta potential and Particle Size Analysis

Zeta potential of the niosome preparations ranged from about -100 to 0 mV, with all formulations F1–F18 exhibiting values from 30.4 to 36.8 mV, indicating sufficient electrostatic repulsion to prevent vesicle aggregation; the negative charge arises from dicetylphosphate, and the addition of Stearylamine (a positively charged agent) further increases surface charge, enhancing stability during storage and administration. Vesicle size trends show an inverse relationship with surfactant-to-cholesterol ratio across all surfactants: increasing from 1:1 to 2:1 generally reduces mean size (e.g., Span-20: 4.86→4.41 μm , Span-60: 4.46→4.11 μm) due to tighter packing and bilayer curvature; Span-60 consistently yields slightly smaller vesicles than Span-20 because its C18 chains promote a denser, more rigid bilayer. Adding Stearylamine to the optimal Span-60 2:1 system (F6–F9) yields a gradual size decrease ($\approx$ 4.07–4.28 μm in F7–F9), attributed to electrostatic repulsion preventing fusion and promoting tighter packing. Tween-60–based formulations (F13–F15) produce markedly smaller vesicles than Tween-20 counterparts (F10–F12) due to the longer C18 chains in Tween-60, which form more rigid, highly curved bilayers; combining Tween-60 with Stearylamine (F16–F18) yields the smallest vesicles overall (e.g., F18 $\approx$ 3.38 μm) thanks to a synergistic effect of rigid bilayer formation and strong electrostatic stabilization, which suppresses aggregation and maintains a discrete vesicle population. Overall, longer alkyl chains (C18) in Span-60/Tween-60 and the inclusion of Stearylamine drive smaller, more stable vesicles, with surfactant-to-cholesterol ratio and charge induction acting as key secondary factors in modulating size and colloidal stability.

Scanning electron microscopy

Scanning electron microscopy (SEM) was employed for morphological study of the optimized formulation. The SEM image of optimized formulation F18 exhibits the spherical nature of niosomal vesicles indicating with smooth surface.

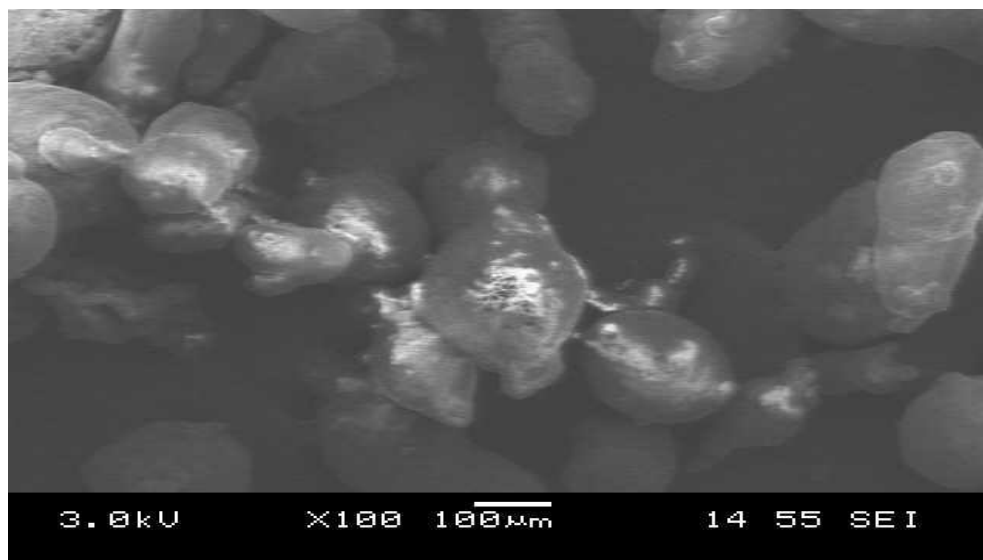


Figure 2: Scanning electron picture of optimized formulation

In vitro release study for niosomal preparation

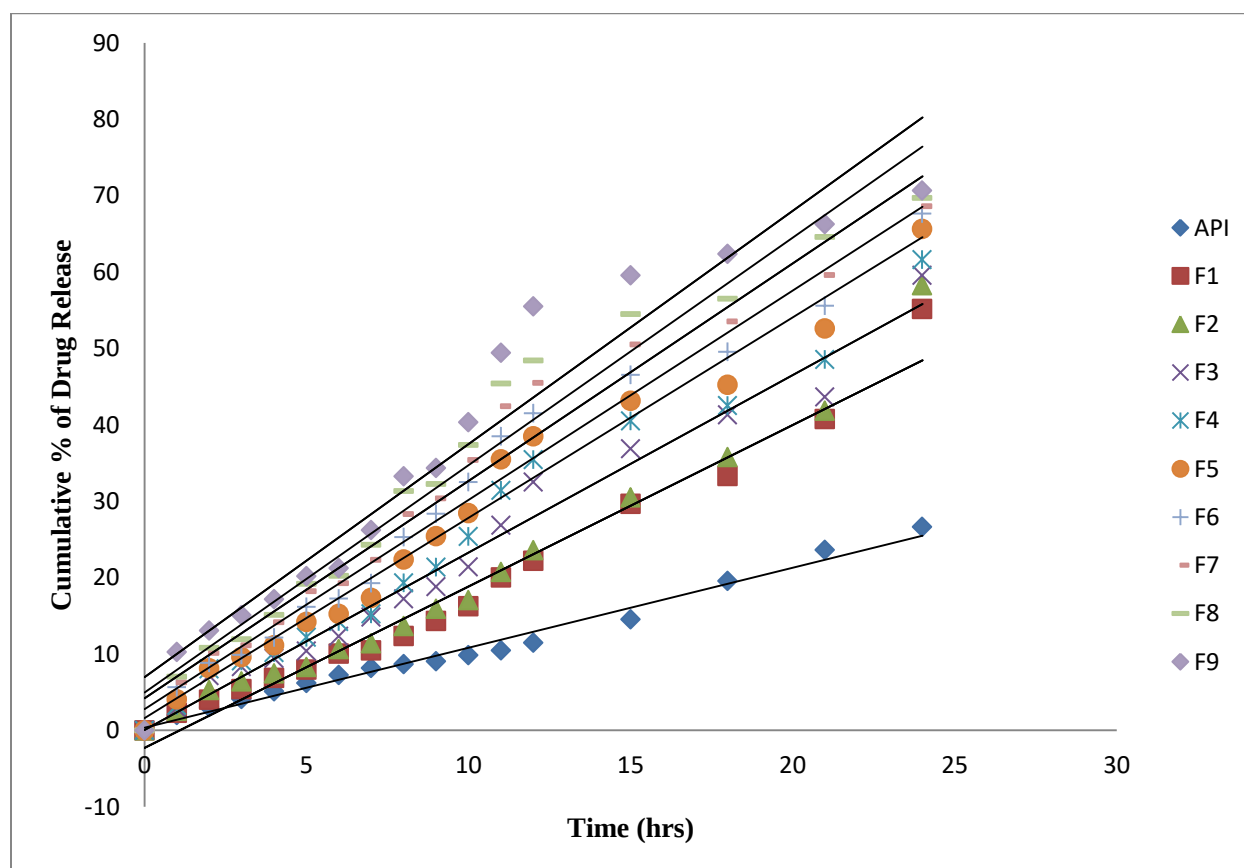


Figure 3: Comparative in-vitro drug release data of the prepared formulation F1 to F9

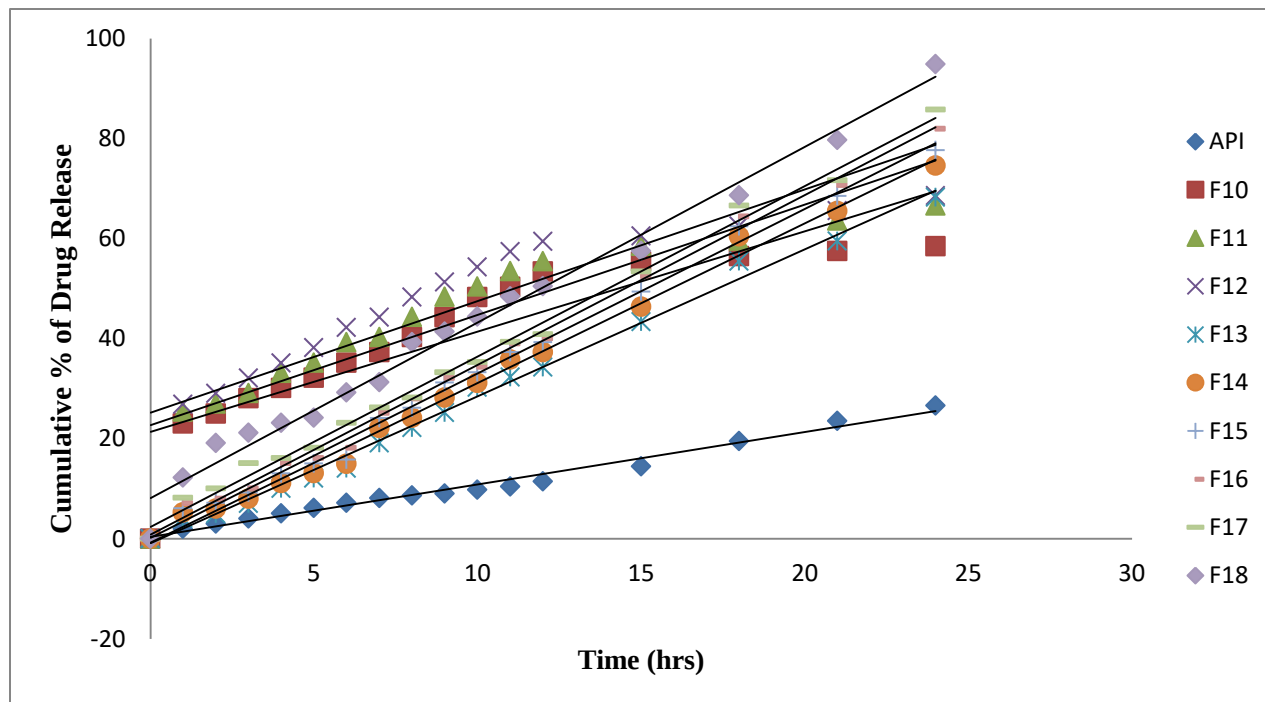


Figure 4: Comparative in-vitro drug release data of the prepared formulation F10 to F18

The study shows that all niosomal formulations (F1–F18) vastly outperform pure Griseofulvin (API) in 24-hour cumulative drug release, with the best formulation (F18) delivering 94.86% versus the API's 26.61%. The core reason is the amphiphilic niosome structure: Griseofulvin's hydrophobic nature limits its aqueous solubility, but encapsulation in the niosomal bilayer boosts apparent solubility and dispersibility. Surfactants act as solubilizers, promoting drug partitioning from vesicles into the medium and markedly improving release kinetics and potential absorption.

Within the formulations, Span-60-based systems (F4–F6) outperform Span-20 ones (F1–F3) at all time points due to longer, saturated C18 chains that yield a more rigid bilayer, reducing burst release and promoting sustained 24-hour release. Release increases with higher surfactant-to-cholesterol ratios (e.g., Span-60: F4 to F6: 61.58% to 67.64%), as cholesterol stabilizes membranes; lower cholesterol yields more fluid membranes and greater diffusion. Tween-60-based formulations (F13–F15) also deliver superior, well-controlled release (68.25% to 77.65%), thanks to a rigid bilayer from its C18 chain, with higher surfactant-to-cholesterol ratios (F15: 77.65%) further enhancing release. The pinnacle is F18 (Tween-60, 2:1, with 0.75% Stearylamine), achieving 94.86% release at 24 hours, due to electrostatic stabilization, optimized bilayer disruption for permeability, and the combined benefits of Tween-60 rigidity and anti-aggregation effects from Stearylamine. Overall, F18 emerges as the optimized formulation, offering high encapsulation efficiency (72.73%), the smallest mean vesicle size (3.38 μm), and near-complete, controlled 24-hour release, indicating enhanced solubility and bioavailability of Griseofulvin.

Drug Release Kinetic Study

The drug release kinetics of optimized formulation F18 were first evaluated using zero-order and first-order models. The results showed that the correlation coefficient (R^2) for the zero-order model (0.995) was markedly higher than that for the first-order model (0.783). This clearly indicates that the release pattern of F18 follows a zero-order kinetic profile, suggesting a near-constant drug release rate over time rather than a concentration-dependent release.

Further evaluation of the release mechanism was performed using the Higuchi and Hixson–Crowell models. The Higuchi model demonstrated an excellent correlation ($R^2 = 0.999$), while the Hixson–Crowell model also showed a good but slightly lower fit ($R^2 = 0.983$). These results imply that the release of drug from F18 is predominantly

governed by diffusion through the polymeric matrix, although the influence of surface area and geometry changes (erosion/attrition) cannot be entirely ruled out.

To confirm the dominating mechanism, the release data were also analyzed using the Korsmeyer–Peppas equation. The obtained release exponent (n) was 0.80, with an R^2 of 0.999. For cylindrical tablets, an n value in the range of $0.45 < n < 0.89$ corresponds to non-Fickian (anomalous) transport, which indicates a combined mechanism of both Fickian diffusion and polymer relaxation/erosion.

Taken together, these findings suggest that Formulation F18 exhibits a zero-order release profile, primarily driven by diffusion as described by the Higuchi model, while the Korsmeyer–Peppas analysis confirms that the overall release mechanism is non-Fickian (anomalous) transport, resulting from the interplay of diffusion and matrix relaxation processes.

STABILITY STUDIES

Percent Entrapment efficiency:

Temperature	Percentage Entrapment Efficiency (n=3)		
	After one month	After two months	After three months
4°C	74.42±2.11	73.79±1.27	73.22±1.43
Room Temperature (25°C)	72.14±1.54	71.82±1.66	71.13±1.21
45°C/75%RH	71.36±2.16	70.44±1.47	69.36±2.11

Table 3: Percent Entrapment efficiency data of Optimized Formulation (F18)

The optimized formulation F18 exhibits strong physical and chemical stability across a three-month study, with an evident inverse relationship between storage temperature and stability. At 4°C, entrapment efficiency (EE) remains nearly intact, decreasing only from 72.73% to 73.22%—a negligible absolute loss (<1.5%). In contrast, storage at 45°C/75% RH causes the greatest degradation, with EE falling to 69.36% (≈3.4% loss). Elevated temperature increases bilayer fluidity, promoting drug leakage and vesicle instability, while high humidity can drive hydrolytic or other chemical degradations, collectively accelerating drug loss under accelerated conditions. Room temperature (25°C) storage shows particularly practical viability, with EE modestly declining from 72.73% to 71.13% over three months, indicating high ambient stability. The Tween-60–based, cholesterol-stabilized, Stearylamine–modified niosomes benefit from a rigid C18 Tween-60–derived bilayer and electrostatic repulsion preventing aggregation, contributing to robust, long-term stability without cold-chain requirements. Overall, F18 proves highly stable under varied conditions, with refrigeration (4°C) offering the longest shelf life, while room temperature storage still preserves efficacy—both favorable for potential commercial development and patient convenience.

In vitro drug release study

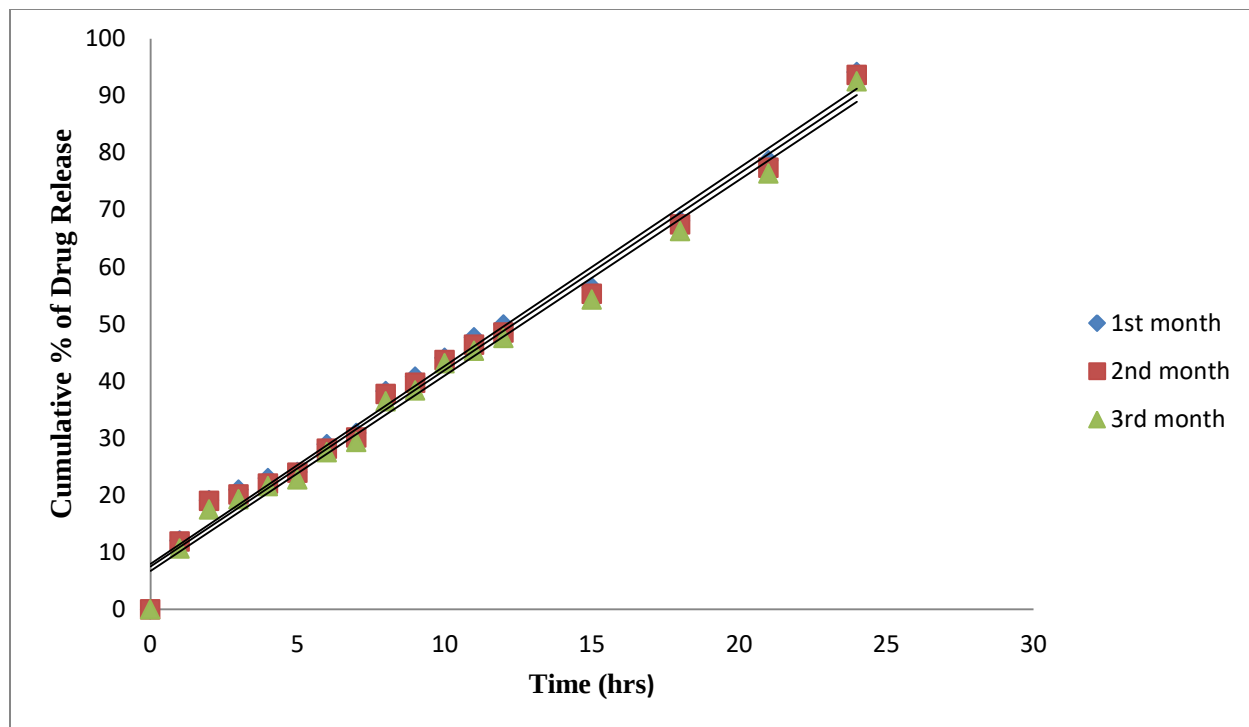


Figure 5: Stability study release data for formulation F18 at room temperature

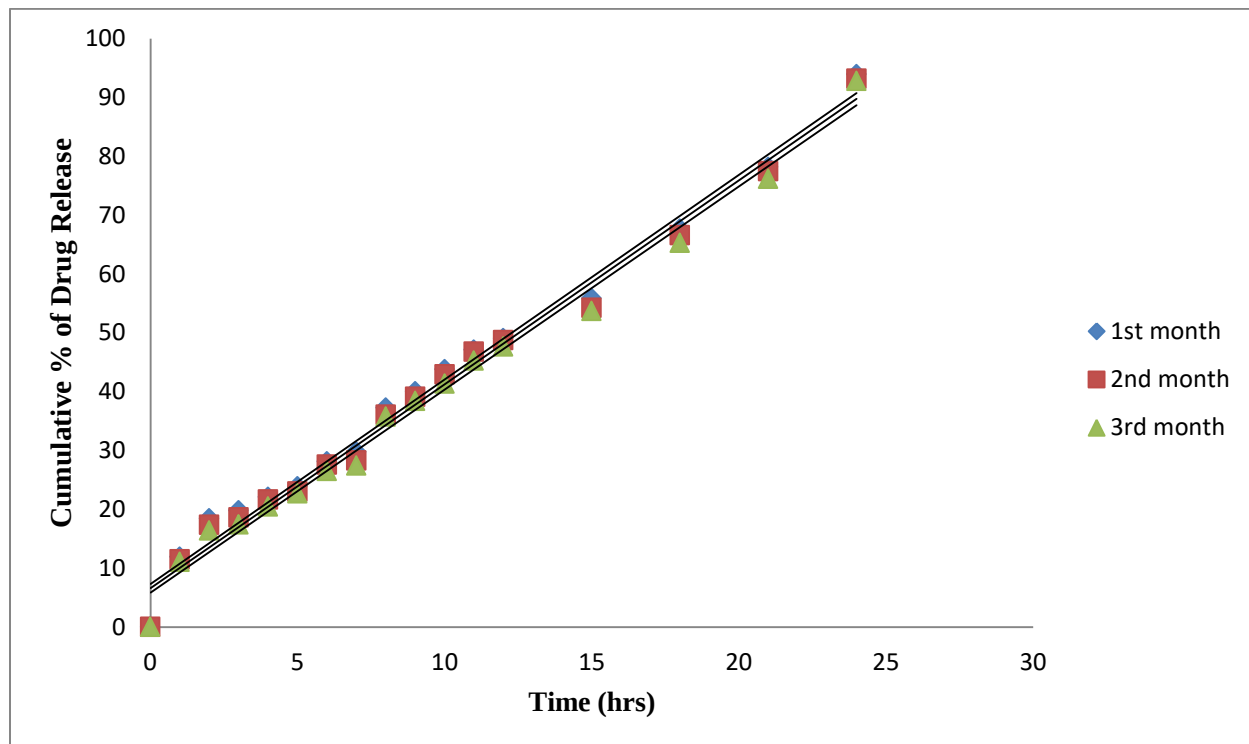


Figure 6: Stability study release data for formulation F18 at 4°C

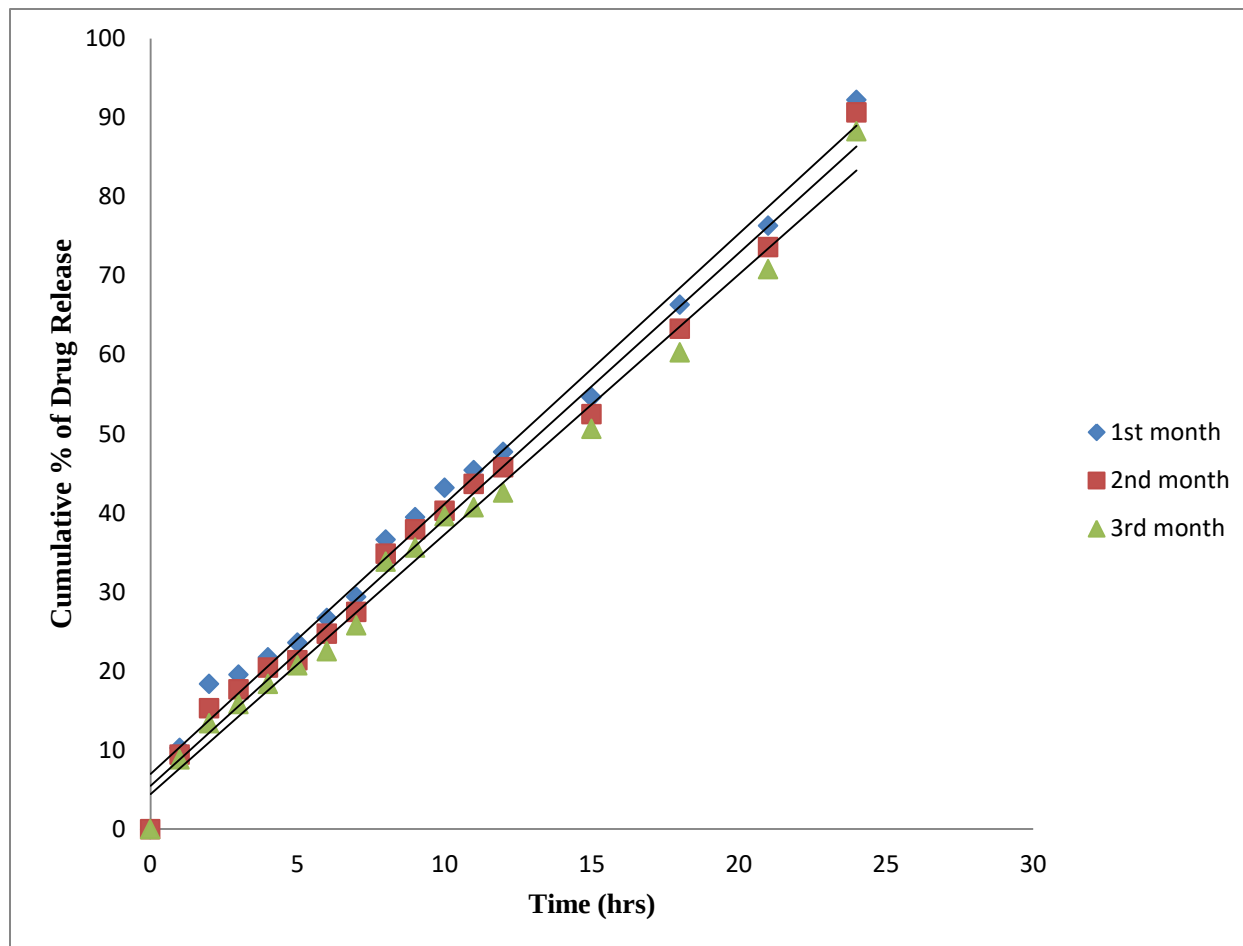


Figure 7: Stability study release data for formulation F18 at 45°C/75%RH

The drug release stability study of Formulation F18 demonstrates that storage at 4°C and 25°C maintains highly consistent release profiles over three months, with only minimal decreases in cumulative drug release (%CDR) at 24 hours (from ~94.14% to ~92.5%), confirming that the anomalous transport mechanism governed by diffusion through the stable Tween-60/cholesterol/Stearylamine matrix remains unchanged. Under accelerated conditions (45°C/75% RH), a moderate reduction in %CDR (to 88.26% at 24 hours) and slightly slower release rates were observed, likely due to increased bilayer rigidity/aggregation and partial drug degradation, though the overall release pattern was preserved without burst effects. These findings confirm that F18 is a robust and reliable delivery system with controlled drug release maintained across storage conditions, supporting room temperature stability and consistent therapeutic efficacy throughout its shelf life.

CONCLUSION

This study successfully developed, optimized, and characterized a niosomal drug delivery system for Griseofulvin, a poorly soluble drug with limited bioavailability. Eighteen formulations were prepared and systematically analyzed based on surfactant type, cholesterol ratio, and the inclusion of Stearylamine. Results showed that surfactants with longer alkyl chains (Tween-60, Span-60) exhibited higher entrapment efficiency and more sustained release compared to shorter-chain surfactants. Optimizing the surfactant-to-cholesterol ratio (2:1) and incorporating Stearylamine further improved stability, reduced particle size, and enhanced drug loading. Among all formulations, F18 (Tween-60:Cholesterol 2:1 with 0.75% Stearylamine) emerged as the optimized candidate, demonstrating the highest entrapment efficiency, smallest vesicle size, and nearly complete sustained release. Kinetic modeling confirmed that drug release was predominantly diffusion-controlled, following Higuchi and Korsmeyer–Peppas

models, while stability studies under different storage conditions verified the robustness and practicality of F18, with room temperature deemed suitable for storage.

The findings establish that niosomal encapsulation is a highly effective strategy to overcome the solubility and bioavailability challenges of Griseofulvin. The optimized formulation (F18) demonstrated superior performance, with high entrapment efficiency (72.73%), ideal vesicle size (3.38 μm), and a complete 24-hour release profile (94.86%). The release followed a non-Fickian transport mechanism, involving both diffusion and matrix relaxation, while stability studies confirmed its integrity and performance under standard storage conditions. Overall, this work highlights the potential of F18 as a reliable and practical niosomal system, and the promising in vitro results justify further in vivo pharmacokinetic and pharmacodynamic studies to advance its clinical application.

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