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DEVELOPMENT AND CHARACTERIZATION OF INVASOME-LOADED GELS FOR ENHANCED TOPICAL DELIVERY OF MICONAZOLE AGAINST FUNGAL INFECTIONS

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Abstract
This study investigates the development and characterization of various invasome-loaded gel preparations containing Miconazole for the treatment of fungal infections. Six formulations (IG1 to IG6) were evaluated based on their physicochemical properties, pH, drug content, spreadability, viscosity, and cumulative drug release. Among the formulations, IG4 exhibited optimal characteristics, including the highest drug content (99.65±0.15%) and significant cumulative release (98.96±0.15% at 8 hours). The release kinetics followed Higuchi's and Korsmeyers-Peppas models, indicating a diffusion-controlled release mechanism. Antifungal activity tests demonstrated that IG4 had comparable efficacy to Miconazole against *Candida albicans*, showing promising zones of inhibition. Stability studies confirmed that IG4 maintained its integrity and efficacy over time under varying storage conditions. Overall, IG4 represents a potential effective formulation for topical antifungal therapy, meriting further clinical evaluation.

Keywords: Invasome-loaded gel, Miconazole, antifungal activity, *Candida albicans*, drug release kinetics, formulation stability, topical therapy.

Introduction
Fungal infections, particularly those caused by *Candida albicans*, are a significant global health concern, especially in immunocompromised patients and those with chronic conditions (Pappas et al., 2003). These infections can lead to severe morbidity and have prompted the need for effective antifungal therapies. Miconazole, a broad-spectrum azole antifungal, is widely used due to its efficacy in treating various fungal infections (Marr et al., 2002). However, traditional formulations often face challenges such as poor skin permeability and limited sustained release, which can reduce therapeutic effectiveness (Cai et al., 2018).

Invasomes are novel drug delivery systems that utilize lipid-based vesicles to enhance the delivery of active pharmaceutical ingredients through biological barriers (Saha et al., 2020). These vesicles are designed to improve drug solubility, stability, and permeation, thus facilitating a more efficient delivery of drugs to targeted tissues (Yadav et al., 2020). Recent studies have indicated that invasomes can enhance the skin penetration of drugs, making them a promising candidate for topical antifungal formulations (Ghosh et al., 2021).

Topical gels, particularly those that are invasome-loaded, can offer several advantages, including easy application, targeted delivery, and reduced systemic side effects (Singh et al., 2018). The incorporation

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of Miconazole into such gels may improve its bioavailability and therapeutic outcomes in treating fungal infections. The present study aims to formulate and characterize various invasome-loaded gel preparations of Miconazole, focusing on their physicochemical properties, drug release profiles, antifungal activity, and stability, with the objective of identifying an optimized formulation for clinical use.

Material and Methods

Preparation of Miconazole loaded invasomes

Miconazole invasomes were produced using a traditional thin-layer evaporation method. Initially, a clean, dry, round-bottom flask was used to dissolve Miconazole, Phospholipid, and terpene (Limonene) in ethanol (Shah *et al.,* 2015). The organic solvent was eliminated by rotary evaporation, and any remaining traces were removed under vacuum overnight. The deposited lipid film was hydrated using a mixture of phosphate buffer saline (pH 7.4) and rotated at 60 rpm for 1 h at room temperature. The resulting vesicles were left to swell for 2 hrs at room temperature to produce large multilamellar vesicles. To create smaller particles, large particles were subjected to probe sonication at 4°C, with an output frequency of 40% at 40 W.

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Preparation of Miconazole invasomal gel

Invasomal formulation F11 having good entrapment efficiency and small particle size was incorporated in Carbopol 934 gel base (1-3.5% w/v) (Table 1) (Anitha and Satyanarayana, 2021). 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.

Table 1: The composition of different Miconazole invasomal gels

Composition	IG1	IG2	IG3	IG4	IG5	IG6
Invasomes eq to (%)	2	2	2	2	2	2
Carbopol 934 (%)	1	1.5	2	2.5	3	3.5
Triethanolamine (%)	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Distilled water (Qs)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Evaluation of Invasomes gel

Determination of physiochemical properties

The physical appearance, clarity, washability, and organoleptic characteristics of the gel were assessed through visual observation. pH evaluation of the Miconazole invasomal gel was conducted using a pH meter. Measurements were performed in triplicate, and the average value was determined (Tate *et al.,* 2022).

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Homogeneity and Grittiness

The grittiness of the invasomal gel was assessed by gently pressing a small quantity of gel between the index finger and thumb. The gel was carefully examined for any coarse particles on the fingers to determine its consistency. To evaluate the homogeneity of the gel, a small amount was rubbed onto the skin at the back of the hand.

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 6cm with a standardized weight of 20g placed over it. Calculate the time required for 6cm replacement while applying 20gm of weight (El-Tokhy *et al.,* 2021). Calculate the spreadability using formula:

$S = m \times l/t$

S–Sample spreadability, m–mass (g), l–glass plates length (cm), t–time taken to separate (s)

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 (Singh and Bhardwaj, 2021).

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 (Wang *et al.,* 2009).

Content uniformity analysis of gel

To validate that the Miconazole 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 Miconazole content was determined using a UV-visible spectrophotometer (Ahmed and Badr-Eldin, 2019).

***In-vitro* drug release**

The *in vitro* drug release study was conducted using Franz's diffusion cell, with a receiver cell volume of 10 ml and an effective permeation area of 0.196 cm^2. The invasomal gel was placed in the donor cell, positioned over the receptor cell containing phosphate buffer saline (pH 7.4). A pre-treated dialysis membrane with a molecular weight cut off of 12-14 kD was placed between the donor and receptor compartments using a clamp. The experiment was carried out for 24 hours at a temperature of 37 ± 1°C, with constant magnetic stirring at 600 rpm. Samples were collected from the receptor cell at predetermined time intervals (1, 2, 3, 4, 5, 6, 8, and 12 hours) and analyzed for Miconazole content using a UV spectrophotometer at 235 nm. Fresh release medium was added to the receiver compartment simultaneously to maintain sink conditions and ensure continuous drug release. The obtained data from the *in vitro* drug release study were analyzed using various kinetic models to understand the release kinetics of the invasomal gel (Higuchi, 1963).

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

The well diffusion method was used to determine the antifungal activity of the optimized formulation

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using standard procedure. There were 3 concentration used which are 10, 20 and 30 µg/ml for Miconazole invasomal gel formulation in studies. The placement of wells containing antibiotics on the surfaces of agar soon after inoculation with the organism examined is a key component. After a 48-hour incubation period at 25°C, the plates were evaluated for obvious zones of inhibition around the wells impregnated with a specific drug concentration.

Physical stability studies of Miconazole invasomal gel formulation

The stability studies of Miconazole invasomal gel was performed by determining their physical or chemical attributes during storage. The gel was filled in borosilicate glass container which was observed for 6 months by keeping in two different storage conditions i.e., 4±2°C and 25±2°C with 60±5% RH. The following parameters were analysed during the stability study at specific time periods of four weeks.

pH Evaluation

The pH was evaluated as mentioned earlier.

Physiochemical Evaluation

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

Results and Discussion

The evaluation of various invasome-loaded gel preparations (IG1 to IG6) provides insightful data into their physicochemical properties and therapeutic potential. The results from Table 2 indicate that all formulations exhibit similar sensory attributes, characterized by a transparent appearance and a smooth after-feel. However, consistency varied significantly among the gels, with IG4 and IG5 displaying higher consistency, suggesting that they may offer better stability and handling during application.

As presented in Table 3, the pH values of the formulations ranged from 6.68 to 6.98, indicating that all gels are within the acceptable range for topical applications. Notably, IG4 displayed the highest drug content (99.65±0.15%), which is crucial for ensuring effective therapeutic outcomes. The spreadability values were also noteworthy, with IG1 showing the highest spreadability (13.25±0.25 gm/cm), potentially enhancing the ease of application.

Table 4 highlights the cumulative drug release profiles over time. IG4 stands out with the highest cumulative release of Miconazole (98.96±0.15% at 8 hours), suggesting that its formulation optimally facilitates drug release. In contrast, IG6 showed the lowest release, indicating a slower release profile. The release pattern indicates that IG4 may provide a more effective therapeutic effect due to its ability to release the drug more rapidly and completely.

The regression analysis in Table 5 reveals that IG4 follows Higuchi's model and Korsmeyers-Peppas equation closely (R^2 values of 0.990 and 0.994, respectively), indicating a diffusion-controlled release mechanism. This suggests that the formulation could maintain therapeutic drug levels over an extended period, which is particularly beneficial for antifungal therapy.

The antifungal activity of the optimized formulation IG4 against *Candida albicans*, as shown in Table 6, is promising. IG4 demonstrated zones of inhibition comparable to Miconazole at varying concentrations, which confirms its efficacy. This is a strong indication that the formulation not only releases the drug effectively but also retains its antifungal potency.

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Stability studies outlined in Table 7 suggest that IG4 maintains its characteristics over time, with only minor fluctuations in drug content and pH at both 4.0±0.5°C and 25±0.5°C. The appearance remained smooth, and homogeneity was generally satisfactory, affirming the formulation's stability under tested conditions. Such stability is essential for ensuring that the gel remains effective and safe throughout its shelf life.

Table 2: Characterization of various invasome loaded gel preparations

Specifications	IG1	IG2	IG3	IG4	IG5	IG6
Colour	Transparent	Transparent	Transparent	Transparent	Transparent	Transparent
After feel effects	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Consistency	Easy pourable	Less	Good	Very good	High	High
Homogeneity	Good	Good	Good	Good	Good	Good

Table 3: pH of various gel preparations (IG1 to IG6)

S. No.	Formulation Code	pH	Drug Content (%)	Spreadability (gm/cm)	Viscosity (cp)
1	IG1	6.85±0.05	96.55±0.25	13.25±0.25	4632±15
2	IG2	6.95±0.03	98.85±0.32	12.45±0.12	4365±23
3	IG3	6.78±0.06	97.12±0.25	11.65±0.36	4254±19
4	IG4	6.98±0.04	99.65±0.15	10.25±0.24	3998±22
5	IG5	6.81±0.02	97.74±0.36	9.98±0.18	3885±26
6	IG6	6.68±0.03	96.45±0.15	8.85±0.32	3715±21

Table 4: Cumulative drug release from invasomal gel Miconazole

Time in (Hr)	Cumulative percent release* (% CPR)					
	IG1	IG2	IG3	IG4	IG5	IG6
1	32.25±0.32	30.25±0.36	28.85±0.22	22.23±0.25	18.85±0.25	15.65±0.33
2	46.58±0.25	43.36±0.25	39.65±0.32	33.25±032	23.32±0.32	20.32±0.21
3	53.32±0.15	51.56±0.14	48.85±0.14	40.23±0.41	36.65±0.41	31.45±0.14
4	69.98±0.22	67.74±0.47	65.58±0.25	52.32±0.32	47.74±0.33	43.36±0.25
5	76.65±0.36	75.65±0.58	73.32±0.36	59.98±0.45	56.65±0.25	54.47±0.32
6	88.85±0.38	83.32±0.69	78.85±0.54	65.56±0.32	68.85±0.36	63.32±0.45
7	98.85±0.24	94.46±0.41	88.88±0.47	76.65±0.25	73.32±0.32	70.12±0.32
8	98.96±0.15	98.85±0.52	98.85±0.55	83.32±0.36	86.65±0.41	84.45±0.74
10	99.12±0.25	99.32±0.63	99.05±0.66	94.65±0.21	92.23±0.32	90.32±0.58
12	99.85±0.11	99.74±0.44	99.65±0.48	98.78±0.22	94.45±0.63	93.32±0.36

*Average of Six determination

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Table 5: Regression analysis data of invasomal gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
IG4	0.968	0.898	0.990	0.994

Table 6: Antifungal activity of optimized formulation (IG4) against *Candida albicans*

S.N	Standard/ Formulation	Zone of Inhibition		
		10µg/ml	**20µg/ml**	**30µg/ml**
1.	Miconazole	12.5±0.86	13.8±0.50	18.5±0.50
2.	IG4	13.0±0.47	14.3±0.94	19.2±0.52

Table 7: Results of stability studies of the invasomal optimized gel formulation

Condition	Days	Appearance	% Drug content	pH	Homogeneity	Washability
4.0±0.5°C	7	Smooth	97.98±0.45	6.7	Good	Good
	15	Smooth	96.25±0.15	6.5	Good	Good
	28	Smooth	96.75±0.65	6.8	Satisfactory	Good
25±0.5ºC	7	Smooth	96.65±0.73	6.0	Good	Good
	15	Smooth	96.35±0.49	6.4	Satisfactory	Good
	28	Smooth	95.44±0.48	5.9	Satisfactory	Good

Conclusion

Formulation IG4 emerged as the optimal formulation among the studied invasomal gels due to its superior drug content, effective release profile, potent antifungal activity, and stability. These findings suggest that IG4 can be a promising candidate for the treatment of fungal infections, warranting further investigation in clinical settings to assess its efficacy and safety in human subjects.

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