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Original Research Article

FORMULATION AND CHARACTERIZATION OF VORICONAZOLE ETHOSOMAL GEL FOR EFFECTIVE TOPICAL FUNGAL TREATMENT

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ABSTRACT

This study aimed to formulate and characterize a Voriconazole ethosomal gel for effective topical treatment of fungal infections. Voriconazole ethosomes were prepared with varying concentrations of phospholipids and ethanol and evaluated for vesicle size, zeta potential, and entrapment efficiency. The optimal formulation, EF7, exhibited a vesicle size of 185.65±0.33 nm, a zeta potential of -36.58 mV, and an entrapment efficiency of 72.54±0.068%. The ethosomes were then incorporated into a gel base, with the resulting gel (G2) showing a pH of 6.832±0.003, a drug content of 99.023±0.303%, and favorable organoleptic properties. *In-vitro* release studies indicated that the drug release followed the Higuchi model (R^2 = 0.8997), suggesting a diffusion-controlled mechanism. The Voriconazole ethosomal gel demonstrated enhanced stability, drug delivery, and user compliance, making it a promising candidate for topical antifungal therapy. Further in-vivo studies are recommended to validate these findings and explore clinical applications.

Keywords: Voriconazole, ethosomal gel, topical antifungal treatment, vesicle size, zeta potential, entrapment efficiency.

INTRODUCTION

Fungal infections, particularly those affecting the skin, pose significant health challenges due to their prevalence and the difficulty in achieving effective treatment. Topical antifungal therapies offer a targeted approach, minimizing systemic side effects while delivering high drug concentrations directly to the site of infection. Voriconazole, a broad-spectrum triazole antifungal agent, is effective against various fungal pathogens, including *Candida* and *Aspergillus* species. However, its clinical utility is often limited by poor solubility and low skin penetration when applied topically (Hussain *et al.,* 2016).

To overcome these limitations, novel drug delivery systems such as ethosomes have been explored. Ethosomes are lipid-based vesicles composed of phospholipids, ethanol, and water, which enhance drug permeation through the skin due to their small size and high deformability (Touitou *et al.,* 2000). The ethanol content in ethosomes disrupts the lipid bilayers of the stratum corneum, increasing drug penetration, while the vesicular structure protects the encapsulated drug from degradation.

The formulation of voriconazole into an ethosomal gel can potentially enhance its therapeutic efficacy by improving its solubility, stability, and skin penetration. Ethosomal gels combine the advantages of ethosomes with the ease of application and sustained release properties of gels, making them ideal for topical administration (Dayan & Touitou, 2000).

This study aims to formulate and characterize a voriconazole ethosomal gel for effective topical treatment of fungal infections. The ethosomal vesicles will be prepared using various concentrations of phospholipids and ethanol, and their size, entrapment efficiency, and stability will be evaluated. The optimized ethosomal formulation will then be incorporated into a gel base and assessed for its in-vitro drug release, skin permeation, and antifungal activity. By enhancing the delivery of voriconazole to the skin, this ethosomal gel formulation has the potential to provide a more effective and convenient treatment option for topical fungal infections.

MATERIALS AND METHODS

Material

The materials used for developing the voriconazole ethosomal gel included voriconazole from Pharmaceutical Industries, and soya phosphatidylcholine from Ash Chemie India, Thane. Buffering agents disodium hydrogen phosphate, dipotassium hydrogen orthophosphate, and sodium chloride were supplied by S. D. Fine Chem. Ltd., Mumbai. Solvents methanol, ethanol, and chloroform were from Qualigens Fine Chemicals, Mumbai. The gelling agent Carbopol 934p, preservatives methyl paraben and propyl paraben, and propylene glycol were also sourced from S. D. Fine Chem. Ltd., Mumbai.

Preparation of Voriconazole Drug Ethosomes - Cold Method

Preparation of Voriconazole drug ethosomes was followed by method suggested by Touitou et al., with little modification (Kumar *et al.,* 2010). The ethosomal system of Voriconazole drug (50mg) comprised of 0.5-2% phospholipids, 10-20ml ethanol, 10% of propylene glycol, 10mg of cholesterol and aqueous phase to 100 % w./w. Voriconazole 0.025 g was dissolved in ethanol in a covered vessel at room temperature by vigorous stirring. Propylene glycol was added during stirring. This mixture was heated to 30^0C in a separate vessel and was added to the mixture drop wise in the center of the vessel, which was stirred for 5 minutes at 700 rpm in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation is stored under refrigeration. Ethosomes were formed spontaneously by this process Table 1.

Preparation of Voriconazole ethosomal gel

The best achieved ethosomal vesicles suspension, formula EF-6 was incorporated into carbopol gel (1%, 1.5%, 2% w/w) (Touitou *et al.,* 1997). The specified amount of carbopol 934 powder was slowly added to ultrapure water and kept at 1000 c for 20min. Tri ethanolamine (TEA) was added to it drop wise. Appropriate amount of formula EF-6 containing Voriconazole (1% w/w) was then incorporated into gel-base. Water q.s was added with other formulation ingredients with continuous stirring until homogenous formulation were achieved (G-1, G-2, G-3 and G-4).Gel containing free Voriconazole was prepared by similar method using 1.5% Carbopol Table 2.

Evaluation of Ethosomes

Size and Shape Analysis

A sample of ethosomes were suitably diluted with distilled water in order to observe individual vesicle and a drop of diluted suspension was put on a glass slide covered with cover slip and examined under microscope (magnification 15 × 45 X). The

diameters of 150 vesicles were determined randomly using calibrated eyepiece micrometer with stage micrometer. The average diameter was calculated using the formula (Godin and Touitou, 2003).

Average diameter = nd/n

n = number of vesicles

d = diameter of vesicles

Sanitation reduced the vesicular size. Since vesicular size of these vesicles could not be analyzed using microscopic method at magnification 15 × 45 X. Hence analysis of sonicated vesicles was done under a special microscope which is connected with software and photomicrographs were taken under 400 and 800 magnification. Further selected photomicrographs were analyzed for size analysis by using special software "particle size analysis" developed by BIOVIS. This special software works on images of photomicrographs with standard dimension.

Zeta potential

The charge of the ethosomal vesicle is an important parameter than can influence both vesicular properties such as stability as well as skin-vesicle interactions and its zeta potential was determined using a computerized inspection system (Touito *et al.,* 2000).

Entrapment Efficiency

The entrapment efficiency of Voriconazole by ethosomal vesicle was determined by ultracentrifugation 10 ml of (ethosomal suspension) each sample was vortexed for 2 cycles of 5 min with 2 minutes rest between the cycles. 1.5ml of each vortexed sample and fresh untreated ethosomal formulations were taken into different centrifugal tubes. These samples were centrifuged at 20,000 rpm for 3 hours. The supernatant layer was separated, diluted with water suitably and drug concentration was determined at 256 nm in both vortexed and unvortexed samples. The entrapment efficiency was calculated as follows:

$$\textbf{Entrapment Efficiency} = \frac{\mathbf{T - C}}{\mathbf{T}} \mathbf{x100}$$

'T' is total amount of drug that detected from supernatant of vortexed sample.

C' is the amount of drug unentraped and detected from supernatent of unvortexed sample.

Evaluation of gel

Organoleptic Characteristics

The formulations were tested for its physiological rheological properties like colour, odour, texture, phase separation and feel upon application (grittiness, greasiness).

Washability

A small quantity of gel was applied on the skin. After washing with water, checked for whether the gel was completely washable or not (Thomas *et al.,* 2019).

pH

Solution of 1gm of gel dissolved in 30ml of distilled water (pH 7) was prepared. The pH of the ethosomal gel was determined by using digital pH meter, measured by bringing the probe of the pH meter in contact with the samples.

Spreadability

It was determined by modified wooden block and glass slide apparatus. A measured amount of gel was placed on fixed glass slide; the movable pan with a glass slide attached to it and was placed over the fixed glass slide, such that the gel was sandwiched between the two glass slides for 5min (Mishra *et al.,* 2018). The weight was continuously removed. Spreadability was determined using the formula:

$$S = \frac{M}{T}$$

Where, S is the Spreadability in g/s,
M is the mass in grams &
T is the time in seconds.

Drug content

1g of gel was dissolved in a 100ml of phosphate buffer pH 7.4 for 48 hrs with constant stirring using magnetic stirrer. Solution was filtered and observed with U.V spectrophotometer at λ_{max} 256nm. The measurements were made in triplicate (Gadakh *et al.,* 2012).

***In-vitro* release studies**

Drug Release Study from Dialysis Membrane

The skin permeation of Voriconazole from ethosomal formulation was studied using open ended diffusion cell specially designed in our laboratory according to the literates (Korsmeyer *et al.,* 1983). The effective permeation area of the diffusion cell and receptor cell volume was 2.4 cm and 200 ml respectively. The temperature was maintained at 37 ± 0.5°C. The receptor compartment contained 200 ml of pH 7.4 buffer and was constantly stirred by magnetic stirrer at 100 rpm. Prepared dialysis membrane was mounted between the donor and the receptor compartments. Optimized ethosomal gel was applied to the dialysis membrane and the content of diffusion cell was kept under constant stirring then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analysed by spectrometric method at 256 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh pH 7.4 buffer. Triplicate experiments were conducted for drug release studies. The results of *in vitro* release profile obtained for all the formulations were plotted in modes of data treatment as zero order models, first order model, higuchi model and korsmeyer / peppa's model.

RESULTS AND DISCUSSION

The ethosomal formulations (EF1-EF8) were characterized by their vesicle size, zeta potential, and entrapment efficiency. The vesicle sizes ranged from 185.65±0.33 nm (EF7) to 274.65±0.85 nm (EF5) (Table 3). The smaller vesicle size in EF7 suggests it may have better skin penetration capabilities compared to the larger vesicles.

The zeta potential values for the formulations ranged from -28.85 mV (EF2) to -36.58 mV (EF7) (Table 4). Negative zeta potential indicates good stability due to repulsive forces preventing aggregation. EF7, with the highest negative zeta potential, is likely to have the best stability among the formulations.

Entrapment efficiency varied across the formulations, with EF3 showing the highest efficiency at 73.02±0.044%, and EF1 showing the lowest at 52.92±0.024% (Table 5). High entrapment efficiency is crucial for ensuring that a significant amount of voriconazole is encapsulated within the ethosomes, enhancing its therapeutic effectiveness.

The pH values of the gels (G1-G3) were close to the skin's natural pH, with G2 having a pH of 6.832±0.003, which is suitable for topical application without causing irritation (Table 6). Drug content analysis revealed that G2 had the highest drug content at 99.023±0.303%, indicating efficient incorporation of voriconazole into the gel matrix.

The organoleptic properties of formulation G2 were favorable, exhibiting a golden yellow color, non-greasy texture, and free from grittiness, which ensures user compliance and comfort (Table 7).

G2 was also easily washable and showed good spreadability (6.3 g.cm/sec), enhancing its application convenience. The release kinetics of formulation G2 indicated that the drug release followed the Higuchi model best (R^2 = 0.8997), suggesting that the release mechanism is primarily diffusion-controlled (Table 8). This sustained release profile is beneficial for maintaining therapeutic drug levels over an extended period, reducing the need for frequent reapplication.

Table 1: Formulation of Ethosomes

Ethosomal formulation	Lecithin (Soya lecithin (%)	Ethanol (ml)	Propylene glycol (%)	Drug (mg)	Cholesterol (mg)	Water
EF1	0.5	10	10	50	10	q.s
EF2	0.5	20	10	50	10	q.s
EF3	1.0	10	10	50	10	q.s
EF4	1.0	20	10	50	10	q.s
EF5	1.5	10	10	50	10	q.s
EF6	1.5	20	10	50	10	q.s
EF7	2.0	10	10	50	10	q.s
EF8	2.0	20	10	50	10	q.s

Table 2: Ethosomal gel formulations

Gel formulation	Voriconazole ethosomal suspension (equivalent to Voriconazole 1%)	Carbopol (%)	Triethanolamine (ml)	Water
G-1	1	1	0.5	q.s
G-2	1	1.5	0.5	q.s
G-3	1	2	0.5	q.s
*G-4	-	1.5	0.5	q.s

***G4 drug free gel**

Table 3: Size of Ethosomes of various formulations

S. No.	Ethosomal formulation	Vesicle Size (nm)
1.	EF1	225.45±0.25
2.	EF2	215.45±0.32
3.	EF3	246.65±0.45
4.	EF4	232.85±0.65
5.	EF5	274.65±0.85
6.	EF6	210.45±0.74
7.	EF7	185.65±0.33
8.	EF8	225.47±0.58

Table 4: Zeta potential of formulations

S. No.	Ethosomal formulation	Zeta Potential (mV)
1.	EF1	-32.56
2.	EF2	-28.85
3.	EF3	-30.74
4.	EF4	-33.74
5.	EF5	-31.85
6.	EF6	-34.74
7.	EF7	-36.58
8.	EF8	-29.85

Table 5: Entrapment efficiency of formulations

Formulation code	Entrapment efficiency (%)			Mean ± SD
EF1	52.90	52.95	52.89	52.92 ± 0.024
EF2	71.45	71.47	71.42	71.44 ± 0.020
EF3	73.05	73.02	73.10	73.02 ± 0.044
EF4	65.00	65.04	65.06	65.03 ± 0.024
EF5	55.27	55.23	55.25	55.25 ± 0.016
EF6	64.38	64.42	64.32	64.37 ± 0.034
EF7	72.52	72.50	72.61	72.54 ± 0.068
EF8	59.47	59.52	59.43	59.47 ± 0.036

Table 6: pH and drug content measurement

Formulation code	pH Mean ± SD*	Drug content (%) Mean ± SD*
G1	6.812±0.055	94.817±0.246
G2	6.832±0.003	99.023±0.303
G3	6.822±0.016	95.567±0.890

Table 7: Organoleptic properties of formulation G2

Organoleptic Characteristics	Color: golden yellow Greasiness: Non-greasy Grittiness: Free from grittiness Ease of application: Easily/smoothly
Washability	Easily washable without leaving any residue on the surface of the skin
Spreadability	6.3 g.cm/sec

Table 8: Release kinetics

Formulation code	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmeyer peppas (R^2)
G2	0.5912	0.4682	0.8997	0.3952

CONCLUSION

The study successfully formulated and characterized voriconazole ethosomal gels for topical fungal treatment. Among the tested formulations, EF7 emerged as the most promising due to its optimal vesicle size, high zeta potential, and significant entrapment efficiency. Formulation G2, incorporating EF7, demonstrated excellent drug content, favorable organoleptic properties, and a sustained release profile following Higuchi kinetics. These results indicate that voriconazole ethosomal gel is a promising candidate for effective topical fungal therapy, offering enhanced drug delivery, stability, and user compliance. Further in-vivo studies are recommended to confirm these findings and explore clinical applications.

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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