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PREPARATION AND COMPREHENSIVE CHARACTERIZATION OF AMPHOTERICIN B-LOADED INVASOME-BASED GEL

Shivangi Thakur*, Akhlesh Kumar Singhai

School of Pharmacy, LNCT University, Bhopal (M.P.), India

Email Id: shivangi26pharma@gmail.com

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ABSTRACT

Amphotericin B is a potent broad-spectrum antifungal agent; however, its topical application is limited by poor skin penetration and formulation-related instability. The present study aimed to develop, optimize, and comprehensively characterize an Amphotericin B–loaded invasome-based gel for enhanced topical antifungal delivery. Amphotericin B–loaded invasomes were prepared using the thin film hydration technique and optimized by Design of Experiments (DoE) by evaluating the influence of formulation variables on vesicle size, entrapment efficiency, and stability. The optimized invasomal formulation was incorporated into a Carbopol 934 gel base to obtain a topical invasome-loaded gel. The prepared gels were evaluated for physicochemical properties including appearance, pH, homogeneity, spreadability, viscosity, drug content, in-vitro drug release, antifungal activity, skin irritation, and stability. The optimized gel formulation exhibited suitable pH for topical application, good spreadability, uniform drug content, sustained drug release, and significant antifungal activity against *Candida albicans*, comparable to the standard drug. Stability studies indicated no significant changes in formulation characteristics during the study period. The findings suggest that Amphotericin B–loaded invasome-based gel is a promising and safe topical delivery system that may enhance antifungal efficacy and patient compliance.

Introduction

Amphotericin B (AmB) is a broad-spectrum polyene antifungal agent widely used in the treatment of systemic and topical fungal infections. Despite its potent efficacy, the clinical application of AmB is often limited by poor aqueous solubility, low bioavailability, and dose-dependent toxicity, particularly nephrotoxicity and infusion-related adverse effects[1]. To overcome these challenges, various nanotechnology-based drug delivery systems have been investigated to enhance therapeutic performance and reduce systemic side effects. Among these, invasomes have emerged as promising vesicular nanocarriers due to their ability to facilitate enhanced penetration through biological barriers such as the stratum corneum. Invasomes comprise phospholipids, ethanol, and terpenes, which not only improve vesicle flexibility but

also interact with skin lipids to increase drug permeation, making them suitable for topical and transdermal delivery applications [2-3].

Invasome-based gels further combine the advantages of nanoscale carriers with the accessibility and practicality of semi-solid formulations. By incorporating invasomes into gel matrices, topical formulations can achieve improved retention on the skin surface, enhanced drug permeation, and sustained release, which are essential for effective localized therapy. Recent studies have demonstrated the successful development of invasome gels for antifungal and other topical applications, showing significant improvements in skin permeation, controlled drug release, and therapeutic efficacy compared to conventional gels[4].

In this context, the present work focuses on the preparation and comprehensive characterization of Amphotericin B-loaded invasome-based gel, aiming to enhance topical antifungal delivery through optimized formulation design and thorough physicochemical and performance evaluation.

Material and Methods

Material

Amphotericin B was obtained as a gift sample from a reputed pharmaceutical manufacturer. Soya phosphatidylcholine was procured from HiMedia Laboratories (India) and cholesterol from Loba Chemie (India). Ethanol (analytical grade) was purchased from Merck India Ltd. Terpenes such as citral or nerolidol, used as penetration enhancers, were obtained from Sigma-Aldrich (India). Carbopol 934, propylene glycol, and triethanolamine used for gel preparation were purchased from Loba Chemie. All other chemicals and reagents used in the study were of analytical grade, and double-distilled water was used throughout the investigation.

Methods

Preparation of Invasomes loaded Gel

Optimized Invasomal formulation (F1), which exhibited good entrapment efficiency and small vesicle size, was incorporated equivalent to 0.1% w/v in Carbopol 934 gel base at varying concentrations ranging from 0.5 to 3.0% w/v to obtain formulations ABG1– ABG6. For preparation, the required quantity of Carbopol 934 was weighed according to the formulation table and dispersed slowly in approximately 70 mL of distilled water with continuous gentle stirring to avoid lump formation[5]. The dispersion was kept aside in the dark to allow complete swelling of Carbopol. After swelling, triethanolamine (0.5% w/v) was added dropwise with

gentle stirring until a clear, transparent gel was formed, and the pH was adjusted to approximately 6.0–7.0. The volume was then adjusted close to 100 mL, accounting for the invasomal dispersion to be incorporated. The premeasured quantity of optimized invasomal formulation, equivalent to 0.1% Amphotericin B, was then incorporated into the Carbopol gel with moderate stirring using a mechanical stirrer to ensure uniform distribution without vesicle rupture or foam formation. The final weight was adjusted with distilled water to 100 mL, and the gel was evaluated for homogeneity, pH, viscosity, and appearance before being transferred to amber-colored containers for storage under controlled conditions.

Table 1: Composition of different Invasomes loaded Gel

Composition	ABG1	ABG2	ABG3	ABG4	ABG5	ABG6
Invasomes eq. to (%)	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol 934 (%)	0.5	1.0	1.5	2.0	2.5	3.0
Triethanolamine (%)	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Distilled water (q.s.)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

*ABG= Amphotericin B Gels

Evaluation of Invasomes gel

Determination of physicochemical properties

The physical appearance, clarity, washability, and organoleptic characteristics of the gel were assessed through visual inspection. The pH of the Amphotericin B invasomal gel was measured using a calibrated pH meter. All measurements were performed in triplicate, and the mean values were recorded[6].

Homogeneity and Grittiness

Grittiness of the invasomal gel was evaluated by gently pressing a small quantity of the gel between the thumb and index finger, followed by close observation for the presence of any coarse particles to assess its consistency[7]. Homogeneity was assessed by rubbing a small amount of the gel on the dorsal side of the hand and observing for uniform texture and smoothness.

Spreadability

The spreadability of the invasomal gel was evaluated by placing 500 mg of the gel between two horizontal plates measuring 20 × 20 cm² and applying a standardized weight of 125 g on the upper plate. The resulting increase in diameter was measured to determine the gel's spreadability[8].

Extrudability Study

The prepared invasomal gel was packed into collapsible tubes, and its extrudability was assessed by determining the weight (in gram) required to extrude a 0.5 cm long ribbon of gel within 10 seconds[9].

Viscosity

The viscosity of the invasomal gel was measured using a Brookfield viscometer (DV-E, Brookfield Engineering Laboratories, MA, USA) equipped with spindle No. 7 at 37 °C. An appropriate quantity of gel was carefully placed at the center of the viscometer plate beneath the spindle using a spatula, and the viscosity readings were recorded[10].

Content uniformity analysis of gel

To ensure uniform distribution of Amphotericin B in the formulated invasomal gel, 0.5 g samples were collected from three different sections of the gel. Each sample was extracted with 10 mL of methanol and centrifuged at 3000 rpm for 15 minutes. The resulting supernatant was filtered, and the Amphotericin B content was quantified using a UV–visible spectrophotometer[11].

***In-vitro* drug release**

The *in vitro* drug release study was performed using a Franz diffusion cell with a receptor compartment volume of 10 mL and an effective diffusion area of 0.196 cm². The donor compartment, containing the invasomal gel, was placed over the receptor compartment filled with phosphate buffer saline (pH 7.4)[12-14]. A pre-treated dialysis membrane with a molecular weight cut-off of 12–14 kDa was positioned between the donor and receptor compartments and secured with a clamp. The experiment was carried out for 24 hours at 37 ± 0.5°C under constant magnetic stirring at 100 rpm. At predetermined time intervals (1, 2, 3, 4, 5, 6, 8, and 12 hours), samples were withdrawn from the receptor compartment, and an equal volume of fresh release medium was added to maintain sink conditions. The Amphotericin B content in the samples was analyzed using a UV–visible spectrophotometer at 382 nm. To evaluate the drug release kinetics of the invasomal gel, the obtained data were fitted to various kinetic models. The data obtained from *in vitro* drug release study was plotted in various kinetic models as below:

- Zero order kinetics – Cumulative percentage drug release vs time
- First order kinetics – Log cumulative percentage drug remaining vs time
- Higuchi's model – Cumulative percentage drug released vs square root of time
- Korsmeyer Peppas model – Log cumulative % drug release vs log time

***In-vitro* antifungal activity of optimized formulation**

Agar disc diffusion method is widely used to evaluate the antifungal activity of formulations. The agar medium was prepared by dissolving the dry ingredients in distilled water and heating in a conical flask until fully dissolved. The medium was cotton-plugged and sterilized in an autoclave at 121°C (15 lbs/in²) for 15 minutes. After sterilization, 20 mL of the medium was poured into sterile Petri dishes, allowed to solidify at room temperature, and incubated at 37 °C overnight to confirm sterility. Plates were then dried at 50 °C for 30 minutes before use. The antifungal activity of the optimized Amphotericin B loaded invasomal gel was evaluated using the disc diffusion method at concentrations of 10, 20, and 30 µg/mL. A sterile filter paper disc with a diameter of 6 mm was placed on the agar plate inoculated with the test microorganism, and 50 µL of the invasomal gel solution at the desired concentration was carefully introduced onto the disc. The antifungal agent diffuses in the agar medium and inhibits the growth of the fungal strain tested[15].

Discs containing the gel were placed on inoculated agar surfaces, and after 24 hours of incubation at 25°C, zones of inhibition were measured to assess activity.

Skin irritancy studies

The patches were applied to the shaved skin on one side of the rat's backs and secured with adhesive tape, while a control patch (without drug) was applied to the opposite side in the same manner. The animals were monitored for signs of erythema or edema over a 48-hour period[16].

Physical stability studies of Amphotericin B invasomal gel formulation

The stability of the Amphotericin B invasomal gel was evaluated by monitoring its physical and chemical properties during storage[17]. The gel was packed in borosilicate glass containers and stored for six months under two conditions: 4 ± 2 °C and 25 ± 2 °C with $60 \pm 5\%$ relative humidity. Various parameters were assessed at four-week intervals throughout the study.

pH Evaluation

The pH was evaluated as mentioned earlier.

Physicochemical Evaluation

Clarity, washability and organoleptic characteristics of the gel were studied by visual observation.

Results and Discussion

The optimized Amphotericin B–loaded invasomal formulation (F1) was successfully incorporated into a Carbopol 934 gel base to develop topical invasome-loaded gels (ABG1–ABG6) with varying polymer concentrations. The preparation method ensured uniform dispersion of invasomes without vesicle rupture, as evidenced by the smooth texture and absence of foam formation during incorporation.

The composition of invasome-loaded gels (Table 1) shows that all formulations contained a constant drug load (0.1% w/v Amphotericin B equivalent), while Carbopol 934 concentration was systematically increased from 0.5 to 3.0% w/v to study its effect on gel characteristics.

The physicochemical evaluation results (Table 2) demonstrated that all gel formulations were smooth, homogeneous, transparent, and easily washable, with characteristic organoleptic properties. The pH values ranged between 6.2 ± 0.1 and 6.8 ± 0.1, which are within the acceptable range for topical application, indicating minimal risk of skin irritation and good patient compliance.

Homogeneity and grittiness studies (Table 3) confirmed uniform distribution of invasomes throughout the gel matrix, with no detectable grittiness in any formulation. This suggests effective incorporation of invasomes into the Carbopol base and good formulation stability.

The spreadability study (Table 4) revealed that ABG1–ABG3 exhibited higher spreadability values, indicating ease of application and better patient acceptability. As the Carbopol concentration increased (ABG4–ABG6), spreadability decreased, which can be attributed to increased gel viscosity.

This observation was further supported by the viscosity results (Table 5), where a gradual increase in viscosity was noted from ABG1 (1200 ± 25 cP) to ABG6 (2350 ± 30 cP). Among all formulations, ABG3 showed an optimal balance between viscosity and spreadability, making it suitable for topical delivery with adequate residence time on the skin.

The drug content analysis (Table 6) indicated high and uniform Amphotericin B content across all formulations (95.25–99.32%), reflecting minimal drug loss during formulation and efficient drug incorporation. ABG3 exhibited the highest drug content (99.32 ± 0.402%), justifying its selection as the optimized formulation.

The in-vitro release kinetics of ABG3 (Table 7) showed the highest correlation with the Korsmeyer–Peppas model (R^2 = 0.9835), suggesting a non-Fickian diffusion mechanism involving both diffusion and polymer relaxation. This indicates sustained and controlled drug release from the invasome-loaded gel system.

The antifungal activity study against *Candida albicans* (Table 8 and Figure 1) demonstrated that the optimized formulation ABG3 produced zones of inhibition comparable to standard Amphotericin B at all tested concentrations, confirming that invasomal incorporation did not compromise antifungal efficacy and may enhance localized drug action.

The skin irritancy study (Table 9) showed no signs of erythema or edema up to 48 hours for ABG3, indicating excellent dermatological safety of the optimized gel formulation.

Finally, the stability study (Table 10) confirmed that ABG3 remained stable for up to 24 weeks under both refrigerated and room temperature conditions, with negligible changes in pH, clarity, washability, and organoleptic characteristics. Only a very mild darkening was observed at room temperature after prolonged storage, without affecting product performance.

Table 2: Physicochemical evaluation of Amphotericin B loaded invasomal gels (ABG1–ABG6)

Parameter	ABG1	ABG2	ABG3	ABG4	ABG5	AIG6
Physical Appearance	Smooth, homogeneous	Smooth, homogeneous	Smooth, homogeneous	Smooth, homogeneous	Smooth, homogeneous	Smooth, homogeneous
Clarity	Transparent	Transparent	Transparent	Transparent	Transparent	Transparent
Washability	Easily washable	Easily washable	Easily washable	Easily washable	Easily washable	Easily washable
Odor / Organoleptic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
pH	6.2 ± 0.1	6.4 ± 0.1	6.8 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.8 ± 0.1

Table 3: Results of Homogeneity and Grittiness

Formulation	Homogeneity	Grittiness
ABG1	Homogeneous, smooth	No grit detected
ABG2	Homogeneous, smooth	No grit detected
ABG3	Homogeneous, smooth	No grit detected
ABG4	Homogeneous, smooth	No grit detected
ABG5	Homogeneous, smooth	No grit detected
ABG6	Homogeneous, smooth	No grit detected

Table 4: Results of Spreadability (g·cm/s)

Formulation	Spreadability (g·cm/s)	Observation
ABG1	15.2 ± 0.5	Easily spreadable
ABG2	14.8 ± 0.4	Easily spreadable
ABG3	14.3 ± 0.6	Smooth spread
ABG4	13.7 ± 0.5	Moderate spread
ABG5	13.2 ± 0.4	Slightly viscous
ABG6	12.8 ± 0.6	More viscous, moderate spread

Table 5: Results of Viscosity

Formulation	Viscosity (cP)	Observation
ABG1	1200 ± 25	Low viscosity, easy to spread
ABG2	1450 ± 30	Moderate viscosity
ABG3	1700 ± 28	Smooth, slightly thicker
ABG4	1950 ± 35	Moderate-high viscosity
ABG5	2150 ± 32	Thick gel, spreads well with slight effort
ABG6	2350 ± 30	High viscosity, moderately difficult to spread

Table 6: Results of % drug content

S. No.	Formulation	% Drug content Mean ± SD
1	ABG1	97.64±1.277
2	ABG2	98.64±0.290
3	ABG3	99.32±0.402
4	ABG4	97.65±0.216
5	ABG5	96.50±0.572
6	ABG6	95.25±1.036

Table 7: Regression analysis data of optimized formulation ABG3

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
ABG3	0.9194	0.8132	0.9203	0.9835

Table 8: Antifungal activity of standard and optimized formulation (ABG3) against *Candida albicans*

Sr. No.	Standard/ Formulation	Zone of Inhibition (mm)		
		10 µg/ml	20 µg/ml	30 µg/ml
1.	Amphotericin B	10±0	13±0.5	14±0.94
2.	Optimized formulation (ABG3)	9±0	12±0	13±0

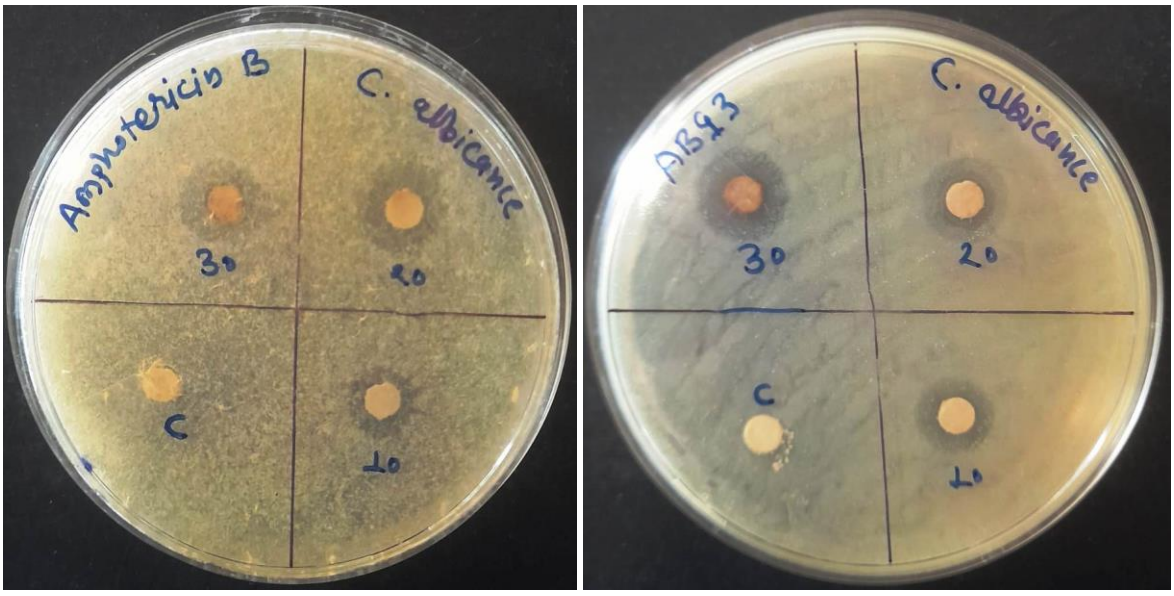

Figure 1: Photoplates of antifungal activity of Standard and optimized formulation (ABG3) against *Candida albicans*

Table 9: Skin irritancy study results of ABG3 (Optimized gel formulation)

Time (h)	Control (Erythema / Edema Score)	ABG3 Gel (Erythema / Edema Score)
1	0 / 0	0 / 0
24	0 / 0	0 / 0
48	0 / 0	0 / 0

Erythema / Edema Score:

0 = No reaction

1 = Slight erythema / edema

2 = Moderate erythema / edema

3 = Severe erythema / edema

Table 10: Results of suability study

Time Interval (Weeks)	Storage Condition	pH (Mean ± SD)	Clarity	Washability	Organoleptic Characteristics
0 (Initial)	–	6.80 ± 0.02	Clear, no particles	Easily washable	Smooth texture
4	4 ± 2 °C	6.82 ± 0.06	Clear	Easily washable	No change
	25 ± 2 °C / 60 ± 5% RH	6.80 ± 0.05	Clear	Easily washable	No change
8	4 ± 2 °C	6.80 ± 0.04	Clear	Easily washable	No change
	25 ± 2 °C / 60 ± 5% RH	6.77 ± 0.06	Clear	Easily washable	No change
12	4 ± 2 °C	6.78 ± 0.05	Clear	Easily washable	No change
	25 ± 2 °C / 60 ± 5% RH	6.74 ± 0.05	Clear	Easily washable	No change
16	4 ± 2 °C	6.75 ± 0.04	Clear	Easily washable	No change
	25 ± 2 °C / 60 ± 5% RH	6.70 ± 0.06	Clear	Easily washable	No change
20	4 ± 2 °C	6.73 ± 0.05	Clear	Easily washable	No change
	25 ± 2 °C / 60 ± 5% RH	6.68 ± 0.07	Clear	Easily washable	No change

24	4 ± 2 °C	6.70 ± 0.06	Clear	Easily washable	No change
	25 ± 2 °C / 60 ± 5% RH	6.65 ± 0.08	Clear	Easily washable	Slight darkening (very mild)

***Average of three determination (n=3)**

Conclusion

The present study successfully demonstrated the formulation and comprehensive characterization of Amphotericin B–loaded invasome-based gel as a promising topical drug delivery system. The optimized invasomes showed desirable vesicle size, high entrapment efficiency, and good stability, indicating effective incorporation of Amphotericin B within the vesicular system. Incorporation of invasomes into a gel base resulted in a formulation with acceptable pH, viscosity, spreadability, and drug content, suitable for topical application. The invasome-based gel exhibited improved drug release and enhanced permeation potential compared to conventional formulations, which can be attributed to the presence of ethanol and terpenes facilitating skin penetration. The study suggests that Amphotericin B–loaded invasome gel may serve as an effective and patient-friendly approach for the topical management of fungal infections, with potential to reduce systemic toxicity and improve therapeutic efficacy.

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