Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 1, 2024, pp. 67-81.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

BIFONAZOLE LOADED TRANSETHOSOMAL GEL: FORMULATION AND OPTIMIZATION BY BOX BEHNKEN DESIGN

Kshema[1], Sandhya Vasanth[2], Sneh Priya[1*]

[1]*Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences, Department of Pharmaceutics, Deralakatte, Mangalore-575018*
[2]*Department of Pharmaceutics, Yenepoya Pharmacy College & Research Centre, Mangalore-575018*

This study was intended to develop and optimize the transethosomal gel of Bifonazole for the treatment of fungal infection. For the optimization purpose, Box-Behnken Design was used and the concentration of soya lecithin, ethanol and sodium cholate were selected as independent variables. The individual and combined effects of independent variables were assessed on vesicle size, PDI and entrapment efficiency. Further, the optimized Bifonazole-loaded transethosomes were evaluated for zeta potential and TEM. The optimized transethosomes were incorporated into 1.5 % Carbopol 940 gel and evaluated for in vitro release, ex vivo permeation studies and antifungal activity. The vesicle size, PDI, %EE and zeta potential of optimized formulation were found to be 104.7nm, 0.356, 86.23% and -27.2mV respectively. The transmission electron microscopy showed that the vesicles were uniform and spherical. The in vitro release of transethosome suspension (73.49%), and transethosomal gel (68.43%) was more when compared to the conventional gel (45.37%), marketed cream (43.31%) and drug suspension (36.49%). For both prepared gel in vitro release data fits well into the Higuchi's model (R^2 > 0.99). From an ex vivo study, transethosomal gel shows a significant increase in the steady state flux to 1.53 times than the conventional gel and 1.54 times than marketed cream. Prepared transethosomal gel of Bifonazole showed better antifungal activity than marked cream. This study concluded that Bifonazole-loaded transethosomal gel showed better permeation and enhancement in antifungal activity.

Keywords: *transethosome, Bifonazole, topical, Carbopol gel, antifungal*

INTRODUCTION

Fungal infections may be superficial or systemic[1]. Approximately 20–25% of the human population shows the incidence of skin fungal infections reportable. Athlete's foot, dermatophytosis, ringworm or tinea corporis, itchiness of jock or tinea cruris and candidiasis are examples of fungal infection[2]. Candida, Aspergillus, Penicillium, Cryptococcus and Coccidioides are the causative organism of fungal infections. Fungal infections are highly contagious and identified simply by visual symptoms like redness, itching, cracking and peeling. Initial-stage fungi attack the surface of the skin and later spread into the deeper layers of the skin by desquamation[1,2]. Azoles, polyenes, echinocandins, allylamines and antimetabolites are five broad categories of antifungal agents[3].

Treatment for fungal infections through a topical route provides several advantages such as patient compliance, targeting the site of infection, enhancement in treatment efficacy and decrease in risk of systemic side effects. Various dermatological skin infections are treated with different types of effective antifungal compounds[4] The oral conventional formulations showed several drawbacks like poor absorption, drug-drug interactions, high metabolism and toxicity. Even the marketed conventional topical dosages such as lotions, sprays, ointment, gels and creams show various challenges like the barrier function of stratum

Received : 27/9/2023 & Accepted : 21/11/2023

*Corresponding author: Sneh Priya, E-mail: snehpriya123@nitte.edu.in

corneum, poor drug penetration, limiting local bioavailability and irritation or allergic reactions[2]. Gels are one of the semisolid topical drug delivery systems and they contain a transparent gelling agent to form three-dimensional colloidal structures. These preparations are transparent and opaque[5]. The limitation of topical conventional dosages can be overcome by formulating drugs in vesicular systems like ethosome, transethosomes, and transferosomes etc[2].

Transethosome was introduced by Chung Kil Song et al in 2012[6]. They are made up of high amounts of ethanol, phospholipids such as phosphatidylcholine, and a permeation enhancer or edge activator. These are deformable and thermodynamically stable vesicles. They have irregular spherical shapes and their size lies between 40 nm to 200 nm, depending on the drug[7]. Transethosomes ensure increased permeation of drug through the skin due to the combined effect of ethanol and edge activator that causes lipid-bilayer rearrangement of these vesicles[6].

Bifonazole is an azole derivative that shows a broad spectrum of activity against fungi, dermatophytes, yeasts, moulds and some Gram-positive bacteria. Creams, solutions, powders and gels having the strength of 1% have been used to treat superficial fungal infections that were applied once a day. They are mainly chosen for the treatment of topical infections such as cutaneous candidiasis and tinea pedis[8] This drug comes under BCS class IV which has low solubility and low permeability drug[9]. The drug is insoluble in water, and sparingly soluble in ethanol. Bifonazole is a highly lipophilic drug (log p:4.77) with a very short half-life (1-2h) and 0.6% of an applied dose is minimally absorbed by dermal application. Therefore, Transethosome load gel was formulated to enhance the permeability and antifungal activity of Bifonazole by topical application.

MATERIALS AND METHODS

Bifonazole was purchased from Yarrow Chem Products, Mumbai. Soya lecithin was obtained from HiMedia Laborites, Mumbai, Ethanol was purchased from Nice Chemicals, Kerala. Sodium cholate and Carbopol 940 were obtained from Loba Chemie, Mumbai. Mycospor (Bifonazole cream IP) manufactured by Bayer Pharmaceuticals Pvt. Ltd was purchased from the local market.

The apparatuses used in the present studies were U V Spectrophotometer (UV-1900i-Shimadzu), Probe Sonicator (Sonics, Model: VCx-130-220), Magnetic stirrer (Remi Electro ltd.), Malvern Zeta sizer (Zeta sizer Malvern UK based-Zen 3600), Brookfield Viscometer (Brookfield, Model: LVDV-II+Pro) etc.

METHODS

Formulation and Characterization of Bifonazole transethosome

Experimental Design

For optimization of the formulation, DOE was performed using Design Expert® software. A Box Behnken statistical design approach was utilized to determine the influence of the three independent variables, namely soya lecithin, ethanol, and surfactant at three different levels low (-1), medium (0), high (1) on dependent variables Vesicle size, Polydispersity index, Entrapment efficiency of the prepared transethosome as shown in **Table 1**. The design presented a total of 17 formulation runs.

Table 1: Factors and Levels.

Factors	Levels			Dependent variable	Desirability
	-1	0	+1		
Soya lecithin (X1, %w/v)	2	3	4	Vesicle size (Y_1, nm)	100 nm
Ethanol (X2, %v/v)	20	27.5	35	Polydispersity Index (Y_2)	Minimum
Sodium cholate (X3, %w/v)	0.1	0.2	0.3	Entrapment efficiency (Y_3, %)	Maximum

Preparation of transethosomes

Bifonazole-loaded transethosomes were prepared by the cold method as the composition given in **Table 2**. The organic phase was obtained by dissolving the drug (100 mg) and soya lecithin in 3 ml of different concentrations of ethanol (20-35% v/v) at 30°C. The aqueous phase was obtained by using dissolving surfactant in distilled water heated to 30°C. Then aqueous phase is added to the organic phase dropwise using a syringe with continuous stirring at 700rpm using a magnetic stirrer. The stirring continued for 45 minutes to get the transethosomal dispersions. Then the dispersion is subjected to size reduction for 5 min using a probe sonicator[10].

Characterization of Bifonazole Loaded Transethosome

Vesicle size, Zeta Potential and PDI

Vesicle size characterization was carried out using a Zeta sizer (Nano ZS, Malvern Instruments, UK) with the Dynamic light scattering principle. PDI indicates the size distribution (polydispersity or monodispersity) of transethosome. Zeta potential is defined as the charged particle obtained when it is present in the medium is the overall charge that the particles obtain in a particular medium. The sample was prepared by diluting 0.5 ml of the formulation with double distilled water up to 10 ml and analyzed with a Zeta sizer instrument[11].

Percentage entrapment Efficiency

Take 1.5 ml of transethosomal suspension in 2 ml Eppendorf's tube and centrifuge at 13,000 rpm for 90 min at 4°C by cold centrifugation. After centrifugation supernatant and the sediment were separated. The Bifonazole concentrations present in the supernatant and sediment were analyzed at 254 nm using the UV spectroscopic method. The %EE was calculated using the formula given below[12].

$$\%\text{Entrapmentfficiency} = \frac{\text{The total amount of drug} - \text{Amount of free drug}}{\text{The total amount of Drug}} \times 100$$

Optimization of Bifonazole Loaded Transethosome

Based on the constraints given as a vesicle size range of 100 nm, minimum PDI and maximum entrapment efficiency, Design Expert® software provided a solution with high desirability which was considered an optimized formulation. The optimized formulation was prepared as per the solution i.e., 2.685% w/v of soya lecithin, 31.844% v/v of ethanol and 0.300% w/v of sodium cholate was used for the formulation.

Transmission Electron Microscopy

The surface appearance and shape of vesicles are determined using Transmission Electron Microscopy. Transethosome suspension is dispersed in distilled water and then 10 µL of this diluted suspension was placed on the carbon-coated grid which was then visualized using Jeol/JM 2100, source LaB6 electron microscope[13].

Formulation and characterization of transethosomal gel

Formulation of different gels

The 1% Conventional gel (CG) and transethosomal gel (TG) were prepared by using Carbopol 940 (1.5%). Carbopol gel was prepared by dissolving 1.5 gm of Carbopol 940 in 25 ml of distilled water and stirring it for 2 hours with a magnetic stirrer. CG and TG were prepared by incorporating 75 ml of ethanolic solution of drug and transethosomal suspension which contained 1g of drug respectively to previously prepared concentrated Carbopol gel. A small amount of triethanolamine was added to achieve the gel-like consistency[10].

Characterization of transethosomal gel

pH

Weigh about 1 g of transethosomal gel which uses distilled water to dilute to 100 ml. The pH of both formulations is determined by placing the electronic pH meter in the solution and equilibrating it for 1 minute[14].

Measurement of viscosity

The Brookfield viscometer, DV-II+pro D220 used for viscosity measurements by choosing the spindle number T-96 at 12 rpm[15].

Table 2: Composition and responses of transethosome as per Box-Behnken design.

Form. code	Independent variables			Responses		
	A: Soya lecithin %w/v	**B: Ethanol %v/v**	**C: Sod. Cholate %w/v**	**Vesicle size nm Y_1**	**PDI Y_2**	**% EE Y_3**
1	2	20	0.2	117.3±1.85	0.408±0.035	84.24±1.24
2	4	20	0.2	154±1.02	0.501±0.013	68.3±1.52
3	2	35	0.2	82.47±1.54	0.365±0.054	92.56±2.35
4	4	35	0.2	50.71±2.01	0.245±0.025	82.5±1.42
5	2	27.5	0.1	131.2±1.22	0.426±0.075	87.13±1.75
6	4	27.5	0.1	143.9±1.57	0.455±0.013	74.18±1.35
7	2	27.5	0.3	124.3±1.35	0.399±0.057	92.62±2.10
8	4	27.5	0.3	87.74±1.47	0.260±0.064	73.96±1.54
9	3	20	0.1	171±1.85	0.448±0.051	76.93±1.74
10	3	35	0.1	64.04±2.11	0.341±0.026	87.75±1.34
11	3	20	0.3	141.1±1.63	0.473±0.021	77.73±1.58
12	3	35	0.3	62.85±1.42	0.348±0.035	86.91±1.86
13	3	27.5	0.2	136.4±2.05	0.430±0.015	77.41±1.02
14	3	27.5	0.2	122.1±1.25	0.470±0.024	77.96±1.42
15	3	27.5	0.2	135.8±1.48	0.390±0.045	82.3±2.33
16	3	27.5	0.2	135.2±2.02	0.391±0.057	79.3±2.05
17	3	27.5	0.2	133.9±1.48	0.412±0.080	80.1±1.45

Spreadability

The spreadability of the gel was measured using a modified wooden block and glass slide equipment. About 1 gm of gel was measured on this ground slide. Then, the gel was sandwiched and fitted with the hook between this slide and another glass slide with a set ground slide length. A 100 g weight was placed on the two slides for 5 minutes to eliminate air and provide a clear gel film between the slides. The excess gel was scraped out from the corners. The top plate subsequently was subjected to a pull of 20 gms. To cover a distance of 6 cm, using the string connected to the handle, and the time (in seconds) taken by the top slide is noted Spreadability was measured using the formula

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

Where S = is the spreadability, M = is the weight in the pan (attached to the upper slide), L = is the length transferred by the glass slide and T = reflects the time required to remove the slide entirely from each other[16].

Drug content

The drug content of the gel was determined by dissolving an accurately weighed 1gm gel (about 1 gm) in about 100 ml of ethanol containing 10% methanol in a 100 ml volumetric flask. Appropriate dilutions were made with the phosphate buffer pH 6.8. The resulting solutions were then filtered and spectrophotometric analyzed at 254nm. Drug content was determined from the standard curve of Bifonazole[13].

FTIR Study

A Shimadzu FTIR 8300 Spectrophotometer was used for Fourier transform infrared (FTIR) spectroscopy, and the spectrum was obtained from the 4000 to 400 cm^{-1} range. The compatibility of the prepared gel with the formulation ingredients was determined and compared with the FTIR peak of Bifonazole[16].

***In vitro* drug release study**

The *in vitro* experiment was performed utilizing the dialysis bag method. The dialysis membrane was soaked in distilled water for 24 hours before starting the experiment. Add the required volume (equivalent to 10mg of drug) of the transethosome suspension, drug suspension, and amount (equivalent to 10mg of drug) of transethosomal, and conventional gel to each cellophane membrane respectively. The acceptor compartment was filled with 50 ml of

the 6.8 phosphate buffer which contained a small magnetic bead rotated at a constant speed of 50 rpm. The study was carried out at 37± 0.5°C for 8 hrs. 1ml of samples were withdrawn from the acceptor compartment at predetermined time intervals. Which is suitably diluted and absorbance was measured spectrophotometrically at 254 nm. Each time the reservoir compartment was replenished with the same quantity of fresh phosphate buffer of pH 6.8 to maintain sink condition[16].

Ex vivo drug permeation studies using goat skin

The *ex vivo* skin experiments were performed using two compartments containing Franz Diffusion cells. The donor compartment consists of two open ends where one end is covered with goat skin previously harvested from the slaughterhouse, hair separated from the skin and soaked with pH 6.8 phosphate buffer. Add the required volume (equivalent to 10mg of the drug) of transethosome suspension, drug suspension and amount (equivalent to 10mg of the drug) of transethosomal gel and conventional gel in the donor compartment on each dermal side of the skin, respectively. The acceptor compartment was loaded with 12 ml pH 6.8 phosphate buffer comprising a small rotating magnetic bead at a steady 50 rpm speed. The study was performed for 8 hours at 37± 0.5 ° C. At a fixed time, 1 ml of samples were collected from the reservoir compartment. Which was suitably diluted and the absorbance was calculated spectrophotometrically at 254nm. Each period the reservoir compartment was replenished with the same volume of fresh pH 6.8 phosphate buffer to maintain sink condition[16]. The flux was determined from the linear portion of the slope. The relationship established from the first law of Fick's diffusion used for calculating the Bifonazole permeability coefficient (Kp) through goat skin, the equation below:

$$Kp = J/C$$

Where J is the flux, and C is the drug concentration in the donor compartment[16].

In-vitro antifungal activity

The cup plate technique was used to evaluate antifungal action. Marketed cream of Bifonazole was used as standard. 100 µl of *Candida albicans* fungal inoculums were seeded in Petri dishes containing 15 ml medium (sabourd dextrose agar). After solidification 4 wells of 2cm diameter were bored out of the agar plates. Each well was filled with transethosomal gel and 1% marketed cream. Then fungal plates were incubated for two days at 25°C and the zone of inhibition was recorded[17].

RESULTS AND DISCUSSION

Results

Formulation and characterization of Bifonazole Loaded Transethosome

Statistical analysis of the design of the experiment

The Bifonazole loaded transethosomes were successfully formulated by using Box Behnken design to understand the effects of the transethosome constituents i.e., soya lecithin, ethanol, and surfactant on its attributes.

Particle Size, Polydispersity Index and Entrapment efficiency

The effect of soya lecithin, ethanol, surfactant on particle size, polydispersity index, and Entrapment efficiency of the formulation obtained for box Behnken design is shown in **Table 2,** Summary of Regression analysis and ANOVA shown in **Table 3,** the response surface curve **(Fig.s 1, 2 and 3**) also illustrates the effects of soya lecithin, ethanol, surfactant on particle size, PDI, and Entrapment efficiency.

Vesicle size of transethosomes

The independent variables i.e., the concentration of soya lecithin, concentration of ethanol and concentration of sodium cholate showed significant effects on vesicle size as depicted in the 3D graph **(Fig. 1).** The Box Behnken Design showed that increase in soya lecithin concentration from 2 to 4% w/v, and there was an increase in vesicle size at limited concentrations of ethanol, as phospholipids are the main constituents of transethosomes, it acts as a vesicle forming agent forms compact and condense structure[18]. As the concentration of ethanol increased from 20-35% v/v initially there was an increase in vesicle size up to a certain concentration and then the vesicle size decreased. The size of transethosomes reduces due to the reduction in the membrane thickness and also due to the formation of a phase with interpenetrating hydrocarbon chains[11,19]. As the concentration of sodium cholate increases,

vesicle size decreases. This may be because of the anionic nature of sodium cholate, there was an increase in curvature due to the steric repulsion in the nearby charged molecules causes lessens the size of vesicles[13].

The model generated for vesicle size had a p-value of < 0.05 and an F value of 22.36 indicating the Quadratic model to be significant. The value of 4.73 indicates a non-significant lack of fit, implying the model to be appropriate to calculate the vesicle size. The Predicted R^2 of 0.5689 is in reasonable agreement with the Adjusted R^2 of 0.9232; i.e., the difference is more than 0.2. The polynomial equation obtained from the results of the analysis showed a quadratic model:

$$\text{Vesicle size} = +132.68 - 2.36(A) - 40.42(B)^* - 11.77(C)^* - 17.12(AB)^* - 12.32(AC)^* + 7.18(BC) - 9.76(A^2) - 21.80(B^2)^* - 1.13(C^2) \quad \ldots\ldots (1)$$

Where A is the concentration of soya lecithin, B is the concentration of ethanol and C is the concentration of sodium cholate. the coefficient in this equation reflects the standardized beta coefficient and the asterisk symbol implies variable significance. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect.

PDI of transethosomes

PDI is a measure of the width of unimodal size distributions. A value of 0-0.5 indicates homogenous dispersion, while a value of 1 indicates an entirely heterogenous polydisperse population. An acceptable PDI should have a value below 0.5[19,20]. The concentration of soya lecithin, concentration of ethanol and concentration of sodium cholate showed significant effects on PDI as depicted in the 3D graph **(Fig. 2)**. As the concentrations of ethanol and sodium cholate increase PDI decreases, because aggregation between the vesicles reduced due to the negative charges causes the electrostatic repulsion, these charges are provided by ethanol and sodium cholate[10,13,21]. The model generated for PDI had a p-value of < 0.05 and an F value of 5.76 indicating the Quadratic model to be significant. The value of 2.09 indicates a non-significant lack of fit, implying the model to be appropriate to calculate the PDI. The PDI of the prepared transethosomes was not affected by the studied factors.

The Predicted R^2 of 0.1660 is in reasonable agreement with the Adjusted R^2 of 0.6407; i.e., the difference is more than 0.2. The polynomial equation obtained from the results of the analysis. The polynomial equation obtained from the results of the analysis:

$$\text{PDI} = +0.3984 - 0.0159(A) - 0.0676(B)^* - 0.0238(C) - 0.0558(AB)^* - 0.0420(AC) - 0.0045(BC) \quad \ldots\ldots (2)$$

Where A is the concentration of soya lecithin, B is the concentration of ethanol and C is the concentration of sodium cholate, the coefficient in this equation reflects the standardized beta coefficient and the asterisk symbol implies variable significance. The polynomial equation shows that transethosome constituents have an interactive effect on the PDI attributes. However, there was not much effect of the factors on the PDI of formulation.

Table 3: Summary of Regression analysis and ANOVA.

SL No	Factor	Vesicle size (Adjusted R^2 =0.9232)		PDI (Adjusted R^2=0.6407)		%EE (Adjusted R^2=0.8857)	
		Estimated beta coefficient	P value	Estimated beta coefficient	P value	Estimated beta coefficient	P value
1.	Intercept	+132.68	0.0002*	+0.3984	0.0079*	+81.29	< 0.0001*
2.	A-Soya lecithin	-2.36	0.5106	-0.0159	0.3167	-7.20	< 0.0001*
3.	B- Ethanol	-40.42	< 0.0001*	-0.0676	0.0012*	+5.32	< 0.0001*
4.	C- Sodium cholate	-11.77	0.0107*	-0.0238	0.1459	+0.6537	0.4264
5.	AB	-17.12	0.0094*	-0.0558	0.0257*	-	-
6.	AC	-12.32	0.0380*	-0.0420	0.0769	-	-
7.	BC	+7.18	0.1805	-0.0045	0.8369	-	-
8.	A^2	-9.76	0.0766	-	-	-	-
9.	B^2	-21.80	0.0024*	-	-	-	-
10.	C^2	-1.13	0.8164	-	-	-	-

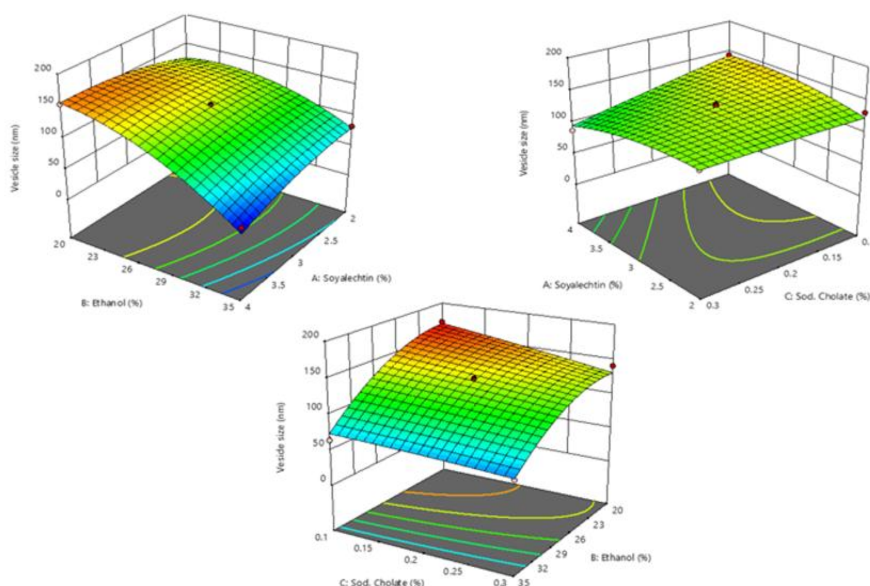

Fig. 1: Response surface curve representing 3D effect of Soya lecithin and ethanol and sodium cholate on Vesicle size.

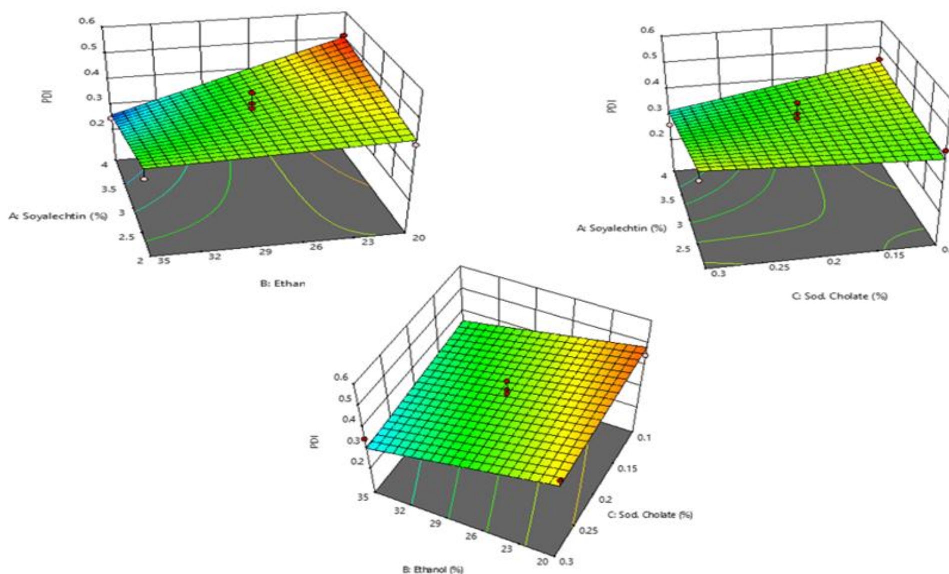

Fig. 2: Response surface curve representing 3D effect of Soya lecithin and ethanol and sodium cholate on PDI.

Percentage entrapment efficiency

The 3D graphs **(Fig. 3)** show that the increased concentration of soya lecithin increased the entrapment efficiency up to 3% upon which there was no significant increase in entrapment efficiency. This may be due to higher encapsulation in the lipid bilayer of formulation[22]. Entrapment efficiency showed a linear relationship with the increase in concentration of ethanol. Increasing drug loading due to ethanol increases the solubility of the lipophilic drug[23]. As the concentration of sodium cholate increases there is an increase in entrapment efficiency, which could be due to electrostatic repulsive force leading to high interlayer distance[24].

The model generated for entrapment efficiency had a p-value of < 0.05 and an F value of 42.34 indicating the linear model to be significant. The value of 1.52 indicates a non-significant lack of fit, implying the model to be appropriate to calculate the entrapment

efficiency. The Predicted R^2 of 0.8857 is in reasonable agreement with the Adjusted R^2 of 0.8498; i.e., the difference is less than 0.2. The polynomial equation obtained from the results of the analysis:

$$\%EE = + 81.29 -7.20 (A)^* +5.32 (B)^* +0.6537 (C) \ldots\ldots(3)$$

Where A is the concentration of soya lecithin, B is the concentration of ethanol and C is the concentration of sodium cholate the coefficient in this equation reflects the standardized beta coefficient and the asterisk symbol implies variable significance.

Optimization of Bifonazole loaded transethosome.

Transethosomes were optimized based on constraints such as 100nm vesicle size, minimum PDI and maximum entrapment efficiency, and desirability of more than 0.76. The optimized formula was prepared as per the suggestion given by the software, which contains 2.685 %w/v of soya lecithin, 31.844 %v/v of ethanol and 0.300 %w/v of sodium cholate. The vesicle size, PDI and % entrapment efficiency values given by the software were 100nm, 0.361 and 87.29%, respectively. The observed values were found to be within ± 5% error of the predicted value, which is acceptable as shown in **Table 4 and Fig. 4.**

The zeta potential of the optimized batch was found to be -27.2 mV, as shown in **Fig. 5**. The zeta potential of transethosomes showed a negative value due to the presence of ethanol and sodium cholate. The charge of transethosomes is an important parameter that can influence both vesicular properties such as stability and skin-vesicle interactions[13].

Transmission Electron Microscopy

The TEM image in **Fig. 6** showed the surface morphology of vesicles with a unilamellar vesicular structure. The formed vesicles were spherical and had a vesicle size less than 200nm[13].

Formulation and Characterization of Bifonazole Loaded Transethosomal Gel

Transethosomal gel (TG) was prepared by using Carbopol 940 as the polymer, and the prepared gel was off-white in colour and smooth. It was characterized by measuring pH, viscosity spreadability, and drug content. The results are shown in **Table 5.**

The pH of TG and CG was found to be closer to the skin pH ie., 6.4, which is considered safe when applied to the ski. If the pH was varied from the skin, it might damage the skin. The drug content of both gels was found to be greater than 97%. The results described that drug loss during the hydrogel preparation was minimal and it was evenly distributed throughout the formulation. Spreadability values indicate that with a small amount of shear, the prepared gel can be spread easily, showing good spreadability. The TG had a higher viscosity than the CG, which could be due to the presence of transethosomal suspension that provides more viscosity to the preparation.

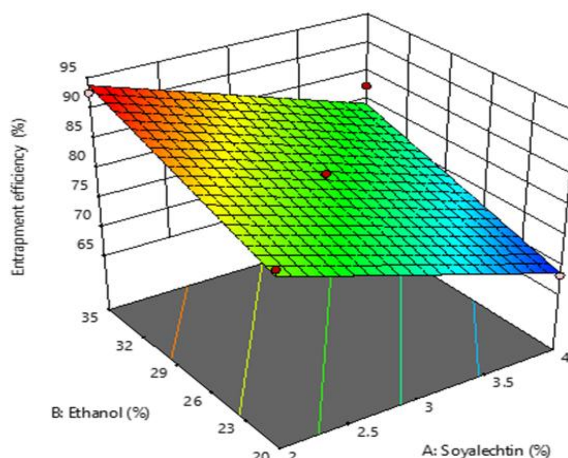

Fig. 3: Response surface curve showing effect of different factors on % Entrapment Efficiency.

Table 4: Comparison of experimental and theoretical values for the optimization**.**

Independent Factors			Responses		
Soya lecithin (%w/v)	Ethanol (%v/v)	Sodium Cholate (%w/v)	Vesicle size (nm)	PDI	Entrapmen t Efficiency (%)
			Predicted Mean		
2.685	31.844	0.300	100	0.361	87.29
			Observed Mean		
			104.7	0.356	86.23
% Error			4. 7	-1.38	-1.21

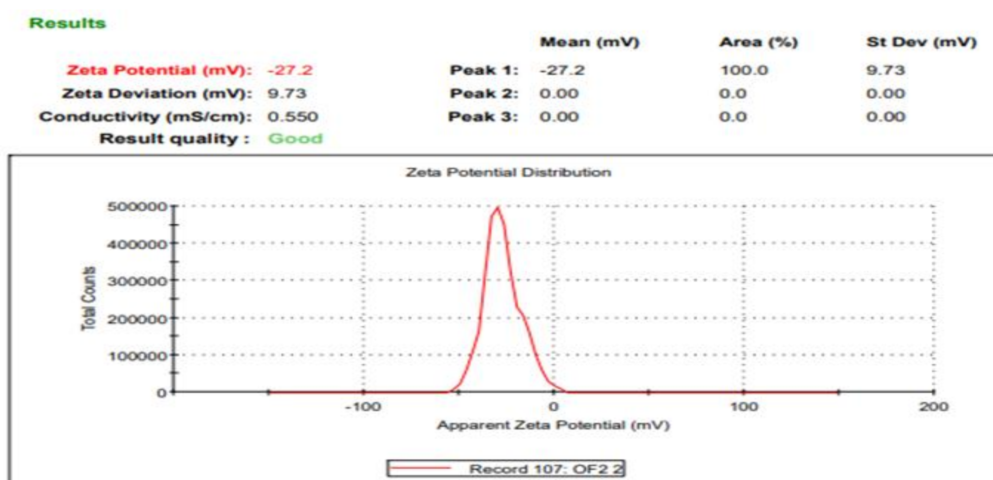

Fig. 4: Size distribution of optimized formulation of transethosome

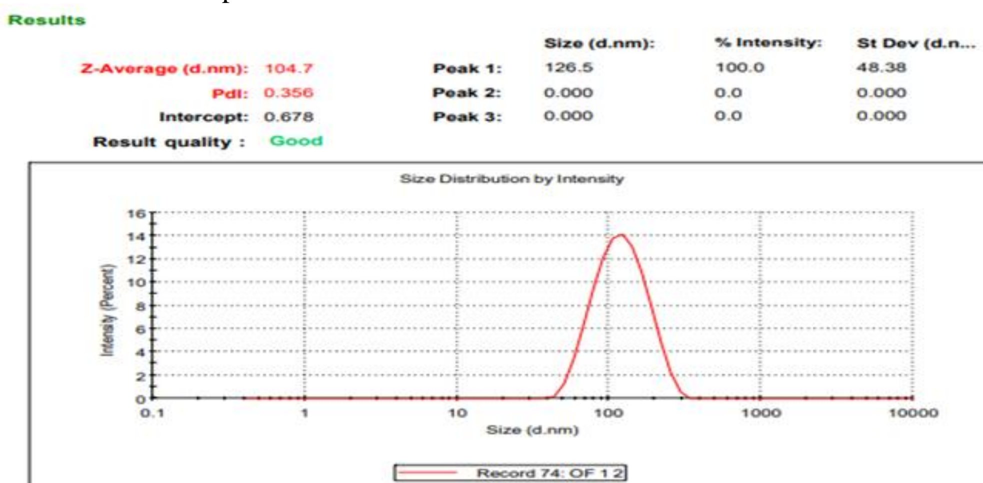

Fig. 5: Zeta potential of optimized formulation of transethosome

Table 5: Characterization of different gels**.**

Form.	Appearance	pH	Viscosity(cps)	Spreadability (g.cm/sec)	% Drug content
TG	Off-white, Smooth	6.62	43256	13.87	98.02±1.06
CG	Transparent, Smooth	6.61	32984.4	13.46	97.63±1.43

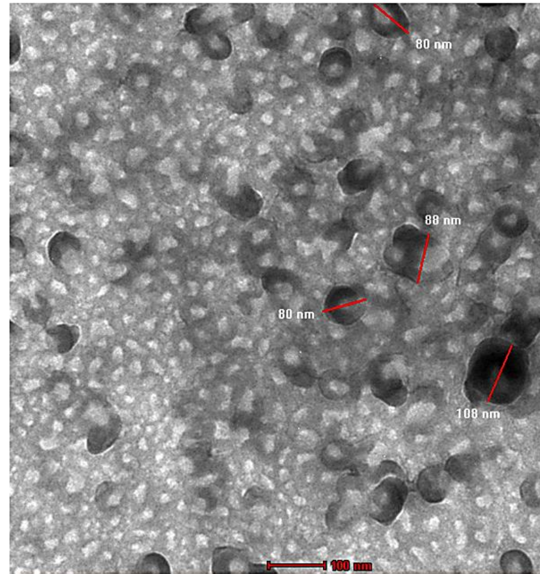

Fig. 6: TEM of optimized transethosomal vesicle.

FTIR Study

The bifonazole showed his principle peaks at 3029.97cm-1 due to Aromatic C-H stretching, 2881.98cm-1 due to Aliphatic C-H stretching, 1487.95cm-1 due to Aromatic C-C stretching, 1453.91cm-1 due to C-H deformation, 1383.65cm-1 due to Aromatic amine C-N stretching and 760.62 due to Aromatic C-H bending. Most of the major peaks of the drug were present in the final formulation shown in **Fig. 7**, which indicates that there is no incompatibility between the drug and the excipient used[16].

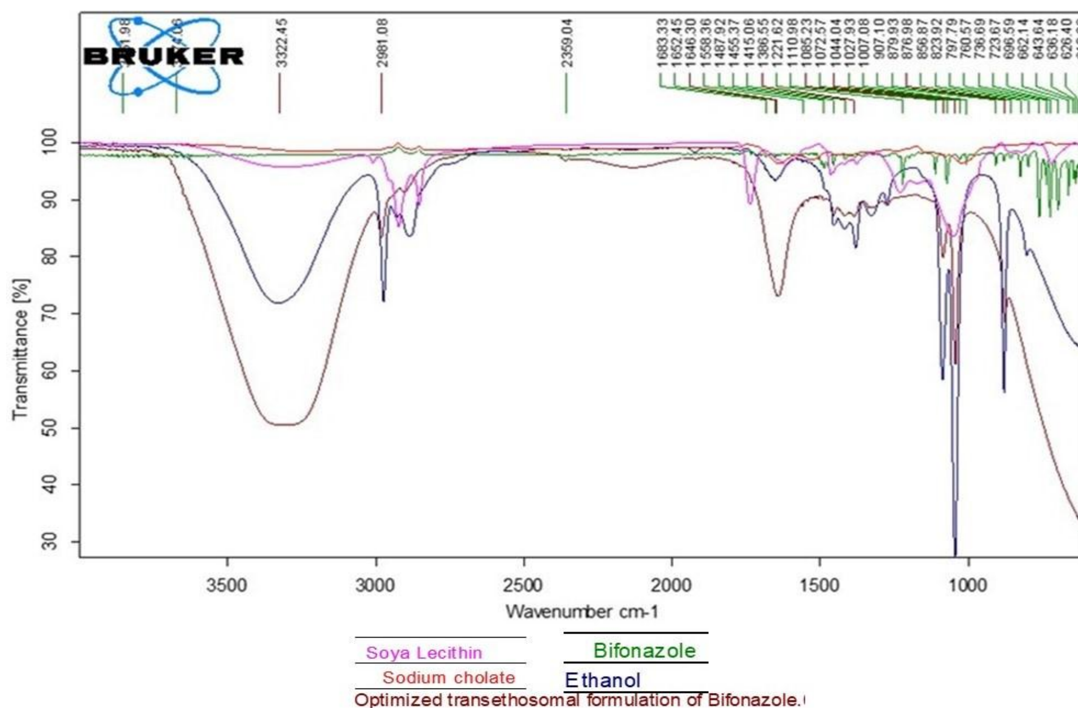

Fig. 7: FTIR spectra of Bifonazole, excipients and optimized transethosomal formulation.

In-vitro drug release of different formulations

The *in vitro* drug release profile of different formulations is given in **Fig. 8**. The release from drug suspension was less when compared to transethosome suspension, this may be due to the Bifonazole comes under a BCS class IV drug which is low soluble, and low permeable. But in the transethosomal suspension, the drug is entrapped in the vesicular carrier which contains ethanol and surfactant which improves the solubility of the drug. The drug release was greater for transethosome suspension relative to gel formulation. In the gels, the drug should have to first diffuse from the vesicle in which it is entrapped followed by diffusion through the gel. The transethosomal gel showed greater release when compared to the conventional gel, this may be due to the flexible nature of the transethosomes. The release from drug suspension is lower compared to the prepared conventional gel this may be due to, in suspension drug being suspended in distilled water, but in the case of conventional gel drug is dissolved in ethanol[13,16].

Ex vivo drug permeation using goat skin

An *ex vivo* study has been performed to evaluate the amount of drug permeated through the goat skin. The results are shown in **Table 6 and Fig. 9**. The cumulative amount of drug permeated by goat skin after 480 mins for drug suspension, marketed cream and conventional gel were significantly lower compared to transethosomal gel and transethosome suspension, suggesting that vesicular systems could improve the delivery of hydrophobic drugs such as Bifonazole to the skin due its flexible nature. The cumulative amount of drug permeated, steady state flux and permeability coefficient were found to be less in the case of transethosomal gel when compared to vesicular suspensions. It may be due to the viscous nature of the gel; retarded the release of the drug from formulation. The steady-state flux and permeability coefficient was higher in the case of transethosome suspension and transethosomal gel than in drug suspension, conventional gel and marketed cream. The results could be attributed to the high deformability and flexibility of transethosomes, which allowed them to overcome skin barrier properties[10,16,24].

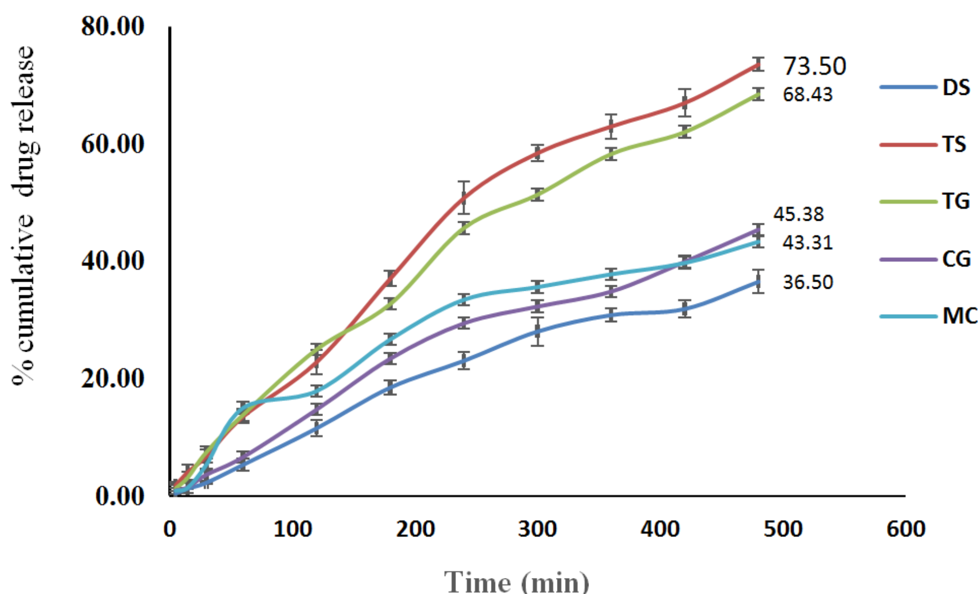

Fig. 8: Comparative in vitro drug release study of different formulations (DS=Drug Suspension, TS=Transethosome Suspension TG= Transethosomal Gel, CG= Conventional Gel and MC=Marketed Cream).

Table 6: The permeated amount of Bifonazole at 480 mins, flux & permeability coefficient.

Form. Code	Permeated amount at 480 min ($\mu g/cm^2$)	Flux ($\mu g/cm^2/min$)	Permeability constant (K_p) × 10^{-3} (cm/min)
DS	691.847	1.4580	0.0145
TS	1380.779	3.095	0.0309
TG	1349.514	2.923	0.0292
CG	847.965	1.902	0.0192
MC	844.153	1.887	0.0188

DS=Drug Suspension, TS=Transethosome Suspension TG= Transethosomal Gel, CG= Conventional Gel and MC=Marketed Cream.

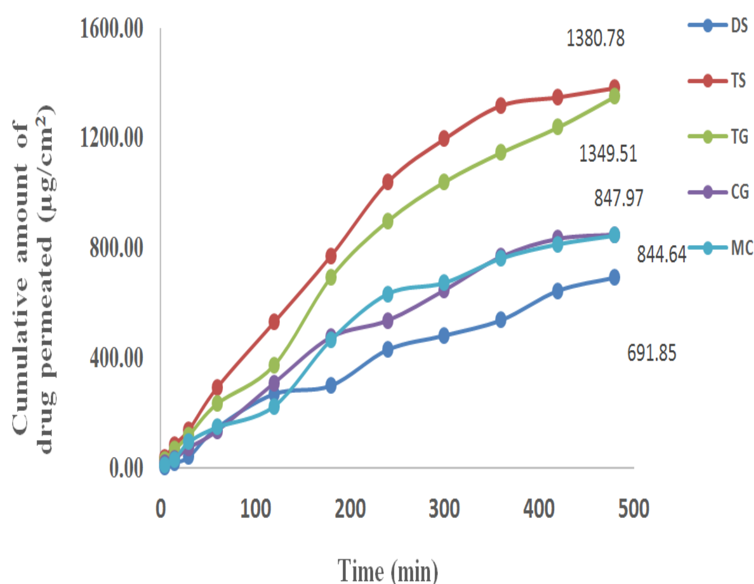

Fig. 9: *Ex vivo* drug penetration from the different formulations through goat skin.

In-vitro Antifungal Studies

The antifungal study was performed by the cup plate method. The report given in **table 7 and Fig. 10**; shows that transethosomal gels have a zone of inhibition of 22.8 mm, compared to marketed cream of 20.1mm, respectively. This shows that there is enhancement of antifungal activity of Bifonazole compared to the marketed cream[17].

Table 7: Zone of inhibition of Bifonazole transethosomal compared with marketed cream.

Sl. no	Formulation	Zone of inhibition (mm)
1	Transethosomal gel	22.8mm
2	Marketed Cream	20.1mm

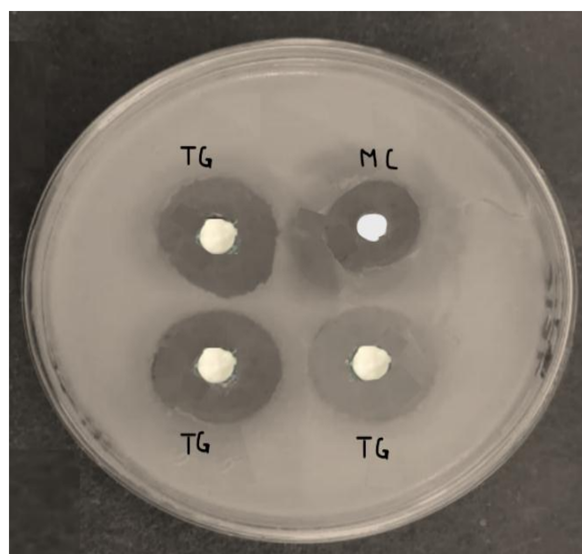

Fig. 10: Zone of inhibition of A(Marketed cream B) Transethosomal gel.

Conclusion

Bifonazole-loaded transethosome was successfully prepared and showed better permeability and antifungal activity compared to marketed cream. Based on the results, it is reasonable to conclude that the transethosome system could be a promising drug delivery carrier for the topical delivery of Bifonazole.

Acknowledgement

The authors are thankful to NGSM Institute of Pharmaceutical Sciences, Mangalore for providing the necessary facilities to carry out the research work.

REFERENCES

1. I.P. Kaur and S. Kakkar, "Topical delivery of antifungal agents", ***Expert Opin Drug Deliv,***7(11),1303–1327 (2010).
2. M. Nagpal and M Kaur, "Nanomaterials for skin antifungal therapy: An updated review", ***J Appl Pharm Sci,*** 11(1), 15–25 (2021).
3. V. Mishra, M. Singh, Y. Mishra, N. Charbe, P. Nayak, *et al.,* "Nanoarchitectures in management of fungal diseases: An overview", ***Appl Sci,*** 11(15),1–19 (2021).
4. S. Güngör, M.S. Erdal, B.Aksu, "New Formulation Strategies in Topical Antifungal Therapy", ***J Cosmet Dermatological Sci Appl,*** 03(01), 56–65(2013).
5. R Viswanath V, Shiva M, Narasimha Rao B, Gnana Prakash K. Formulation Development and Invitro Evaluation of Clarithromycin Topical gel. Int. J. Pharm. Sci. Rev. Res. 2017;42(1):91-6.
6. Ascenso A, Raposo S, C. Batista, P. Cardoso, *et al.,* "Development, characterization, and skin delivery studies of related ultradeformable vesicles: Transfersomes, ethosomes, and transethosomes", ***Int J Nanomedicine,*** 10, 5837–5851 (2015).
7. M. Gupta, V. Sharma and N.S.Chauhan, "Promising Novel Nanopharmaceuticals for Improving Topical Antifungal Drug Delivery. Nano- and Microscale Drug Delivery Systems: Design and Fabrication", ***Elsevier Inc***, 197–228 (2017).
8. "Antifungal azoles and other antifungal drugs for topical use", ***Meyler's Side Effects of Drugs***, 602–605 (2016).
9. D. Patel, D. Patel, J. Prajapati, U. Patel and V. Patel, "Formulation of thermoresponsive and buccal adhesive in situ gel for treatment of oral thrush containing poorly water soluble drug bifonazole", ***J Pharm Bioallied Sci,*** 4(Suppl 1), S116–S117 (2012).
10. I.M. Abdulbaqi, Y. Darwis, R.A. Assi and N. A. K. Khan, "Transethosomal gels as carriers for the transdermal delivery of colchicine: Statistical optimization, characterization, and ex vivo evaluation", ***Drug Des Devel Ther***, 12,795–813 (2018).

11. D. Nayak, R.M. Tawale, J.M. Aranjani and V.K.Tippavajhala, "Formulation, Optimization and Evaluation of Novel Ultra-deformable Vesicular Drug Delivery System for an Anti-fungal Drug", ***AAPS PharmSciTech,*** 21(5),1–10 (2020).
12. Z.X. Chen, B. Li, T. Liu, X. Wang, Y. Zhu, *et al.,* "Evaluation of paeonol-loaded transethosomes as transdermal delivery carriers", ***Eur J Pharm Sci***, 99,240–245 (2017).
13. M. Rahangdale and P. Pandey, "Development and characterization of apremilast transethosomal gel for transdermal delivery", ***Int J Pharm Sci Nanotechnol***, 14(3),5508–5518 (2021).
14. S. Shetty, J. Jose and L. CR. Kumar, "Novel ethosomal gel of clove oil for the treatment of cutaneous candidiasis", ***J Cosmet Dermatol,*** 18(3),1–10 (2018).
15. V.D. Sundar, P. Divya and M. D. Dhanaraju, "Design development and characterisation of tramadol hydrochloride loaded transethosomal gel formulation for effective pain management", ***Indian J Pharm Educ Res,*** 54(2), S88–S97 (2020).
16. H.A. Pawar, V.B. Attarde and G. Parag Subhash, "Optimization of Bifonazole-Loaded Nisomal Formulation Using Plackett-Burman Design and 2 Factorial Design", ***Open Pharm Sci J***, 3(1), 31–48 (2016).
17. P. P. Botre, M. G. Maniyar, "Formulation and evaluation of solid lipid nanoparticles of Bifonazole", ***Int J Sci Res Sci Technol***, 7(5),105–120 (2020).
18. R. Albash, A. A. Abdelbary, H. Refai, M. A. El-Nabarawi, "Use of transethosomes for enhancing the transdermal delivery of olmesartan medoxomil: *In vitro, ex vivo*, and *in vivo* evaluation", ***Int J Nanomedicine,*** 2019,1953–6198 (2019).
19. M. Shitole, S. Nangare, U. Patil and N. R. Jadhav, "Review on drug delivery applications of ethosomes: Current developments and prospects", ***Thai J Pharm Sci***, 46(3), 251–265 (2022).
20. S.R. Mita, M. Abdassah, U. Supratman, Y. Shiono, D. Rahayu, *et al.,* "Nanoparticulate System for the Transdermal Delivery of Catechin as an Antihypercholesterol : *In Vitro* and *In Vivo* Evaluations", ***Pharmaceutics,*** 15(9), 1142 (2022).
21. M. Farooq, F. Usman, S. Zaib, H.S. Shah, Q.A. Jamil, *et al.,* "Fabrication and Evaluation of Voriconazole Loaded Transethosomal Gel for Enhanced Antifungal and Antileishmanial Activity", ***Molecules,*** 27(10), 3347 (2022).
22. N. B. Shaik, S. D. Mortha, P. K. Lakshmi and Latha Kukati, "Formulation and evaluation of tizanidine hydrochloride loaded ethosomes for transdermal delivery", ***J Pharm Sci Res,*** 12(11),1400–1410(2020).
23. K. Anju, S. Priya, D. S. Sandeep, P. Nayak, P. Kumar, *et al.,* "Formulation and Optimization of Zaltoprofen Loaded Ethosomal Gel by using 23 Full Factorial Designs", ***J Pharm Res Int***, 17, 30–44 (2021).
24. I. M. Abdulbaqi, Y. Darwis, N. A. K. Khan, R. A. Assi and A. A. Khan, "Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, *in vivo* studies, and clinical trials", ***Int J Nanomedicine***, 11,2279–304 (2016).
25. N.Y. Hegdekar, S. Priya, S.S. Shetty and D. Jyothi, "Formulation and Evaluation of Niosomal Gel Loaded with Asparagus racemosus Extract for Anti-inflammatory Activity", ***Ind J Pharm Edu Res,*** 57(1s), s63–s74 (2023).

Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 1, 2024, pp. 67-81.

نشرة العلوم الصيدليـــة
جامعة أسيوط

ترانزيثوسومات البيفونازول المحملة على هلام : الصياغة والتقويم بواسطة تصميم بوكس بينكن الإحصائى

كشيما[١] – سانديا فاسانث[٢] – سنيه بريا[١]*

[١]جامعة نيتى ، معهد NGSM للعلوم الصيدلانية ، قسم الصيدلانيات ، ديرالاكات ، مانجالور ، ٥٧٥٠١٨

[٢]قسم الصيدلانيات ، كلية ينيبويا للصيدلة ، مانجالور ،٥٧٥٠١٨

تهدف هذه الدراسة إلى تطوير وتحسين هلام الترانزيثوسومات للبيفونازول لعلاج العدوى الفطرية ، وقد تم استخدام تصميم Box-Behnken وتم اختيار تركيز ليسيثين الصويا والإيثانول وكولات الصوديوم كمتغيرات مستقلة لتحسين أداء الهلام . وتم تقييم التأثيرات الفردية والجماعية للمتغيرات المستقلة على حجم الحويصلات ومؤشر توزيع الجسيمات وكفاءة التغليف . علاوة على ذلك، تم تقييم الصياغة المختارة من الترانزيثوسومات المحملة بالبيفونازول من حيث قياس جهد الزيتا والتصوير بالميكرسكوب الإلكترونى النافذ.

وتم تحميل الصياغة المختارة من الترانزيثوسومات المُحسّنة على جل كاربوبول ٩٤٠ بنسبة ١.٥% وتم تقييمها بغرض دراسة إنطلاق العقار في المعمل ، وكذلك إجراء دراسات التغلغل للجسم الحي و كذلك النشاط المضاد للفطريات . وكان حجم الحويصلات ومعامل توزيع الجسيمات وكفاءة التغليف ومقدار جهد الزيتا كالتالى ١٠٤.٧ نانوميتر ، ٠.٣٥٦ ، ٨٦.٢٣ % ، ٢٧.٢ - mv على الترتيب . وأظهر فحص الميكروسكوب الإلكترونى النافذ للعينات أشكال كروية متجانسة . وكان إنطلاق العقار من الترانزيثوسومات (٧٣.٤٩ %) بينما كان إنطلاقه من الهلام المحمل بالحويصلات (٦٨.٤٣%) وعندما تم مقارنة إنطلاق العقار من الهلام التقليدى كانت نسبة إنطلاقه ٤٥.٣٧ %) ومن مستحضر الكريم المتداول فى السوق (٤٣.٣١%) بينما كان إنطلاق العقار من صورة معلق(٣٦.٤٩%) . وتم تطبيق ميكانيكية إنطلاق العقار على نموذج هيجوشى و أثبتت الصياغات انها تحقق هذا النموذج استنادا الى قيمة (r2) والتى كانت (< ٠.٩٩) . ومن دراسة إنطلاق العقار خلال الجلد الحى أظهر الهلام المحتوى على الترانزيثوسومات زيادة ملحوظة فى الحالة المستقرة بمقدار ١.٥٣ مقارنة بالهلام التقليدى وبقيمة ١.٥٤ مقارنة بالمستحضر الموجود بالسوق. وأظهرت النتائج أن هلام الترانزيثوسومات المحملة بالبيفونازول له نشاط مضاد للفطريات أفضل من المستحضر (الكريم) المتداول فى السوق .

وخلصت هذه الدراسة إلى أن هلام الترانزيثوسومات المحمل بعقارالبيفونازول أظهر تغلغلا ملحوظا للأنسجة و تعزيزا للنشاط المضاد للفطريات.