RESEARCH ARTICLE

Formulation and Characterization of Isoconazole Loaded Invasomal Gel for Effective Antifungal Activity

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ABSTRACT

The characterization of isoconazole invasomal gel formulations (IG1–IG5) aimed to evaluate their physical attributes, drug content, and drug release kinetics for topical application. All formulations exhibited transparency and smooth texture, indicating uniform drug dispersion. While IG1 had an easily pourable consistency, IG4 displayed very good consistency, suggesting viscosity differences. Despite these variations, all formulations showed good homogeneity and high drug content, with IG4 leading in drug content percentage. Spreadability and viscosity varied among formulations, influencing ease of application and adherence to the skin. Cumulative drug release profiles revealed sustained release over 12 hours, with notable differences in release rates and extents among formulations. IG4 exhibited the most sustained release, aligning with pharmacokinetic requirements for antifungal therapy. Its release profile followed a diffusion-controlled mechanism, with a high cumulative drug release percentage of 98.95 ± 0.32% over 12 hours. Antifungal activity against *Candida albicans* was comparable to Isoconazole, suggesting IG4's effectiveness in treating fungal infections. Stability studies confirmed the formulation's stability under tested conditions, supporting its potential for further development and clinical use.

Keywords: Isoconazole, Invasomal gel formulations, Drug release kinetics, Sustained release, Antifungal activity, Stability studies.

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INTRODUCTION

Fungal infections are a major concern in global health, affecting everything from superficial skin ailments to serious systemic diseases. Isoconazole, a broad-spectrum antimycotic, is normally used in topical formulations for the treatment of various fungal skin contagions due to its potent activity and favorable safety profile.[1] However, optimizing the formulation of isoconazole to ensure effective delivery and sustained antifungal activity remains a priority in dermatological therapy. In recent years, invasomal gel formulations have emerged as promising vehicles for the delivery of antifungal agents. Improved drug absorption and sustained release features are offered by invasomes, which are innovative vesicular systems that can encapsulate hydrophilic and lipophilic medicines,[2] by formulating isoconazole into invasomal gels, it is possible to enhance its bioavailability, prolong its retention at the site of infection, and improve therapeutic outcomes.

Effective formulation of isoconazole-loaded invasomal gels requires a comprehensive understanding of their physicochemical properties and drug release kinetics. Characterizing these formulations allows for the evaluation of their suitability for topical application and their potential to combat fungal infections effectively. Previous research has demonstrated the importance of factors such as drug content, homogeneity, and rheological properties in determining the performance of topical formulations.[3]

Moreover, assessing the antifungal activity of isoconazole-loaded invasomal gels against clinically relevant fungal pathogens, such as *Candida albicans*, provides critical insights into their therapeutic efficacy. Comparing their activity to that of standard antifungal agents allows for the evaluation of their clinical relevance and potential as alternative treatment options.

Stability studies are also essential to ensure the long-term integrity and efficacy of isoconazole-loaded invasomal gels under various storage conditions. Maintaining stability is important for the practicality and clinical utility of these formulations, especially in resource-limited settings.[4]

By formulating and characterizing isoconazole-loaded invasomal gels, this study goals to contribute to the development of effective and stable topical antifungal formulations with enhanced therapeutic efficacy and improved patient outcomes.

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MATERIAL AND METHODS

Materials

Isoconazole, a potent antifungal agent, was generously provided as a gift sample by Bioplus Life Sciences, located in Bangalore. Carbopol 934p, a polymer commonly used as a gelling agent in topical formulations, was obtained from S. D. Fine Chem. Ltd. in Mumbai. Triethanolamine was used to maintain the desired viscosity of the formulation and was procured from S.D Fine Chem. Methyl paraben and propyl paraben, preservatives commonly used to enhance the shelf life of pharmaceutical products, were obtained from the same supplier, S. D. Fine Chem. Ltd. Propylene glycol, a versatile solvent and humectant often used in topical formulations, was also obtained from S. D. Fine Chem. Ltd.

Methods

Isoconazole invasomal gel preparation

The invasomal formulation F11, which has a tiny particle size and good entrapment effectiveness, was mixed with the carbopol 934 gel base at a concentration of 1 to 3% by weight (Table 1). To make a carbopol gel base, the gelling ingredient was dissolved in distilled water and then let to fully expand in the dark. To make a translucent, viscous gel, triethanolamine was slowly added to the dispersion. The last step was to combine the optimized invasomal formulation with carbopol gel base, stirring the mixture gradually using a mechanical stirrer.[5,6]

Evaluation of Invasomes gel

Determination of physiochemical properties

The organoleptic properties, clarity, washability, occlusion, and physical appearance of the gel were examined visually. The isoconazole intravenous gel's pH was measured using pH meter. Measurements were noted three times, and the mean was calculated.[7]

Homogeneity and grittiness

A small quantity of gel was pressed between the index finger and thumb to test how gritty the invasomal gel was. For the purpose of establishing its consistency, the gel was meticulously examined for the presence of slightly rough particles on the fingertips. A small quantity of gel was rubbed onto the skin on the backside of the hand to determine the gel's homogeneity.[8]

Spreadability

The spreadability of the invasomal gel was tested by measuring the change in diameter when 125 g of standardized weight was placed on top of 500 mg of gel placed among two 20×20 cm^2 horizontal plates.[9]

Table 1: Composition of isoconazole invasomal gels

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

Extrudability study

The extrudability of prepared gel was evaluated by filling collapsible tubes with it and measuring the weight in grams needed to create 0.5 cm ribbon of gel in 10 seconds.[10]

Viscosity

For determining the viscosity of invasomal gel Brookfield viscometer at 37°C with spindle No.7 was used. An appropriate amount of gel was placed onto the center of the viscometer plate directly below the spindle using the spatula and viscosities were measured.[11]

Content uniformity analysis of gel

We took 0.5 g samples from 3 different areas of generated invasomal gel to ensure that the isoconazole was evenly distributed. Centrifugation at 3000 rpm for 15 minutes was performed after methanol (10 mL) was used to extract the samples. After filtering the liquid, the isoconazole concentration was measured with a UV-visible spectrophotometer.[12]

In-vitro drug release

In-vitro pharmacological investigations were conducted using Franz's diffusion cells, which possessed a potent permeation area of 0.196 cm^2 and a receiver cell volume of 10 mL. The process involved layering the donor cell containing the invasomal gel onto the receptor cell that was previously filled *via* phosphate buffer saline (pH 7.4). A pre-treated dialysis membrane having a molecular weight cutoff of 12 to 14 kD was held in place by a clamp to divide the donor and receptor compartments. As stated in reference,[13] the experiment was conducted under constant magnetic stirring at 600 rpm for 24 hours at a temperature of 37 ± 1°C. With the use of a UV spectrophotometer operating at 272 nm, samples were measured for their isoconazole content. To keep the sink condition constant, new release medium was supplied to the receiver compartment simultaneously with these samples taken from receptor cell at 1, 2, 3, 4, 5, 6, 8, and 12-hour intervals. To ascertain gel release kinetics, a number of release kinetics models were used to the data.

The data gathered from *in-vitro* drug release experiment was plotted using many kinetic models, as shown below:

- In zero-order kinetics, the cumulative percentage of drug release is plotted against time.
- First-order kinetics - logarithm of cumulative% of remaining drug versus time
- In Higuchi's model, the cumulative% of drugs released is plotted against the square root of time.
- In Korsmeyer Peppa's model, cumulative% of drug release is plotted against log time.[14,15]

In-vitro antifungal activity of optimized formulation (IG4)

The optimized formulation's (IG4) antifungal activity was determined by following normal procedure and utilizing the good diffusion method. Three different concentrations were utilized in the experiments for the formulation of isoconazole

invasomal gel: 10, 20, and 30 µg/mL. Crucial to the process is the rapid insertion of antibiotic-containing wells onto agar surfaces following inoculation with the organism under study. No inoculum should ever be used that was prepared from an undiluted overnight broth culture. After incubating the plates at 25°C for 48 hours, we looked for clear zones of inhibition surrounding wells that a certain concentration of medication[16] had permeated.

Physical stability studies of isoconazole invasomal gel formulation

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

pH evaluation

The pH was evaluated as mentioned earlier.

Physiochemical Evaluation

The gel's organoleptic qualities, washability, and clarity were examined visually.[17]

RESULTS AND DISCUSSION

Characterization of Invasomal Gel Formulations

The characterization of various isoconazole invasomal gel formulations (IG1–IG5) aimed to assess their physical attributes and drug content, which are crucial determinants of formulation quality and efficacy. All formulations exhibited a transparent appearance and smooth after-feel effects, indicating uniform dispersion of the drug and a pleasant sensory experience upon application.IG1 demonstrated an easily pourable consistency, while IG4 exhibited a very good consistency, suggesting differences in the viscosity and texture among the formulations. However, all formulations showed good homogeneity, indicating the uniform distribution of drugs throughout the gel matrix. The pH values of formulations were within acceptable range for topical preparations, ensuring compatibility with the skin. Moreover, all formulations exhibited high drug content, with IG4 showing the highest drug content percentage, indicating efficient drug incorporation and uniformity. Spreadability and viscosity are essential factors influencing the ease of application and adherence of the gel to the skin surface. IG1 had the highest spreadability, whereas IG4 exhibited the highest viscosity among the formulations, suggesting differences in their rheological properties Table 2.

Cumulative Drug Release

The cumulative drug release profiles from the Isoconazole invasomal gel formulations (IG1-IG5) provide valuable insights into the formulations' drug release kinetics and potential for therapeutic efficacy over time. Overall, all formulations exhibited sustained drug release profiles over the 12-hour testing period. However, notable variances were observed among formulations in terms of extent and rate of drug release. IG1 and IG2 demonstrated relatively lower cumulative percent release compared to IG3, IG4, and IG5, indicating differences in the formulations' drug release characteristics. Variances in drug release profiles among formulations could be ascribed to variations in their composition, such as the type and concentration of excipients, which influence factors like gel matrix integrity, drug diffusion, and release kinetics. Formulations with higher drug content or modifications in gel consistency may have resulted in enhanced drug release rates and extents. IG4 exhibited the most sustained drug release profile among the formulations, with the slowest rate of release over the 12-hour period. This sustained release behavior is desirable for topical formulations intended for prolonged therapeutic action, as it ensures continuous drug

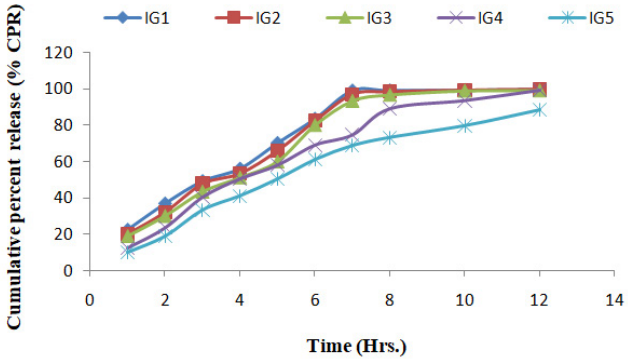

Figure 1: *In-vitro* drug release from invasomal gels of isoconazole

Table 2: Characterization of various gel preparations of isoconazole invasomal gel formulations

Specifications	*IG1*	*IG2*	*IG3*	*IG4*	*IG5*
After feel effects	Smooth	Smooth	Smooth	Smooth	Smooth
Color	Transparent	Transparent	Transparent	Transparent	Transparent
Consistency	Easy pourable	Less	Good	Very good	High
Homogeneity	Decent	Decent	Decent	Decent	Decent
pH	6.3 ± 0.58	6.1 ± 0.49	6.1 ± 0.19	6.2 ± 0.49	6.0 ± 0.76
Drug content (%)	96.04 ± 0.58	94.59 ± 0.73	96.84 ± 0.46	98.73 ± 0.53	96.28 ± 0.27
Spreadability	22.08 ± 4.42	16.75 ± 3.59	15.36 ± 5.27	12.53 ± 3.27	13.28 ± 4.28
Viscosity	1639 ± 1.74	1773 ± 1.86	1863 ± 1.26	1895 ± 1.38	1886 ± 1.71

availability at the application site, possibly enlightening treatment consequences and patient amenability. The sustained drug release profiles observed in the isoconazole invasomal gel formulations align well with the pharmacokinetic requirements for antifungal therapy, where prolonged drug exposure at effective concentrations is essential for combating fungal infections effectively Table 3 and Figure 1.

In-vitro Drug Release Data of Optimum Invasome Gel Formulation (IG4)

IG4 demonstrated a sustained release profile over the 12-hour testing period, with the cumulative percent drug release steadily increasing with time. The release profile exhibited a characteristic sigmoidal pattern, indicating a controlled and prolonged release of isoconazole from the gel matrix. As the medicine slowly seeps out of the gel matrix and into the surrounding medium, the release kinetics of IG4 can be characterized as mostly diffusion-controlled. The gradual increase in cumulative drug release reflects the gradual dissolution and diffusion of Isoconazole from the gel matrix. IG4 exhibited a high cumulative percent drug release of 98.95 ± 0.32% at the end of 12-hour testing period, indicating the formulation's ability to sustain drug release over an extended period. This sustained release behavior is desirable for topical formulations, as it ensures prolonged drug exposure at the application site, potentially improving therapeutic efficacy and patient compliance Table 4 and Figure 2.

Regression Analysis Data

The Korsmeyers Peppas Equation, with an R^2 value of 0.977, also demonstrated a good fit to the experimental data. This equation is normally used to designate drug release from

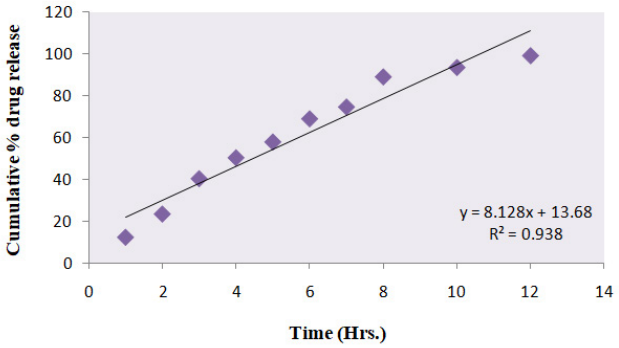

Figure 2: Zero order release kinetics (Cumulative %Drug Release *vs* time (Hours)

Table 3: Cumulative drug release from Isoconazole invasomal gel formulations

Time in (Hr)	*Cumulative percent release* (%CPR)*				
	IG1	*IG2*	*IG3*	*IG4*	*IG5*
1	22.23 ± 0.12	20.32 ± 0.22	18.85 ± 0.32	12.25 ± 0.25	9.85 ± 0.32
2	36.65 ± 0.25	32.25 ± 0.32	29.98 ± 0.25	23.36 ± 0.32	18.83 ± 0.25
3	48.98 ± 0.18	47.78 ± 0.15	43.32 ± 0.23	40.23 ± 0.15	33.32 ± 0.32
4	55.86 ± 0.22	53.36 ± 0.20	51.12 ± 0.32	50.25 ± 0.36	41.12 ± 0.18
5	69.98 ± 0.18	65.85 ± 0.22	59.95 ± 0.18	57.78 ± 0.22	50.41 ± 0.32
6	83.32 ± 0.26	82.12 ± 0.45	79.95 ± 0.33	68.85 ± 0.41	61.14 ± 0.22
7	98.85 ± 0.32	96.65 ± 0.36	93.32 ± 0.25	74.45 ± 0.23	68.85 ± 0.36
8	98.93 ± 0.19	98.12 ± 0.22	96.65 ± 0.32	88.85 ± 0.32	73.32 ± 0.25
10	99.12 ± 0.22	98.95 ± 0.32	98.85 ± 0.26	93.32 ± 0.26	79.85 ± 0.22
12	99.48 ± 0.18	99.45 ± 0.14	99.05 ± 0.18	98.95 ± 0.32	88.56 ± 0.45

*Average of Six determination

Table 4: *In-vitro* drug release for IG4

Time (hours)	*Square root of time (hours)$^{1/2}$*	*Log time*	*Cumulative* %drug release*	*Log cumulative %drug release*	*Cumulative %drug remaining*	*Log dumulative %drug remaining*
1	1.000	0.000	12.25 ± 0.25	1.088	87.75	1.943
2	1.414	0.301	23.36 ± 0.32	1.368	76.64	1.884
3	1.732	0.477	40.23 ± 0.15	1.605	59.77	1.776
4	2.000	0.602	50.25 ± 0.36	1.701	49.75	1.697
5	2.236	0.699	57.78 ± 0.22	1.762	42.22	1.626
6	2.449	0.778	68.85 ± 0.41	1.838	31.15	1.493
7	2.646	0.845	74.45 ± 0.23	1.872	25.55	1.407
8	2.828	0.903	88.85 ± 0.32	1.949	11.15	1.047
10	3.162	1.000	93.32 ± 0.26	1.970	6.68	0.825
12	3.464	1.079	98.95 ± 0.32	1.995	1.05	0.021

Table 5: Regression investigation of invasomal gel optimized formulation IG4

Batch	*Zero order*	*First order*	*Higuchi's Model*	*Korsmeyers Peppas equation*
IG4	0.938	0.909	0.984	0.977

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

S.N	*Standard/ Formulation*	*Zone of inhibition*		
		10 µg/mL	*20 µg/mL*	*30µg/mL*
1.	Isoconazole	11.0 ± 0.86	14.0 ± 0.74	19.0 ± 0.50
2.	IG4	10.5 ± 0.47	15.5 ± 0.94	19.5 ± 0.57

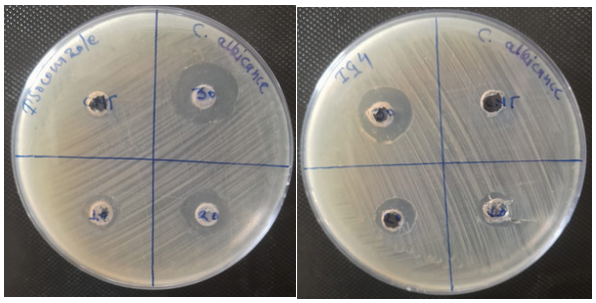

Figure 3: Antimicrobial activity

polymeric systems exhibiting non-Fickian diffusion, which includes both diffusion and polymer relaxation mechanisms. A non-Fickian diffusion mechanism, suggesting an integration of drug diffusion and polymer relaxation contributing to the release process, is supported by the high R^2 value, which suggests that drug release from IG4 follows this mechanism (Table 5).

Antifungal Activity

The antifungal activity of IG4 formulation against *Candida albicans*, compared to the standard isoconazole, is assessed based on the zone of inhibition observed at different concentrations. At all three concentrations (10, 20, and 30 µg/mL), the IG4 formulation demonstrated comparable or slightly larger zones of inhibition compared to Isoconazole. Specifically, at 10 µg/mL concentration IG4 showed a slightly smaller zone of inhibition than isoconazole, whereas at 20 and 30 µg/mL concentrations, IG4 exhibited a slightly larger zone of inhibition compared to isoconazole. The observed zones of inhibition for both isoconazole and IG4 indicate their ability to inhibit the growth of *Candida albicans*, a common fungal pathogen responsible for various infections in humans. While the differences in the zones of inhibition between isoconazole and IG4 are minimal, they suggest that IG4 formulation retains the antifungal activity of Isoconazole and may be effective in treating fungal infections caused by *C. albicans* Table 6, Figure 3.

Stability Studies

The stability studies demonstrate that the invasomal optimized gel formulation maintains its physical and chemical stability under the tested storage conditions over the study period. These findings provide confidence in the formulation's stability and suitability for further development and potential clinical use. However, continued monitoring of stability parameters is recommended to ensure the formulation's long-term stability and efficacy Table 7.

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

Condition	*Days*	*Appearance*	*%Drug content*	*pH*	*Homogeneity*	*Wash-ability*
4.0 ± 0.5°C	7	Smooth	97.43 ± 0.53	6.2	Good	Good
	15	Smooth	97.21 ± 0.19	6.1	Good	Good
	28	Smooth	96.82 ± 0.73	5.9	Satisfactory	Good
25 ± 0.5ºC	7	Smooth	96.83 ± 0.73	6.1	Good	Good
	15	Smooth	96.46 ± 0.49	6.1	Satisfactory	Good
	28	Smooth	95.84 ± 0.48	5.8	Satisfactory	Good

CONCLUSION

The study aimed to characterize Isoconazole invasomal gel formulations (IG1–IG5) to understand their physical attributes, drug content, and release kinetics. All formulations were transparent, smooth, and homogeneous, with differences in consistency and viscosity. Despite variations, all maintained high drug content, with IG4 exhibiting the highest. Drug release profiles showed sustained release over 12 hours, with IG4 displaying the most sustained profile, ideal for antifungal therapy. IG4's release mechanism was diffusion-controlled, supported by a non-Fickian diffusion model.

The antifungal activity of IG4 against *C. albicans* was comparable to Isoconazole, indicating its efficacy in treating fungal infections. Stability studies confirmed the formulation's stability under various conditions, bolstering confidence in its potential clinical use. Overall, the study's findings provide valuable insights into formulation development, suggesting IG4 as a promising candidate for further research and potential commercialization in antifungal therapy.

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