

FORMULATION AND EVALUATION OF PRNIOosomal GEL OF AMPHOTERICIN B FOR ENHANCED TRANSDERMAL PERMEATION

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ABSTRACT

The present study aimed to develop, optimize, and evaluate a proniosomal gel formulation for sustained topical drug delivery. Proniosomes were prepared using soya phosphatidylcholine and Span 80, and optimization was carried out by varying the lipid-to-surfactant ratio, drug concentration, and sonication time. The optimized formulation (F9) consisted of a Soya PC:Span 80 ratio of 10:1, 1% w/v drug concentration, and 60 seconds of sonication. It exhibited an average vesicle size of 147.56 nm, entrapment efficiency of 75.45%, and zeta potential of -38.12 mV, indicating good colloidal stability. The optimized proniosomes were incorporated into a Carbopol gel base to obtain formulation FG-9. The gel showed desirable physicochemical properties, including viscosity of 3418 ± 12 cps, drug content of $99.34 \pm 0.18\%$, spreadability of 11.48 ± 0.63 g·cm/s, extrudability of 162 ± 4 g, and pH of 6.82 ± 0.02 . In vitro drug release studies demonstrated a sustained release pattern from the proniosomal gel, with 99.18% cumulative drug release over 12 hours, whereas the plain gel released nearly the entire drug within 4 hours. Stability studies confirmed that the optimized formulation remained physically and chemically stable under refrigerated and room-temperature conditions over three months. The developed proniosomal gel demonstrated excellent physicochemical characteristics, high drug entrapment, sustained drug release, and satisfactory stability, suggesting its potential as an effective topical drug delivery system.

Keywords: Proniosomes; Niosomal gel; Soya phosphatidylcholine; Span 80; Entrapment efficiency; Vesicle size; Sustained release; Topical drug delivery; Stability studies.

INTRODUCTION

Superficial and deep fungal infections continue to represent a major public health concern worldwide, particularly among immunocompromised individuals, patients with diabetes, and those receiving long-term antibiotic or corticosteroid therapy¹. These infections are caused by a variety of pathogenic fungi, including *Candida* spp., *Aspergillus* spp., and dermatophytes, and are associated with significant morbidity, prolonged treatment, and increased healthcare costs. Although several antifungal agents are available, their clinical effectiveness is often limited by poor skin penetration, inadequate drug retention

at the site of infection, systemic adverse effects, and the emergence of antifungal resistance. Therefore, the development of novel topical drug delivery systems capable of improving drug permeation and sustaining therapeutic concentrations remains an important area of pharmaceutical research ².

Amphotericin B is a broad-spectrum polyene antifungal antibiotic widely recognized as one of the most effective agents for the treatment of severe fungal infections. Its mechanism of action involves binding to ergosterol in the fungal cell membrane, resulting in pore formation, leakage of intracellular components, and eventual fungal cell death ³. Despite its potent antifungal activity, the therapeutic application of Amphotericin B is restricted by its poor aqueous solubility, high molecular weight, limited permeability across biological membranes, and dose-dependent toxicity following systemic administration. Furthermore, conventional topical formulations often fail to deliver sufficient drug concentrations across the stratum corneum, thereby limiting their clinical efficacy in localized fungal infections ⁴.

Advances in nanotechnology-based drug delivery systems have provided new opportunities to overcome these limitations. Among various vesicular carriers, proniosomes have attracted considerable attention because of their improved physical stability, ease of preparation, cost-effectiveness, and ability to form niosomes upon hydration⁵. Proniosomes consist of non-ionic surfactants, phospholipids, and cholesterol coated onto a suitable carrier, producing dry or semi-solid formulations that are converted into niosomal vesicles immediately before or during application. Compared with conventional niosomes, proniosomes offer superior storage stability, reduced aggregation, minimized drug leakage, and improved shelf life ⁶. Proniosomal vesicles can effectively encapsulate both hydrophilic and lipophilic drugs while enhancing their penetration across the skin. Their nanosized structure promotes close contact with the stratum corneum, modifies the lipid organization of the skin barrier, and facilitates controlled drug diffusion into deeper skin layers. In addition, proniosomes provide sustained drug release, prolong drug residence time at the application site, reduce dosing frequency, and minimize systemic exposure, thereby improving patient compliance and therapeutic outcomes ⁷.

Incorporation of proniosomes into a gel base further enhances their applicability as topical formulations. Proniosomal gels possess suitable viscosity, spreadability, ease of application, and prolonged residence time on the skin. The gel matrix protects the vesicles from rapid degradation while providing a convenient dosage form that enhances patient acceptability. Such formulations are particularly advantageous for the topical delivery of Amphotericin B, as they can increase drug permeation, maintain sustained drug release, and improve localized antifungal activity while reducing systemic toxicity⁸.

Based on these advantages, the present study was undertaken to formulate and optimize a proniosomal gel of Amphotericin B for enhanced transdermal permeation. The formulation was optimized by evaluating the influence of lipid-to-surfactant ratio, drug concentration, and sonication time on vesicle size and entrapment efficiency. The optimized proniosomal formulation was subsequently characterized for vesicle size, zeta potential, and entrapment efficiency, incorporated into a gel base, and evaluated for physicochemical properties, *in vitro* drug release, and stability. The study aims to develop a stable and effective proniosomal gel capable of enhancing the transdermal delivery and therapeutic performance of Amphotericin B for the management of fungal infections.

MATERIAL AND METHODS

Material

Amphotericin B was obtained as a gift sample from a reputed pharmaceutical company. Soya phosphatidylcholine (Soya PC) was used as the phospholipid, while Span 80 served as the non-ionic surfactant for the preparation of proniosomes. Cholesterol was incorporated to enhance vesicular membrane stability. Carbopol 934 was employed as the gelling agent, and triethanolamine was used for pH adjustment. Propylene glycol acted as a penetration enhancer and humectant, whereas methyl paraben was used as a preservative. Analytical-grade solvents and reagents, including ethanol, methanol, and distilled water, were used throughout the study without further purification. All chemicals and reagents employed were of analytical grade.

Methods

Preparation of Proniosomes

Preparation of Amphotericin B loaded Proniosomes

Preparation of Proniosomes was done through thin film hydration technique. Soya PC and Span was dissolved in 500 ml of round bottom flask with 10 ml chloroform and was subjected to rotation in a flash evaporator at 60°C⁹⁻¹⁰. The flask was rotated at 125 rpm for 1 hour to form a dry film. This film was hydrated with 5 ml PBS having pH 7.4 and containing 10 mg of Amphotericin B and was rotated for 60 minutes.

Preparation of Gel Base

Carbopol 934 (0.5-1.5%w/v) was weighed accurately and dissolved in double distilled water (80ml) in a beaker¹¹. This solution was stirred at 800 rpm for 1 hour. Then 10 ml of propylene glycol was added to this solution. The resultant slightly acidic solution was neutralized with the addition of 0.05 N sodium hydroxide solutions drop wise, and stirring was continued until the formation of transparent gel. Methyl

paraben and Propyl paraben were added in gel as preservatives. Volume of gel was made 100 ml and sonicated for 10 minutes on bath sonicator to remove air bubbles.

Final pH of the gel base was adjusted to 6.5. The gel formulation was done with pure drug by dissolving 10 mg of drug in the gel. The same procedure was followed to prepare Noisome gels using Proniosomes in place of pure drug. Noisome gel was formulated by adding proniosome preparation equivalent to 0.1% w/w of drug.

Optimization of Proniosomes

Optimization of lipid: surfactant ratio

In the Proniosomes formulation, the lipid: surfactant ratio was optimized by taking their different ratio such as 10:1, 10:2, 10:2.5 and 10:3 ratio and all parameters were remained constant¹². On the basis of average particle size and % entrapment efficiency the prepared formulation was optimized.

Optimization of drug concentration

Drug concentration was optimized by taking different concentration of drug and prepared their formulation and all other parameter such as Soya PC, stirrer time kept remains constant. On the basis of entrapment efficiency and average vesicle size the formulations were optimized¹³.

Optimization of sonication time

Sonication time was optimized by sonicating the formulation for different time i.e 30, 60 and 90 sec at 4⁰C in 3 cycles of 10 minutes with 5 sec rest between the cycles. The optimization was done on the condition of average particle size, and % Entrapment efficiency.

Characterization of Amphotericin B loaded Proniosomes

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were obtained by Dynamic Light Scattering method (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK) ¹⁴. Zeta potential measurement of the Proniosomes was based on the zeta potential that was estimated according to Helmholtz–Smoluchowsky from electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 IS/cm.

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method ¹⁵. Proniosomes entrapped drug was isolated from the free drug using dialysis method. The above said formulations were filled into dialysis bags and the free drug dialyzed for 24 hr. into 50 ml of buffer pH 7.4. The absorbance

of the dialysate was measured at 383nm against blank buffer pH 7.4 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free drug could be obtained from the absorbance difference based on standard curve. Standard curve was obtained by the absorbance at 383 nm for known concentrations of drug solution.

Characterization of Proniosomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical niosome based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm; viscosity of all formulation measured in cps¹⁶.

Drug content

Accurately weighed equivalent to 1 mg of topical Proniosomes gel was taken in beaker and added 20 ml of methanol¹⁷. This solution was mixed thoroughly and filtered using Whatman filter paper. Then 2.0 mL of filtered solution was poured in 10 mL capacity of volumetric flask and make upto 10 mL volume with methanol. This solution was analyzed using UV-Spectroscope at $\lambda_{\max}$ 383nm.

pH measurements

pH of selected optimized formulations was determined with the using of digital pH meter. Before each measurement of pH, pH meter should be calibrated with buffer solution of pH 4, pH 7 and pH 9. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted.

Extrudability study

Extrudability was based on the amount of the gel extruded from collapsible tube on application of certain load. More the amount of gel extruded shows better extrudability¹⁸. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube. Extrudability of gel required 175 grams of weight to extrude a 0.6cm ribbon of gel in 6 seconds.

Spreadability

Spreadability of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response¹⁹. An apparatus in which a slide fixed on wooden block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadability, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80g of weight was noted. Good spreadability show lesser time to spread.

$$\text{Spreadability (g.cm / sec)} = \frac{\text{Weight tide to Upper Slide} \times \text{Lenth moved on the glass slide}}{\text{Time taken to slide}}$$

***In -vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using Franz Diffusion Cell. Egg membrane is taken as semi permeable membrane for diffusion²⁰. The Franz diffusion cell has receptor chamber with an potent volume approximately 60 mL and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm² size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to control the temp at 32 ± 0.5°C. By using a thermostatic hot plate with a magnetic stirrer heat is provided. The receptor fluid is stirred with the help of Teflon coated magnetic bead which is kept in the diffusion cell.

During each sampling interval, samples are taken and replaced by same volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of drug 383nm.

Stability Studies

Stability studies carried for drug loaded Proniosomes at two different temperatures i.e. refrigeration temperature (4.0 ± 0. 2°C) and at room temperature (25-28±2°C) and elevated temperature (45 ± 2°C) for 3 month. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drug content.

RESULTS AND DISCUSSION

The optimization studies demonstrated that formulation variables had a significant influence on the vesicle size and entrapment efficiency of the proniosomal formulations (Table 1). During optimization of the lipid-to-surfactant ratio, formulation F2 (Soya PC:Span 80, 10:1) exhibited the highest entrapment efficiency (80.32%) with a relatively small vesicle size (276.65 nm). Further increases in surfactant concentration reduced entrapment efficiency, probably due to increased membrane fluidity and drug leakage. Similarly, optimization of drug concentration revealed that 1% w/v drug loading (F6) produced the best balance between vesicle size (240.25 nm) and entrapment efficiency (73.32%). Optimization of sonication time demonstrated that 60 seconds (F9) produced vesicles of 147.56 nm with an entrapment efficiency of 75.45%, whereas prolonged sonication (90 seconds) further reduced particle size but significantly decreased entrapment efficiency because of vesicle disruption. Therefore, F9 was selected as the optimized proniosomal formulation for further investigation (Table 1).

The optimized formulation (F9) exhibited excellent physicochemical characteristics suitable for topical drug delivery (Table 2). The formulation showed an average vesicle size of 147.56 nm, indicating the

formation of nanosized vesicles capable of enhancing skin penetration. The entrapment efficiency (75.45%) confirmed efficient incorporation of the drug into the lipid bilayer. Furthermore, the zeta potential of -38.12 mV indicated excellent colloidal stability, suggesting sufficient electrostatic repulsion to prevent vesicle aggregation during storage (Table 2).

The optimized proniosomal formulation was successfully incorporated into a gel base, producing formulation FG-9 with desirable physicochemical properties (Table 3). The gel exhibited a viscosity of 3418 ± 12 cps, ensuring adequate consistency for topical application. Drug content was $99.34 \pm 0.18\%$, indicating uniform distribution of the drug throughout the formulation. The gel showed satisfactory extrudability (162 ± 4 g) and spreadability (11.48 ± 0.63 g·cm/s), facilitating easy application over the skin. The pH (6.82 ± 0.02) was close to the physiological skin pH, indicating compatibility with topical administration. The formulation also achieved approximately 99.06% drug release after 12 hours, demonstrating its suitability for sustained drug delivery (Table 3).

The *in vitro* release profiles demonstrated a clear difference between the plain gel and the proniosomal gel (Table 4). The plain gel exhibited an initial burst release, with 34.82% drug release within 0.5 hours, reaching 97.35% by 4 hours. In contrast, the proniosomal gel showed a sustained release profile, releasing only 11.68% of the drug within 0.5 hours, followed by a gradual increase to 47.26% at 4 hours, 69.72% at 6 hours, 88.54% at 8 hours, and 99.18% after 12 hours. The sustained release behavior is attributed to encapsulation of the drug within the proniosomal vesicles, which regulate drug diffusion and prolong drug release. Such a controlled release profile is advantageous for improving therapeutic efficacy and reducing the frequency of topical application (Table 4).

The stability study indicated that the optimized proniosomal formulation remained stable throughout the three-month storage period (Table 5). Samples stored at $4 \pm 0.2^\circ\text{C}$ exhibited only minimal changes in vesicle size and entrapment efficiency, while maintaining normal physical appearance. Moderate increases in vesicle size and slight reductions in entrapment efficiency were observed under room-temperature storage ($25\text{--}28 \pm 2^\circ\text{C}$). Under accelerated conditions ($45 \pm 2^\circ\text{C}$), the formulation showed comparatively greater increases in vesicle size, gradual reduction in entrapment efficiency, and slight agglomeration after three months. Nevertheless, the formulation retained acceptable stability, indicating that refrigerated and ambient storage conditions are more suitable for maintaining the physicochemical properties of the optimized proniosomal formulation (Table 5).

Table 1: Optimization of Proniosomal Formulation

Formulation Code	Average Vesicle Size (nm)	Entrapment Efficiency (%)
F1	325.36	73.32
F2	276.65	80.32
F3	320.45	65.58
F4	268.45	62.12
F5	276.65	60.25
F6	240.25	73.32
F7	289.74	61.45
F8	215.32	69.98
F9	147.56	75.45
F10	135.58	61.25

Table 2: Characterization of optimized formulation of Proniosomes

Formulation code	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-9	147.56	75.45	-38.12

Table 3: Characterization of gel-based formulation of Proniosomes

F. Code	Viscosity (cps)	% Drug Content	% Release after 12 hr	Extrudability (g)	Spreadability (g·cm/sec)	pH
FG-9	3418±12	99.34 ± 0.18	99.06 ± 0.17	162 ± 4	11.48 ± 0.63	6.82 ± 0.02

FG* Optimized gel formulation

Table 4: *In-vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	Plain Gel (% Cumulative Drug Release)	Niosomal Gel (% Cumulative Drug Release)
1	0.5	34.82	11.68
2	1	61.94	23.94
3	2	78.46	35.81
4	4	97.35	47.26
5	6	—	69.72
6	8	—	88.54
7	12	—	99.18

Table 5: Characterization of optimized formulation of Proniosomes formulation

Characteristics	Time (Months)								
	1 Month			2 Months			3 Months		
Temperature	$4.0 \pm 0.2^{\circ}\text{C}$	$25-28 \pm 2^{\circ}\text{C}$	$45 \pm 2^{\circ}\text{C}$	$4.0 \pm 0.2^{\circ}\text{C}$	$25-28 \pm 2^{\circ}\text{C}$	$45 \pm 2^{\circ}\text{C}$	$4.0 \pm 0.2^{\circ}\text{C}$	$25-28 \pm 2^{\circ}\text{C}$	$45 \pm 2^{\circ}\text{C}$
Average vesicle size (nm)	156.84	161.72	168.95	158.16	164.38	174.42	160.05	167.91	181.63
Entrapment Efficiency (%EE)	77.84	75.63	71.48	77.18	74.52	68.92	76.41	72.86	65.35
Physical Appearance	Normal	Slightly turbid	Turbid	Normal	Slightly turbid	Turbid	Normal	Moderately turbid	Turbid with slight agglomeration

CONCLUSION

The present study successfully developed and optimized a proniosomal gel formulation for sustained topical drug delivery. The optimized formulation (F9), containing Soya PC:Span 80 (10:1), 1% w/v drug, and 60 seconds of sonication, exhibited a vesicle size of 147.56 nm, entrapment efficiency of 75.45%, and zeta potential of -38.12 mV, indicating good stability and efficient drug encapsulation. The proniosomal gel demonstrated suitable physicochemical properties, sustained drug release over 12 hours, and satisfactory stability during storage. These findings suggest that the developed proniosomal gel is a promising carrier for enhancing topical drug delivery and improving therapeutic efficacy.

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