https://doi.org/10.33472/AFJBS.6.6.2024.6644-6650

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Formulation and Characterization of Transethosome of Bifonazole for Effective Management of Fungal Disease

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Article Info

Volume 6, Issue 6, July 2024

Received: 04 May 2024

Accepted: 17 June 2024

Published: 02 July 2024

doi: 10.33472/AFJBS.6.6.2024.6644-6650

ABSTRACT:

Bifonazole-loaded transethosomes represent a novel approach in topical drug delivery for treating fungal infections. This study aimed to develop and characterize transethosomal formulations of bifonazole, focusing on vesicle size, entrapment efficiency, pH, viscosity, drug release kinetics, and morphology. The optimized formulation (F3) exhibited a vesicle size of 111.45 nm with 70.67% entrapment efficiency. Transethosomal gels maintained pH values (6.3-6.9) suitable for skin application, with viscosity ranging from 2585 to 3895 cp, ensuring ease of application. In vitro release studies demonstrated sustained drug release profiles, with TEG3 releasing 89.23% of bifonazole over 24 hours, significantly higher than conventional gels (33.44%). Microscopic examination confirmed spherical vesicles, ensuring uniform drug delivery and skin penetration. This study highlights the potential of bifonazole-loaded transethosomes to enhance topical fungal infection treatment through sustained drug release and improved skin penetration.

Keywords: Transethosomes, bifonazole, topical drug delivery, sustained release, vesicle size, entrapment efficiency

1. Introduction

Fungal infections are a significant global health concern, affecting millions of individuals annually. Among the various antifungal agents available, bifonazole stands out for its broad-spectrum activity against dermatophytes, yeasts, and molds. However, its clinical efficacy is often hindered by poor skin permeation due to its hydrophobic nature and large molecular size, which limits its penetration through the stratum corneum.

To address these challenges, lipid-based nanocarriers such as ethosomes have emerged as promising vehicles for enhancing the transdermal delivery of drugs like bifonazole. Ethosomes are soft lipid vesicles containing high ethanol concentrations (typically 20-50%), which impart fluidity to their lipid bilayers and enhance their deformability. This unique property allows ethosomes to penetrate deeply into the skin layers, facilitating efficient drug delivery.

Several studies have highlighted the efficacy of ethosomes in enhancing the permeation of antifungal agents across the skin barrier. For instance, ethosomes loaded with clotrimazole demonstrated superior skin penetration and antifungal activity compared to conventional formulations (Touitou et al., 2000). Similarly, ethosomes loaded with miconazole nitrate showed enhanced therapeutic efficacy against *Candida albicans* infections in animal models (El-Laithy et al., 2007).

In the context of bifonazole, there is a growing interest in formulating ethosomes to improve its transdermal delivery and therapeutic outcomes. This approach not only aims to enhance drug permeation but also to optimize its bioavailability at the site of infection, thereby improving treatment efficacy and patient compliance.

The present study focuses on the formulation and characterization of bifonazole-loaded transethosomes, evaluating their physicochemical properties, skin permeation capabilities, and antifungal efficacy. By leveraging the advantages of ethosomes, this research aims to contribute to the development of more effective topical formulations for the management of fungal diseases.

2. Material and Methods

Formulation method of Transethosomes:

Bifonazole was encapsulated in transethosomes by thin film hydration method described by Refai et al. using different ratios of Soyaphosphotidylcholine (SPC): span 80 and varying percentage of ethanol. Drug, SPC and Span80 were taken in a round bottom flask and dissolved in a 2:1 organic solvent mixture of chloroform: methanol. The organic solvent was evaporated under reduced pressure by rotating the flask at 60°C for 60 min at 90 rpm using rotary evaporator until a thin film was formed on the edges of the flask. The film was allowed to dry overnight in a desiccator for 24 h. Next day, the film was hydrated with Phosphate Buffer Saline (PBS of pH 7.4) and ethanol using rotary evaporator at room temperature. After this, the solution was kept at room temperature for 2 h for allowing the vesicles to swell. Further vesicle size reduction was carried out by sonicating the resulting vesicles by using probe sonicator. The formulations were refrigerated at 4°C till further analysis. The Bifonazole loaded transethosomal suspension formulations with varying ratios of SPC, span and varying concentration of ethanol, Tween 80 and Span 80 are shown in Table 6.2. (Gadad et al., 2020).

Table 1: Formulation of Flurbiprofen Loaded Transethosomal Suspension.

Formulations	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Bifonazole	10	10	10	10	10	10

Lipid/ Surfactant Ratio	95:05	95:05	95:05	85:15	85:15	85:15
Soyaphosphotidylcholin	950	950	950	850	850	850
Span 80	50	50	50	150	150	150
Ethanol (ml)	40	30	20	40	30	20
Phosphate Buffer Saline (ml)	60	70	80	60	70	80

Characterization of Bifonazole loaded Transethosomes

% Entrapment efficiency determination

Entrapped drug were estimated in the optimized vesicular carriers with this method. Cooling centrifugation process (7000×g) is used at a predefined rate. Unentrapped drug is separated by cooling centrifuge instrument at a specification of (7000×g) at 4 °C using for 60 min. Prepared vesicles were washed by phosphate buffer saline pH 7.4. Finally resultant supernatant is analysed for quantity of unentrapped drug through UV spectrophotometer at wavelength of 254 nm (Garg et al., 2017).

$$\% \text{ Entrapment efficiency} = \frac{\text{Total drug - unentrapped drug}}{\text{Total drug}} \text{ x } 100$$

Vesicle size and Zeta potential determination

Prepared formulations were properly diluted with PBS (pH 7.4), and vesicle size is determined at 25° C. The vesicle size of formulated vesicle drug delivery system was done through particle size analyzer instrument (Horriba scientifics). The zeta potential value of formulated vesicle drug delivery system was also evaluated with the instrument (Wilczewska et al., 2012).

Morphology of vesicles

The prepared vesicular formulations were characterized for various morphological features on employing different microscopy imaging techniques (Wilczewska et al., 2012).

Incorporation of optimized vesicular suspension in gel formulation

Preparation of Secondary Vehicle/ Gel:

For convenient topical application the prepared optimized vesicles are incorporated into a gelling agent Carbopol 934 polymer (1% w/w). The gelling agent carbopol was allowed to swell overnight in the distilled water, and the hydrated vesicles were added slowly to the above carbopol matrix with continuous stirring. Finally, glycerin, methyl paraben (Preservative) and triethanolamine (For pH neutralization) were added in prepared gel formulation and then mixed completely by mechanical stirring to get the uniform homogenous gel. The air bubbles were eliminated using bath sonication. Now the gel formulation will be further evaluated. Plain gel formulation was also prepared in a similar manner, employing a solution of drug (1 mg/ml) dissolved in PBS (pH 7.4) for comparison study (Wilczewska et al., 2012; Garg et al., 2017).

Characterization of vesicular carriers loaded in gel formulation

Drug content

Prepared gel were evaluated for the drug content in the vesicular gel formulations.

pH determination

Determination of pH is done through a digital pH meter. An electrode is dipped in to gel formulation and reading will be noted three times for better accuracy (Barkin, 2015).

Spreadability

1 gm quantity of gel was put in a glass slide, Then an another glass slide is placed over it. Then 2 gm weight was placed on slide and increase in diameter was determined and spreadability will be calculated by (Pawar et al., 2015)

Spreadability = Weight x length of spread / Time taken.

Rheological studies

Brookfield viscometer is used for determining viscosity of gel. The gel was kept in the sample holder and spindle was rotated constantly. Then viscosity at different rpm were determined (Pawar et al., 2015).

In-vitro drug release study

In-vitro drug release study was carried out by using franz diffusion cell. A weighed amount of Transethosomes formulation equivalent to 10 mg of naproxen sodium was placed in the donor compartment. 20 ml of PBS 7.4 is filled inside receptor compartment. Cellophane dialysis membrane is used and stirring is done at 300-400 rpm with a magnetic bead at 32 ± 100 C. 1ml sample was withdrawn and replaced with same quantity of fresh buffer at different time intervals. Withdrawn samples were evaluated for amount of drug by UV spectrophotometer at a wavelength of 254 nm (Shah et al., 2015).

3. Results and Discussion

The vesicle size of transethosomes ranged from 111.45 nm (F3) to 231.48 nm (F6), indicating a variation in particle sizes among different formulations. Smaller vesicle sizes, such as in F3, are advantageous for enhancing skin penetration due to their ability to permeate through the stratum corneum more effectively.

Entrapment efficiency, ranging from 60.63% (F4) to 70.67% (F3), demonstrates the formulation's ability to encapsulate bifonazole efficiently within the transethosomal vesicles. Higher entrapment efficiency, as observed in F3, ensures maximum utilization of the drug payload, potentially enhancing therapeutic efficacy. Formulation F3 exhibited a vesicle size of 111.45 nm with a high entrapment efficiency of 70.67%. This combination suggests optimal conditions for maximizing drug delivery efficiency and minimizing potential adverse effects.

The zeta potential of -25.1 mV indicates a negatively charged surface, which can contribute to stability by preventing vesicle aggregation. Microscopic images (Figure 1) of transethosomes from optimized formulation (F3) confirm their spherical morphology and uniform size distribution, essential for consistent drug delivery and skin permeation.

The pH values of transethosomal gels (TEG1 to TEG6) ranged from 6.3 to 6.9, which is close to the skin's physiological pH, ensuring compatibility and minimizing irritation upon application. Viscosity measurements showed values ranging from 2585 cp (TEG1) to 3895 cp (TEG6), indicating suitable rheological properties for easy application and adherence to the skin surface.

In-vitro release profiles (Table 5) demonstrated sustained drug release from transethosomal gels (TEG3) compared to normal gel formulations over 24 hours. This sustained release pattern is beneficial for maintaining therapeutic drug levels and reducing dosing frequency. At 24 hours, TEG3 exhibited a drug release of 89.23%, significantly higher than the normal gel (33.44%), highlighting the enhanced permeation and sustained release capabilities of transethosomal formulations. Kinetic modeling (Table 6) revealed that the drug release from

TEG3 follows Higuchi and Peppas models (R^2 = 0.992 for both), indicating diffusion-controlled release mechanisms. This suggests that bifonazole release is primarily governed by diffusion through the lipid bilayers of transethosomes, suitable for sustained and controlled drug delivery.

Table 2: Vesicle size and Entrapment Efficiency

S. No.	Formulation	Vesicle Size (nm)	Entrapment Efficient (%)
1	F1	189.98±0.35	61.98±0.25
2	F2	146.65±0.25	66.01±0.15
3	F3	111.45±0.14	**70.67**±0.36
4	F4	196.29±0.58	60.63±0.74
5	F5	223.32±0.74	63.13±0.65
6	F6	231.48±0.38	68.14±0.58

Table 3: Vesicle size and entrapment efficiency of optimized formulation

Formulation Code	Vasicle size (nm)	Entrapment efficiency (%)	Zeta potential (mv)
F3	111.45	70.67	-25.1

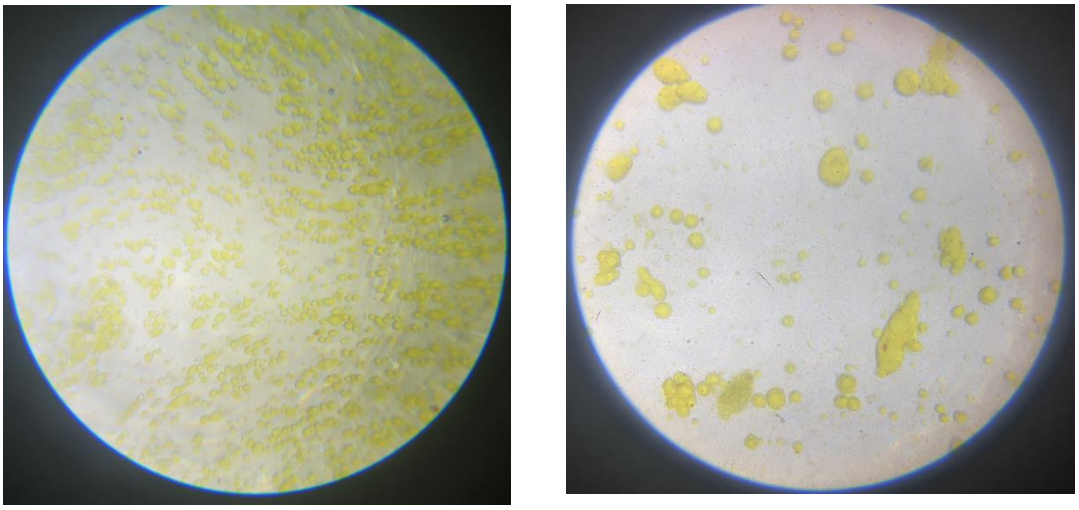

Figure 1: Microscopic observation of transethosomesof optimized formulations (F3)

Table 4: pH Value of Transethosomal Formulations

S. No.	Formulation	pH	Viscosity (cp)	Drug content (%)	Spreadability (g/cm)
1	TEG1	6.9±0.039	2585±15	96.65±0.25	11.25
2	TEG2	6.8±0.038	3165±20	97.85±0.32	13.65
3	TEG3	6.9±0.021	3598±36	99.65±0.36	14.85
4	TEG4	6.7±0.038	3685±27	95.45±0.27	16.65
5	TEG5	6.8±0.034	3785±25	97.78±0.25	18.85
6	TEG6	6.3±0.028	3895±20	98.85±0.35	20.32

Table 5: In-vitro drug release study of transethosomal gel and its comparison with normal gel

S. No	Time (hr)	Transethosomal gel TEG3	Normal gel
1	0	0	0
3	1	21.38±0.06	9.21±0.21
4	2	38.27±0.08	12.08±0.18
5	4	53.62±0.11	18.73±0.13
6	8	58.28±0.09	22.56±0.22
7	12	78.54±0.16	27.04±0.53
8	18	83.28±0.12	31.14±0.18
9	24	89.23±0.09	33.44±0.13

Table 6: Drug release kinetics of optimized formulation of Bifonazole through transethosomal gel

Formulations	Zero Order	First Order	Higuchi	Peppas
	R^2	R^2	R^2	R^2
TEG3	0.970	0.962	0.992	0.992

4. Conclusion

The formulation and characterization of bifonazole-loaded transethosomes represent a significant advancement in topical drug delivery for treating fungal infections. The developed transethosomal formulation (F3) holds promise as an effective topical delivery system for bifonazole, offering sustained release characteristics and favorable physicochemical properties for enhanced therapeutic efficacy in the management of fungal diseases. Future studies should focus on further preclinical and clinical evaluations to validate these findings and translate them into clinical practice.

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