International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

FORMULATION AND EVALUATION OF NYSTATIN LOADED INVASOMES GEL FOR MANAGEMENT OF TOPICAL DISEASES

Gunjan Harode*, Sweety Tiwari, Akhlesh Kumar Singhai
School of Pharmacy, LNCT University, Bhopal (M.P.)

***Correspondence Info:**
Gunjan Harode
School of Pharmacy, LNCT University, Bhopal (M.P.)
Email: gunjanharode@gmail.com

***Article History:**

Received: 13/02/2025
Revised: 27/02/2025
Accepted: 20/03/2025

ABSTRACT

The present study focuses on the formulation and evaluation of a Nystatin-loaded invasomal gel for the effective treatment of topical fungal infections. Invasomes, flexible vesicular carriers composed of phospholipids, ethanol, and terpenes, were employed to enhance the skin penetration and bioavailability of Nystatin. Six different invasomal formulations (N1–N6) were prepared and characterized for vesicle size and entrapment efficiency, with formulation N2 showing the best results. The optimized invasomal formulation was then incorporated into a Carbopol 934P-based gel, and further evaluated for physicochemical parameters, drug content, spreadability, viscosity, and in vitro drug release. The optimized gel formulation (IG2) exhibited controlled drug release over 12 hours, following first-order kinetics, and showed good stability over six months. The findings suggest that invasomal gel is a promising topical delivery system for antifungal therapy, offering better patient compliance and therapeutic outcomes compared to conventional formulations.

Keywords: Nystatin, Invasomes, Topical gel, Controlled release, Antifungal agent, Skin delivery, Vesicular system, Entrapment efficiency, In vitro drug release.

INTRODUCTION

Topical fungal infections are widespread and often challenging to manage due to the barrier function of the skin, poor drug penetration, and recurrent nature of infections. Nystatin, a polyene antifungal antibiotic, has been widely used for the treatment of cutaneous and mucosal fungal infections caused by *Candida* species. However, its therapeutic efficacy is limited by poor skin permeability and local irritation at high concentrations (Patel *et al.*, 2019).

To enhance the efficacy of nystatin for topical application, novel vesicular systems such as invasomes have been explored. Invasomes are modified liposomes composed of phospholipids, ethanol, and terpenes, which significantly enhance drug penetration through the stratum corneum by disrupting lipid packing and increasing fluidity (Verma *et al.*, 2003). This system not only improves skin delivery but also prolongs drug retention at the target site, potentially reducing dosing frequency and side effects.

The incorporation of nystatin into an invasome-based gel offers the advantage of sustained release, enhanced antifungal activity, and better patient compliance due to ease of application. Gelling agents such as Carbopol or HPMC further aid in improving the stability and spreadability of the formulation (Mourya & Inamdar, 2017).

This study aims to formulate and evaluate a nystatin-loaded invasome gel for topical application, focusing on physicochemical properties, drug release profile, and antifungal

efficacy to provide an effective alternative for the management of topical fungal diseases.

MATERIALS AND METHODS

Materials

The materials used for the formulation of the nystatin-loaded invasome gel were procured from reputed suppliers to ensure quality and reliability. Nystatin, the active antifungal agent, was obtained from Bioplus Life Science, Bangalore. Soya phosphatidylcholine, a key component for invasome formation, was sourced from Ash Chemie India, Thane. Buffer salts like disodium hydrogen phosphate, dipotassium hydrogen orthophosphate, and sodium chloride, along with gelling agent Carbopol 934P and solvents such as methanol, ethanol, and chloroform were purchased from S.D. Fine Chem. Ltd. and Qualigens Fine Chemicals, Mumbai. Propylene glycol was also obtained from S.D. Fine Chem. Ltd., serving as a penetration enhancer and humectant in the gel formulation.

Methods

Formulation of Invasome of Nystatin

Invasomes of Nystatin were prepared by mechanical dispersion technique (Table 7.1). Soya phosphatidylcholine was added to ethanol and the mixture was vortexed for 5 minutes. Nystatin and terpenes were added while the mixture was constantly vortexed and sonicated for 5 minutes. Under constant vortexing, a fine stream of distilled water (up to 10% v/v) was added with a syringe to the mixture. To obtain the final invasomal preparation, the formulation was vortexed for an additional 5 minutes (Dragicevic-Curic *et al.,* 2010).

Characterization of Nystatin-loaded invasomes

Entrapment Efficiency

Ultracentrifugation method was used for determining the percentage drug entrapment of the invasomal formulation. 1 ml of invasomal formulation was centrifuged for 40 minutes in an ultra-centrifuge (at 15000 rpm). The supernatant was further diluted with ethanol. UV-visible spectrophotometry was used for analysing the Nystatin content at a wavelength of 298 nm (Aggarwal and Kaur, 2005). Percentage drug entrapment was calculated using the equation:

$$\% \text{ Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Amount of Free Drug}}{\text{total amount of drug}} \times 100$$

Vesicle Size

Microscopic analysis was performed to determine the average size of prepared invasomes. Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip (Ayman *et al.,* 2001; Amnuaikit *et al.,* 2018; Manchanda and Sahoo, 2018).

Preparation of Nystatin loaded Invasomal Gel

Invasomal formulation having good entrapment efficiency, small particle sizewas incorporated in Carbopol 934 gel base. 1%, 2% and 3% i.e IG-1 (1%), IG-2 (2%) and IG-3 (3%) Carbopol gel base was prepared by mixing carbopol 934 with distilled water and leaving it in the dark to allow the gelling agent to completely swell. Triethanolamine was added to the dispersion drop by drop to create a transparent viscous gel. Finally, the optimised invasomal formulation was gently mixed with Carbopol gel base which was moderately stirred with a mechanical stirrer (Singh *et al.,* 2020).

Evaluation of invasomal gel

Determination of physiochemical properties

Physical appearance, clarity, washability, occlusiveness and organoleptic characteristics of the gel were studied by visual observation. A pH metre was used to evaluate the pH of Nystatin invasomal gel. The measurements were taken in triplicate, and the average value was determined (Kumar *et al.,* 2021).

Homogeneity and Grittiness

Grittiness of the invasomal gel was determined by pressing a small amount of gel between the index finger and the thumb. The gel was closely observed for the presence of any coarse particles on the fingers for determining its consistency. The homogeneity of the gel under evaluation was detected by rubbing a small proportion of gel on the skin at the backside of the hand (Chandra *et al.*, 2019).

Spreadability

The spreadability of the invasomal gel was studied by measuring the change in diameter when 500 mg of gel was placed between two horizontal plates of 20×20 cm2 with a standardized weight of 125 g placed over it (Bachhav and Patravale, 2009).

Extrudability Study

The prepared invasomal gel was filled in collapsible tubes and its extrudability was estimated in terms of weight in grams required to produce a 0.5 cm ribbon of gel in 10 seconds (Sareen *et al.*, 2011).

Viscosity

For determining the viscosity of the invasomal gel Brookfield viscometer (DV-E Brookfield Engineering Laboratories, MA, USA) at 37 °C with spindle No.7 was used. An appropriate amount of gel was placed onto the centre of the viscometer plate directly below the spindle using the spatula and viscosities were measured.

Content uniformity analysis of gel

To validate that the Nystatin in the developed invasomal gel was homogeneous, 0.5 g samples were drawn from three separate sections of the gel. Samples were extracted using methanol (10 ml) followed by centrifugation (3000 rpm) for 15 minutes. The supernatant was filtered, and Nystatin content was determined using a UV-visible spectrophotometer with a λ_{max} at 298 nm.

***In vitro* drug release**

In vitro drug release study was conducted using Franz's diffusion cell with receiver cell volume and effective permeation area of 10 ml and 0.196 cm^2 respectively. The donor cell containing the invasomal gel was placed over the receptor cell in which phosphate buffer saline (pH 7.4) was filled. A pre-treated dialysis membrane of molecular weight cut off 12-14 kD was placed between the donor and receptor compartments using a clamp. The experiment was conducted for 24 hours at a temperature of 37 ± 1°C with constant magnetic stirring at 600 rpm. Samples were estimated for Nystatin content using UV spectrophotometer at 298 nm which were withdrawn from the receptor cell at premediated time gaps i.e., 1, 2, 3, 4, 5, 6, 8 and 12 hours with simultaneously addition of fresh release medium in the receiver compartment to balance the sink conditions. To know the release kinetics of invasomal gel, the data was treated according to different release kinetics models (Kumar *et al.*, 2021).

Physical stability studies of Nystatin invasomal gel formulation

The stability studies of Nystatin invasomal gel weas performed by determining their physical or chemical attributes during storage. The gel was filled in borosilicate glass container which was observed for 4 months by keeping in two different storage conditions i.e., 4±2°C and 25±2°C with 60±5% RH (Garg *et al.*, 2010). The following parameters were analysed during the stability study at specific time periods of 0, 1, 3 and 6 months.

pH Evaluation

The pH was evaluated as mentioned earlier.

Physiochemical Evaluation

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

RESULTS AND DISCUSSION

The study focused on the formulation and evaluation of a Nystatin-loaded invasomal gel for improved topical delivery and sustained antifungal activity. Invasomes, known for enhanced skin penetration due to ethanol and terpenes, were successfully formulated with varying vesicle sizes and entrapment efficiencies (Tables 2 and 3). Among the formulations, N2 displayed optimal vesicle size (195.45 nm) and highest entrapment efficiency (83.32%), making it suitable for gel formulation. The invasomal gel formulation IG-2, prepared using N2, exhibited favorable physicochemical characteristics including suitable viscosity (3674 cps), pH (5.8), high drug content (99.45%), and good spreadability and extrudability, confirming its applicability for topical use (Table 4).

In-vitro drug release studies demonstrated a sustained release profile of Nystatin from the invasomal gel, with 89.98% cumulative release over 12 hours, significantly better than the pure drug, which released 89.98% within just 4 hours (Table 5). This controlled release behavior is crucial for prolonged therapeutic action and reduced dosing frequency.

Release kinetics analysis (Table 6 and 7) revealed that the drug release from IG-2 followed first-order kinetics (R^2 = 0.9796), indicating a concentration-dependent release. The high R^2 values for Higuchi (0.9535) and Korsmeyer-Peppas (0.9506) models also suggest diffusion-controlled mechanisms, common for vesicular and gel-based systems.

Stability analysis over 6 months (Table 8) indicated no significant change in physical properties, pH, or homogeneity, confirming the formulation's stability under both refrigerated and room temperature conditions. The developed Nystatin-loaded invasomal gel showed enhanced drug entrapment, sustained release, good topical application properties, and excellent stability, making it a promising formulation for effective management of topical fungal infections.

Table 1: Composition of different invasomal formulation

Formulation	Drug (% w/v)	Terpene (%v/v)	Ethanol (ml)	Phosphatidylcholine (%w/v)
N1	50	0.25	10	0.25
N2	50	0.50	10	0.25
N3	50	0.75	10	0.50
N4	50	0.25	10	0.50
N5	50	0.50	10	0.75
N6	50	0.75	10	0.75

Table 2: Characterization of average vesicle size of Invasome

Invasomal Formulation	Vesicle Size* (nm)
N1	225.65
N2	195.45
N3	210.32
N4	245.65
N5	236.65
N6	240.65

Table 3: Characterization of Entrapment Efficiency of Invasome

Invasomal Formulation	Entrapment Efficiency
N1	74.45±0.15
N2	83.32±0.32
N3	70.12±0.22
N4	70.32±0.16
N5	69.98±0.36
N6	70.33±0.32

Table 4: Characterization of Invasomes gel based formulation

Invasomal Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
IG-1	3565±15	5.6±0.3	98.45±0.45	168±8	11.65±0.35
IG-2	3674±20	5.8±0.2	99.45±0.35	175±10	9.85±0.25
IG-3	3748±42	6.4±0.5	97.63±0.36	185±13	8.75±0.15

Table 5: Cumulative drug release from invasomal gel (IG-2) and pure drug of Nystatin

Time (hrs.)	Cumulative drug release (%)	
	Nystatin Invasomal Gel	Pure Drug
1	8.45	25.65
2	13.36	45.56
3	26.65	68.85
4	48.98	89.98
5	53.32	-
6	65.58	-
7	72.23	-
8	82.25	-
12	89.98	-

Table 6: Release Kinetics of Optimized invasomal gel formulation IG-2

S. No	Time (Hrs.)	Root Time	Log time	% CDR	% Drug Remain	Log % CDR	Log % CDR Remain
1	1	1.000	0.000	8.45	91.55	0.927	1.962
2	2	1.414	0.301	13.36	86.64	1.126	1.938
3	3	1.732	0.477	26.65	73.35	1.426	1.865
4	4	2.000	0.602	48.98	51.02	1.690	1.708
5	5	2.236	0.699	53.32	46.68	1.727	1.669
6	6	2.449	0.778	65.58	34.42	1.817	1.537
7	7	2.646	0.845	72.23	27.77	1.859	1.444
8	8	2.828	0.903	82.25	17.75	1.915	1.249
9	12	3.464	1.079	89.98	10.02	1.954	1.001

Table 7: Regression analysis of data for invasomal gel formulation IG2

F. Code	Zero order	First order	Higuchi	Pappas
IG2 (R^2)	0.891	0.9796	0.9535	0.9506

Table 8: Stability analysis of Nystatin invasomal gel formulation IG2

Parameters	1 months		3 months		6 months	
Temperature (°C)	4±2°C	25±2°C	4±2°C	25±2°C	4±2°C	25±2°C
Colour	White	White	White	White	White	White
Odour	No	No	No	No	No	No
Appearance	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Clarity	Clear	Clear	Clear	Clear	Clear	Clear
pH	6.82	6.72	6.76	6.65	6.73	6.15
Homogeneity	Excellent	Excellent	Good	Good	Satisfactory	Satisfactory
Washability	Washable	Washable	Washable	Washable	Washable	Washable

CONCLUSION

The study successfully formulated and evaluated a Nystatin-loaded invasomal gel for the effective management of topical fungal infections. The optimized invasomal formulation (IG-2) demonstrated desirable vesicle size**,** high drug entrapment efficiency, and sustained drug release over 12 hours, outperforming the pure drug in release rate and duration. The gel exhibited acceptable pH, spreadability, viscosity, and extrudability, indicating good applicability for topical use. Release kinetics followed first-order and diffusion-based models, confirming controlled drug delivery. Moreover, stability studies confirmed that the formulation remained physically and chemically stable over six months under various storage conditions. In conclusion, the Nystatin-loaded invasomal gel presents a novel, stable, and effective topical drug delivery system with enhanced therapeutic potential for the treatment of superficial fungal infections.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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