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FORMULATION AND CHARACTERIZATION OF VORICONAZOLE-LOADED INVASOMES FOR EFFECTIVE ANTI - FUNGAL ACTIVITY

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ABSTRACT

This study focuses on the formulation and characterization of Voriconazole-loaded invasomes to enhance antifungal activity. Voriconazole, an effective antifungal agent, was encapsulated using soya phosphatidylcholine and characterized for entrapment efficiency, vesicle size, and gel properties. The formulations exhibited high entrapment efficiency, with the optimized formulation (F6) achieving 88.95% and a vesicle size of 225.45 nm. The invasomes gel demonstrated suitable viscosity, pH, drug content, and spreadability, confirming its potential for topical applications. In vitro drug release studies indicated a sustained release profile, with 99.44% of the drug released after 12 hours, suggesting effective delivery. Release kinetics analysis revealed a diffusion-controlled mechanism. Overall, voriconazole-loaded invasomes present a promising approach for improved antifungal therapy.

Keywords: Voriconazole, Invasomes, Antifungal Activity, Entrapment Efficiency, Drug Release, Topical Formulation, Phosphatidylcholine, Sustained Release.

INTRODUCTION

Invasomes are composed of phospholipids, which facilitate the incorporation of drugs while ensuring stability and biocompatibility. The unique structure of invasomes allows for improved drug permeation through skin and mucosal membranes, potentially enhancing antifungal efficacy against localized infections. Additionally, the use of surfactants in invasome formulation can further improve drug solubility and penetration.

Voriconazole, a triazole antifungal agent, is widely used for treating serious fungal infections, particularly in immunocompromised patients. However, its clinical effectiveness is often limited by its poor solubility and bioavailability, leading to the need for innovative delivery systems. Invasomes, novel lipid-based nanocarriers, have emerged as promising vehicles to enhance the delivery of hydrophobic drugs. These carriers are characterized by their ability to penetrate biological barriers, providing a targeted and sustained release of the encapsulated drug.

Several studies have highlighted the advantages of using invasomes for drug delivery. For instance, research has shown that invasomes can significantly improve the antifungal activity of various agents by enhancing their permeability and maintaining therapeutic concentrations over extended periods (Sharma *et al.,* 2020). Moreover, the safety profile of invasomes, owing to their biocompatible components, makes them suitable for systemic and topical applications (Patel *et al.,* 2021).

This study aims to formulate and characterize Voriconazole-loaded invasomes to evaluate their potential for enhanced antifungal activity. The effectiveness of these formulations will be assessed through *in vitro* release studies and antifungal susceptibility tests against common fungal strains.

MATERIALS AND METHODS

Materials

The materials used for the preparation and evaluation of voriconazole-loaded invasomes gel include a variety of chemicals sourced from reputable suppliers. Voriconazole, the active pharmaceutical ingredient, was obtained as a gift sample from a pharmaceutical company. Soya Phosphatidylcholine, a key lipid for forming invasomes, was supplied by Ash Chemie India in Thane. Solvents such as Methanol, Ethanol, and Chloroform, all from Qualigens Fine Chemicals, were utilized in various formulation processes. Carbopol 934P from S. D. Fine Chem. Ltd. was employed as a gelling agent. Methyl Paraben and Propyl Paraben, also from S. D. Fine Chem. Ltd., served as preservatives.

Methods

Formulation and optimization of Voriconazole loaded Invasomes

Voriconazole (1%) was loaded in to invasomes by mechanical dispersion technique. Soya Phosphatidylcholine (0.5 to 1.5% w/v) was added to ethanol and vortexed for 5 minutes (El-Kayal *et al.,* 2024). Drug (equivalent to 1%) and terpenes (0.25 to 0.5%) were added under constant vortexing, this mixture was sonicated for 5 minutes. Fine stream of Phosphate buffer saline was added with syringe under constant vortexing. It was vortexed for additional 5 minutes to obtain final invasomal preparation.

Preparation of gel base

Carbopol 934 (1-3%w/v Invasome based gel formulation i.e. G-1 of 1% w/v, G-2 of 2% w/v, G-3 of 3%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution (Gupta *et al.,* 2022). The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. The same procedure was used to formulate Invasomes containing gel in which previously prepared Invasomes suspension was added. Invasomes preparation corresponding to 1% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Evaluation of Invasomes

Entrapment efficiency

Entrapment efficiency of Voriconazole Invasomes formulation was determined using centrifugation method (Lakshmi *et al.,* 2014). The entrapment efficiency of Voriconazole in invasomes vesicle was determined by ultracentrifugation, 10mL of invasomes formulation were collect in test tube. The amount of drug not entrapped in the invasomes was determined by centrifuging at 3,000 rpm and collect the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was

determined at 286nm using UV spectrophotometer.

$$\%\ Entrapment\ Efficiency = \frac{Theroticaldrugcontent - Practicaldrugcontent}{\text{Therotical drug content}} \times 100$$

Vesicle Size

Microscopic analysis was performed to determine the average size of prepared invasomes (Lakshmi *et al.,* 2014). Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer. The average diameter was calculated using the following formula:

$$AverageDiameter = \frac{\Sigma n.d}{\Sigma\, n}$$

Where n = number of vesicles; d = diameter of the vesicles

Evaluation of Invasomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical Invasomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. 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 (Salih *et al.,* 2024).

Drug content

Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max}286 nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube (Anitha *et al.,* 2021).

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer *et al.* An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan.To determine spreadibility, 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 spreadibility show lesser time to spread (Zafar *et al.,* 2024).

$$Spreadability = \frac{Weight\ tide\ to\ Upper\ Slide\ x\ Length\ moved\ on\ the\ glass\ slide}{Time\ taken\ to\ slide}$$

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 diffusion. The franz diffusion cell has receptor compartment with an effective 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 cm^2 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 maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn and analyzed spectrophotometrically at wavelength of 286nm (Sailaja *et al.,* 2024).

RESULTS AND DISCUSSION

The study focused on the formulation and characterization of voriconazole-loaded invasomes, highlighting their potential for enhanced antifungal activity.

The entrapment efficiency varied across different formulations, with F6 achieving the highest efficiency of 88.95%. This suggests that the composition and formulation parameters significantly influence the ability to encapsulate voriconazole within the vesicles. The average vesicle size ranged from 225.45 nm to 283.32 nm, with F6 presenting the smallest size, which is favorable for improved skin penetration and absorption.

The viscosity, pH, drug content, extrudability, and spreadability were evaluated for the gel formulations. IG-2 exhibited a pH of 6.85, which is close to physiological pH, making it suitable for topical applications. The viscosity values indicate good consistency for a gel formulation, while the drug content of IG-2 (99.65%) confirms efficient incorporation of voriconazole. Furthermore, the extrudability and spreadability results suggest that IG-2 possesses desirable characteristics for application ease.

The *in vitro* release profile of IG-2 demonstrated a sustained release of voriconazole, with a cumulative drug release of 99.44% after 12 hours. This indicates that the invasomal gel formulation can maintain drug release over an extended period, which is beneficial for effective treatment.

The regression analysis data suggest that the drug release from the optimized gel formulation IG-2 follows Higuchi and Korsmeyer-Peppas models, with R^2 values of 0.9846 and 0.9855, respectively. This indicates that the release mechanism is likely diffusion-controlled, suggesting the potential for sustained antifungal action.

Table 1: Formulation optimization of Voriconazole loaded Invasomes

Ingredient (%)	F1	F2	F3	F4	F5	F6
Voriconazole (%)	1	1	1	1	1	1
Phosphotidylcholine (%)	1.0	1.5	2.0	1.0	1.5	2.0
Terpenes (%)	0.25	0.25	0.25	0.5	0.5	0.5
Ethanol (ml)	10	10	10	10	10	10

Table 2: Formulation optimization of gel base

Ingredient (%)	G-1	G-2	G-3
Drug (Invasomes equivalent to 1%)	1	1	1
Carbopol 934	1	2	3
Propylene glycol	0.2	0.2	0.2
Water (ml)	100	100	100

Table 3: Entrapment efficiency and average vesicle size of Invasomes

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F1	69.98±0.25	265.45±0.35
F2	73.32±0.32	253.25±0.22
F3	65.85±.15	265.45±0.14
F4	76.65±0.37	283.32±0.23
F5	82.25±0.45	273.45±0.32
F6	88.95±0.33	225.45±0.25

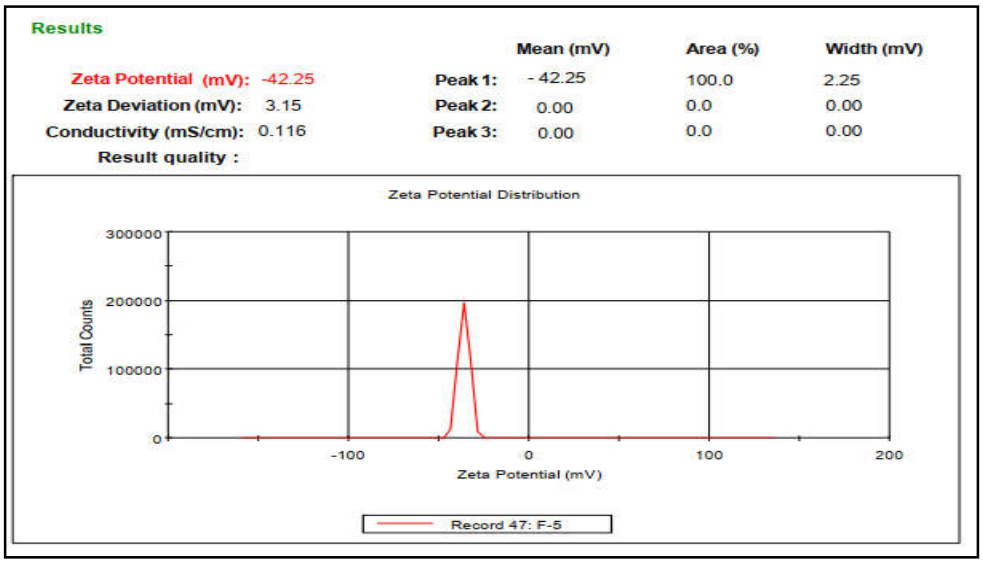

Figure 1: Graph of zeta Potential (mV) optimized formulation F5

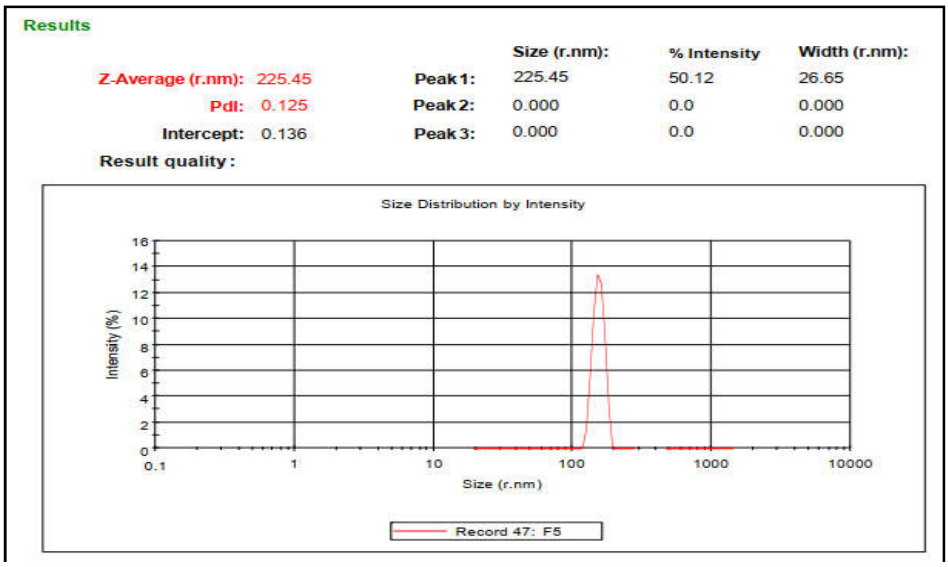

Figure 2: Graph of average vesicle size (nm) of optimized formulation F5

Table 4: Characterization of Invasomes gel

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
IG-1	3255±13	6.78±0.08	97.85±0.15	165±6	12.25±0.23
IG-2	3165±25	6.85±0.02	99.65±0.26	175±5	11.32±0.25
IG-3	3065±20	6.58±0.04	98.85±0.32	183±7	10.36±0.14

Table 5: *In vitro* drug release study of optimized gel formulation IG-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	29.98
2	1	43.32
3	2	56.65
4	4	63.32
5	6	76.65
6	8	88.98
7	10	94.45
8	12	99.44

Table 6: Regression analysis data of optimized gel formulation IG-2

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	0.9376	0.8729	0.9846	0.9855

CONCLUSION

The formulation of voriconazole-loaded invasomes successfully enhanced the antifungal activity of the drug while improving its delivery profile. The optimized formulation exhibited high entrapment efficiency and favorable vesicle size, indicating effective encapsulation and stability. Characterization studies confirmed that the invasomes gel possesses suitable physicochemical properties for topical application, including appropriate viscosity, pH, and spreadability. In vitro drug release studies demonstrated a sustained release mechanism, suggesting the potential for prolonged therapeutic effects. Overall, voriconazole-loaded invasomes represent a promising strategy for effective antifungal therapy, potentially improving patient outcomes in treating fungal infections. Further studies are warranted to evaluate the in vivo efficacy and safety of this formulation.

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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