RESEARCH PAPER

Development and evaluation of Terbinafine-Loaded Ethosomal Gel for Enhanced Transdermal Delivery

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ABSTRACT

The present study aimed to develop and evaluate Terbinafine hydrochloride-loaded ethosomal gel for enhanced transdermal delivery and improved antifungal activity. Ethosomes were prepared using varying concentrations of ethanol and phospholipids by the cold method followed by sonication, and incorporated into a suitable gel base. The formulations were evaluated for vesicle size, entrapment efficiency, drug content, in-vitro drug diffusion, and antifungal activity. The developed formulations exhibited vesicle size around 248 nm, high entrapment efficiency (~88%), and drug content (~98–100%), indicating efficient vesicular characteristics. In-vitro diffusion studies showed drug release in the range of ~70–90%, with formulation F6 showing optimum release (~85–88% at 12 h). Antifungal studies demonstrated enhanced activity of ethosomal gel compared to pure drug and marketed formulation, with F6 showing maximum inhibition against Candida albicans and Aspergillus niger. The results indicated that ethanol improved drug diffusion by increasing vesicle fluidity, while excess phospholipid reduced release due to increased rigidity. Stability studies confirmed that the optimized formulation remained stable under refrigerated conditions. Overall, the developed ethosomal gel showed enhanced permeation, sustained release, and improved antifungal efficacy, suggesting its potential as an effective transdermal delivery system for Terbinafine.

Keywords: Ethosomes, Terbinafine hydrochloride, Transdermal delivery, Drug diffusion, Stability studies, Vesicular systems.

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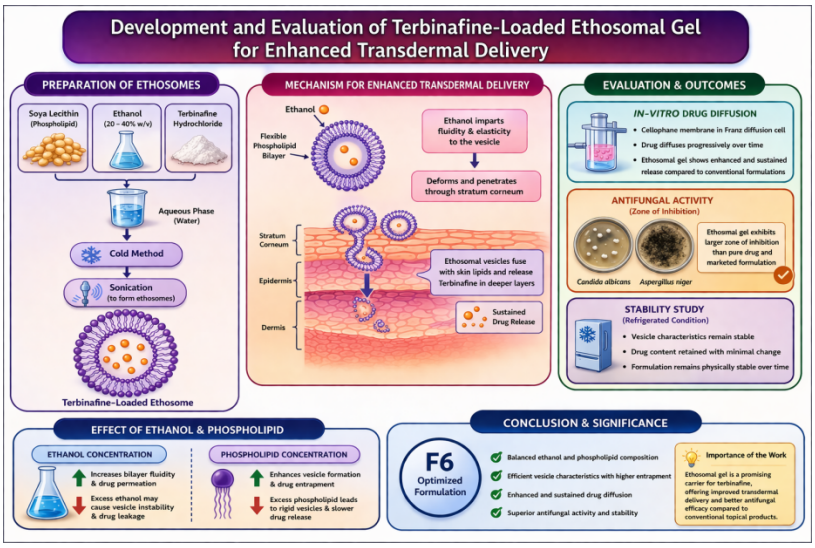
GRAPHICAL ABSTRACT



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INTRODUCTION

The progress in pharmaceutical nanotechnology has seriously changed the transdermal drug delivery system especially by coming up with vesicular carriers to defeat the barrier property of the skin. The modified liposomal systems including transfersomes and ethosomes have received significant attention because of their increased capacity to promote drug permeation through the stratum corneum. The vesicles systems are made of biocompatible and biodegradable compounds, which allow enhanced pharmacokinetic properties, longer retention of the drugs and controlled release properties[1,2].

Ethosomes are deformable vesicles that are soft and mainly consist of phospholipids, comparatively high level of ethanol as well as water. The ethanol in ethosomes, unlike the traditional liposomes, gives ethosomes special physicochemical properties, such as enhanced membrane fluidity and elasticity which greatly contribute to the ability of skin permeation of any product. Ethosomes are non-invasive delivery agents that have the ability to deliver therapeutic agents to deeper skin layers, increasing drug bioavailability at the site of treatment [3,4].

The enhanced transdermal delivery associated with ethosomal systems is attributed to a synergistic action of ethanol, vesicular structure, and skin lipids. Ethanol reacts with the polar head groups of the lipid molecules within the stratum corneum and causes the lipid transition temperature to decrease and the lipid fluidity to rise. The destabilization of the very organized lipid bilayer leads to a reduced barrier resistance, which enhances penetration of vesicles[5]. The flexible ethosomal vesicles then enter the intercellular lipid matrix, which may then be fused with skin lipids, facilitating the easier release of drugs to the deepest dermal layers[6].

Ethosomal systems have also proved to be quite efficient in improving the depth and the amount of drug delivery through the improvement of lipid fluidity and cellular permeability. Other studies have also indicated better performance of ethosomes over traditional topical formulations7,8. An example is an ethosomal preparation of acyclovir demonstrated much better treatment efficacy than traditional cream in a randomized clinical trial. Moreover, it has been established that fluorescence-based techniques can be used to improve penetration depth and accumulation of drugs. Comparative studies have also brought out the benefits of using ethosomes over liposomes in terms of enhanced skin permeation and therapeutic effects[9,10] .

TH is a widely used allylamine antifungal agent that is used to treat superficial or systemic fungus. It displays a high level of antifungal activity against the dermatophytes and Aspergillus sp. by inhibiting the enzyme squalene epoxidase which is essential in the ergosterol synthesis[11,12]. Conventional topical formulations are however usually limited by poor penetration through the skin and decreased word of action Figure 1.

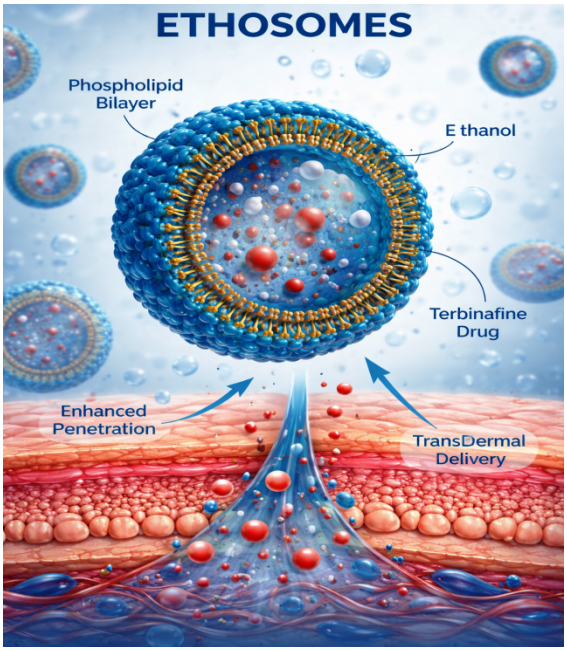

Figure 1: Structure of ethosomes for enhanced dermal drug transport

In this regard, the addition of terbinafine in the ethosomal vesicles is a potential approach to increase the dermal absorption, localized drug delivery and the antifungal effect. Thus, the current research concentrates on the creation, characterization, and testing of terbinafine-loaded ethosomes to be used as a transdermal delivery.

MATERIALS AND METHODS

Materials

TH received as a gift sample from Glenmark Pharmaceuticals Ltd, Mumbai, India. Carbopl934 was provided by Oryan Health Care LLP, Ahmedabad, Gujarat, Idia. Soya lecithin,Ethanol, Propylene glycol, Triethanolamine were bought in LOBA CHEMIE PVT, LTD. Mumbai.i.

Preparation of TH loaded ethosomal gel

An ethosomal suspension of Terbinafine was developed by an adapted cold method. In short, phospholipid (soya lecithin) and TH were dissolved in ethanol (20-30% v/v) in a closed container continuously stirred by stirring. Propylene glycol was inserted to act as a penetration enhancer. The mixture was left at 30-40°C and stirred. Several drops of distilled water were then added with constant stirring at 800 rpm leading to the generation of vesicular dispersion. The mixture was stirred by 30 minutes to bring to full vesicle formation. The obtained dispersion was sonicated using a probe sonicator (Sonics Vibra-Cell, 20 kHz, USA) for 10 minutes to reduce vesicle size and obtain a homogeneous ethosomal suspension [5].

Preparation of gel base

The gel base was made by dissolving Carbopol 934 (1% w/w) in distilled water through constant stirring in magnetic stirrer (Remi Equipment 2MLH) and left to hydrate between 60 minutes. Triethanolamine was then dropped by drop to neutralize the dispersion in order to get a clear and homogeneous gel [10].

Incorporation of TH loaded ethosomes into gel

The prepared ethosomal suspension was added to the gel base in a (1:1 w/w) ratio with a gentle stirring of the mixture, using the mechanical stirrer with low speed so that the vesicles could not be destroyed. The mixture was swirled until the desired even ethosomal gel was received. PH of the final formulation was manipulated to 5.5-6.5 to make it compatible with skin[13,,14].

Table 1: Formulation of ethosomes

Formulation Code	Terbinafine (mg)	Soya Lecithin (% w/v)	Ethanol (% v/v)	Propylene Glycol (% v/v)	Carbopol 934 (% w/w)	Triethanol amine	Distilled Water (q.s. to 100 mL)
F1	10	1.0	20	5	1.0	q.s.	q.s.
F2	10	1.5	20	5	1.0	q.s.	q.s.
F3	10	2.0	20	5	1.0	q.s.	q.s.
F4	10	1.0	25	5	1.0	q.s.	q.s.
F5	10	1.5	25	5	1.0	q.s.	q.s.
F6	10	2.0	25	5	1.0	q.s.	q.s.
F7	10	1.0	30	5	1.0	q.s.	q.s.
F8	10	1.5	30	5	1.0	q.s.	q.s.
F9	10	2.0	30	5	1.0	q.s.	q.s.

EVALUATION PARAMETERS

Vesicle Size and PDI

The vesicle size and polydispersity index (PDI) of the prepared ethosomal formulations were measured using dynamic light scattering (DLS) technique (Zetasizer Nano ZS (Malvern, UK)). Samples were appropriately diluted with a mixture of ethanol and distilled water (1:1 v/v) to obtain suitable scattering intensity before analysis. Measurements were performed at ambient temperature (25 ± 2°C), and each sample was analyzed in triplicate to ensure reproducibility. The mean hydrodynamic diameter was considered as the average vesicle size. The homogeneity of vesicle size distribution was evaluated based on the PDI value, where a value below 0.3 indicates a homogeneous system. Smaller vesicle size is considered desirable for enhanced skin penetration, while a low PDI indicates better stability and uniformity of the formulation [15].

Zeta Potential

The zeta potential of the prepared ethosomal formulations was measured to evaluate the surface charge and physical stability of the vesicles using electrophoretic light scattering (ELS) technique (Zetasizer Nano ZS, Malvern Instruments Ltd., UK). Samples were appropriately diluted with distilled water to avoid multiple scattering effects prior to analysis. Measurements were carried out at ambient temperature (25 ± 2°C), and each sample was analyzed in triplicate to ensure reproducibility. The zeta potential values were expressed in millivolts (mV). Higher absolute values of zeta potential (either positive or negative) indicate better stability of the vesicles due to increased electrostatic repulsion, which prevents aggregation[16].

SEM

Scanning electron microscopy (SEM) was used to study the surface morphology of the ethosomal vesicles. A small amount of sample was placed on an aluminum stub and allowed to air-dry. The dried sample was then coated with a thin layer of gold using a sputter coater. The samples were observed under a scanning electron microscope (JEOL JSM-IT200, Japan) at an accelerating voltage of 15 kV. Micrographs were obtained to examine the shape and surface morphology of the vesicles [17].

FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to study the compatibility between the drug and excipients. Samples were prepared using the KBr pellet method and scanned over a wavelength range of 4000–400 cm^{-1} using an FTIR spectrophotometer (Shimadzu IR Affinity-1, Shimadzu Corporation, Kyoto, Japan). The characteristic peaks were analyzed using the obtained spectra to identify any possible interactions[17,18].

pH Measurement

PH of the ethosomal formulations was determined using digital pH meter under room temperature conditions which was calibrated. The electrode was dipped in the formulation and the readings recorded upon achieving stability. Triplicate measurements were done [19].

Viscosity

The viscosity of the ethosomal gel formulations was determined using a rotational viscometer (Brookfield DV-II+ Pro, Brookfield Engineering Laboratories, USA). The gel sample was placed in the appropriate sample holder, ensuring the absence of air bubbles. Measurements were carried out at 25 ± 1°C using spindle no. 64 at a fixed rotational speed of 10 rpm. The reading was recorded after the instrument attained a constant value. All measurements were performed in triplicate, and the average viscosity was reported in centipoise (cP) [20].

Spreadability

The spreadability of the ethosomal gel formulations was determined using the glass slide method. A known quantity of gel (500 mg) was placed between two clean glass slides. A weight of 500 g was placed on the upper slide for 5 minutes to obtain a uniform thin layer. After that, an additional weight of 100 g was applied to the upper slide, and the time required for the upper slide to move a distance of 5 cm was recorded.

Spreadability was calculated using the following formula:

$S = (M \times L) / T$

Where,

S = Spreadability (g•cm/sec)

M = Weight tied to the upper slide (g)

L = Length moved (cm)

T = Time taken (sec)

All measurements were performed in triplicate, and the average value was reported. A shorter time indicates better spreadability of the formulation [21].

Entrapment Efficiency (%)

The centrifugation method was used to ascertain %EE of the ethosomal formulations. The ethosomal dispersion prepared was hashtagged at 10,000 rpm and the separation between the unentrapped (free) drug and the vesicular system was done in 30 minutes. The supernatant was cautiously pooled and examined on the contents of free drugs through UV visible spectrum at the suitable wavelength. The quantity of drug that was trapped in the vesicles was determined by dividing the total drug content and the quantity of the free drug. To determine the entrapment efficiency the equation was as given below:

Entrapment Efficiency (%) = (Total drug -Free drug)/Total drug × 100

An increase in the entrapping efficiency denotes increased incorporation of drugs within vesicular system currently required to allow the prolonged release of drugs and enhance therapeutic effectiveness[22,23].

Drug content determination

The drug content of the ethosomal formulation was determined using a validated UV-visible spectrophotometric method. An accurately weighed quantity equivalent to 100 mg of TH-loaded ethosomes was dissolved in 100 mL of methanol to ensure complete extraction of the drug. The solution was sonicated, filtered, and appropriately diluted. The absorbance of the resulting solution was measured at 283 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) against a suitable blank.

The drug concentration was calculated using a previously constructed calibration curve. The drug content was expressed as a percentage of the actual drug content with respect to the theoretical drug content. All measurements were performed in triplicate to ensure accuracy and reproducibility[24, 25].

Ex Vivo Skin Permeation Study

A skin permeation experiment in ex vivo was carried out using the skin of the excised animals in Franz diffusion cell with the stratum corneum being exposed to the donor half. A quantity of ethosomal formulation of known quantity was placed on the skin surface. Phosphate buffer (pH 7.4) filled the receptor compartment, was kept at 37 ± 1 °C and stirred with the help of 400 rpm. Samples had been taken after selected intervals and replenished with fresh medium. The drug permeated amount was assayed by a validated UV-Visible spectrophotometric and cumulative drug permeation was determined[26, 27].

In Vitro Drug Diffusion Study

The in vitro drug diffusion study of ethosomal formulations was carried out using a Franz diffusion cell to select the optimized formulation for further ex vivo studies. A cellophane dialysis membrane (grade 110), previously soaked in phosphate buffer (pH 7.4), was mounted between the donor and receptor compartments. Ethosomal formulations (F1–F6), optimized gel formulation (F6), and a marketed cream (1% w/v) were evaluated. An accurately weighed quantity of formulation equivalent to 3 mg of drug was placed in the donor compartment. The receptor compartment was filled with a mixture of phosphate buffer (pH 7.4) and methanol to maintain sink conditions. The system was maintained at 37 ± 1°C with continuous stirring at 400 rpm. At predetermined time intervals, aliquots were withdrawn from the receptor compartment and replaced with an equal volume of fresh medium to maintain constant volume. The amount of drug diffused across the membrane was analyzed using a UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) at 248 nm. The cumulative percentage drug release was calculated and plotted against time [28,29].

In-vitro Antifungal Activity

The agar well diffusion method was used to evaluate the antifungal activity of Terbinafine-loaded ethosomal gel formulations (F1 -F9) against *Candida albicans* and *Aspergillus niger*. On sterile Sabouraud Dextrose Agar plates, fungal suspension brought to 0.5 McFarland standards was put on a uniform lawn by inoculation. Wells (6 mm diameter) were aseptically prepared and the same formulation of each (F1 7) was put into wells. The controls were expressed as pure drug solution and

marketed formulation. The plates were left to diffuse after 1 hour and incubated at 28–30°C after 48-72 hours. ZOI (mental) was measured (mm) and results were in mean SD (n = 3). The comparison of all the formulations in the antifungal activity revealed the optimized formulation[31,32].

Stability Studies

Stability studies of the ethosomal formulations were done to determine their stability in physical and chemical conditions in various modes of storage. The samples were maintained at refrigerated temperature (4 ± 2 °C) and room temperature (25 ± 2 °C) during a duration of up to 3 months. Samples were collected at the specific time points (0, 1, 2, and 3 months) and assessed regarding the size of the vesicles, PDI index, zeta potential and entrapment efficiency. Measurements were done under triplicate and any notable difference in physicochemical properties was recorded to determine the stability of the formulations[30].

RESULTS AND DISCUSSION

Vesicle Size and PDI

Vesicle size of all the prepared ethosomal formulations (F1-F6) were found to have their vesicle size in the nanometric range. Formulation F6 was involved in the smallest size distribution and vesicle size among all the batches as compared to other formulations. The shrinkage of the vesicles in F6 can be explained by the optimal concentration of ethanol and phospholipid increased fluidity of membranes and deformability of the vesicles. The values of PDI displayed agreement of the distribution of the vesicles (ranging between the 0.3-0.5). The F1 and F3 demonstrated the lowest value of PDI which indicated a stable and uniform vesicular system. A low PDI demonstrates increased formulation stability and reproducibility Table **2.**

Table 2: Result of different evaluation parameter

Formulation	Vesicle Size (nm)	Poly. Index	Zeta potential	pH	Viscosity (cP)	Spreadability (g·cm/sec)
F1	6810 ± 0.05	0.214 ± 0.03	+20.1 ± 0.5	5.6 ± 0.02	2850 ± 35	7.8 ± 0.12
F2	7062 ± 1.3	0.43 ± 00.3	+19.7 ± 0.3	5.8 ± 0.03	3015 ± 42	7.2 ± 0.10
F3	7528 ± 0.05	0.123± 00.1	-25.3 ± 1.8	5.7 ± 0.01	2760 ± 30	7.5 ± 0.08
F4	5821 ± 0.5	0.632± 0.02	-35.1 ± 2.8	6.2 ± 0.04	3650 ± 28	6.3 ± 0.09
F5	6788 ± 1.6	0.444 ± 0.08	-32.9 ± 1.0	6.5 ± 0.02	3890 ± 40	5.8 ± 0.07
F6	8746 ± 0.5	0.427 ± 0.01	-21.5 ± 0.6	6.1 ± 0.03	3525 ± 36	6.7 ± 0.11
F7	6654 ± 1.6	0.524 ±0.11	-23.5 ± 0.3	6.3 ± 0.02	3300 ± 25	6.9 ± 0.06
F8	5496 ± 0.8	0.449 ±077	-29.6 ± 1.2	6.8 ± 0.01	4120 ± 45	5.2 ± 0.05
F9	5551 ± 0.8	0.549 ±077	-28.6 ± 1.2	6.7 ± 0.01	4320 ± 45	5.9 ± 0.05

Zeta Potential

The values of zeta potential of all formulations were positive and negative values reporting that the surface charge was varied based on the formulation composition. Majority of the formulations were found to have a negative zeta potential, which could be explained by the existence of phospholipids and ethanol, which helps in stabilizing the vesicles. Recipes that have more absolute zeta potential (> +30 mV) are said to be more stable since there is greater electrostatic repulsion and thus vesicle aggregation does not occur. F4 and F5 have a high negative zeta potential value, which is the reason why they are more physically stable than other formulations used in the present study. Nevertheless, based on the total performance using the characteristics of vesicle size and PDI, and entrapment efficiency, F6 showed good stability with balanced physicochemical characteristics and was thus chosen in further studies as the optimized formulation Table **2**.

Scanning electron microscopy

SEM was carried out to determine the surface morphology of the optimized ethosomal formulation (F6). The micrographs of SMMs showed that the vesicles had smooth surface features and were almost circular in shape which showed successful establishment of ethosomal vesicles. The vesicles were found to be dispersed such that there was slight aggregation hence the formulation was quite stable. The morphology is in agreement with the size of vesicles and PDI outcome, as it demonstrates that the stable and homogeneous vesicular system was formed. Altogether, the structural analysis of the optimized formulation in terms of transdermal drug delivery proved its structural integrity and appropriateness to use Figure **2.**

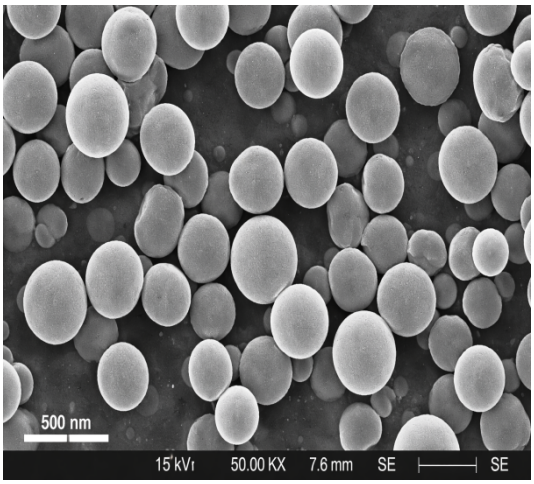

Figure 2: Ethosomal vesicles under electron microscope

FTIR Analysis

This was done using FTIR spectra of pure drug, phospholipids and optimized formulation (F6) to assess potential drug-excipient interaction. Pure drug was on the spectrum with characteristic peaks of functional group such as C=C stretching (~1517 cm $^{-1}$), aromatic C -H stretch (~3038 cm $^{-1}$), alkenyl C -H stretching (~2966 cm $^{-1}$) and C -N stretching (~1134 cm $^{-1}$). The phospholipid spectrum had typical values of aliphatic C H stretching (2860-2920 cm $^{-1}$), carbonyl (C=O) stretching (~1735 $^{-1}$), and the C=C stretching (~1519 -1588 cm $^{-1}$). Under the optimized formulation (F6), all the key characteristic peaks of the drug have been retained with slight changes in the positions of the peaks which can be explained by the fact that there could be physical interactions or hydrogen bonding. There was no major loss, or creation of new heights. The results show that there is no chemical interaction between the drug and the excipients, which proves that the ethosomal formulation is compatible and stable.

Viscosity

The viscosity of ethosomal gel formulations was evaluated to determine their flow behavior and suitability for topical application. The trend of viscosity was found to be between 2850- 4320 **cP**; this showed good topical delivery consistency. There was an increase in viscosity as concentration of polymer increased which could increase the residence time of the formulation on the skin. Of all the formulations, F6 was found to have the best viscosity which provided a balance between application ease and site of application retention Table 2.

Spreadability

Gel formulations were spreadable to determine its ease of application. The values of spreadability were 5.2 - 7.8 g cm/sec and this showed that the formulations were spreadable well. Lower viscosity formulations had better spreadability and high viscosity had lower spreadability. F6 showed the best spreadability value of all the formulations, which is the good balance between the consistency and easiness of application Table **2**.

pH Measurement

All the ethosomal formulations were identified on the basis of the pH to be suitable in cases of topical application. The pH of the formulations was observed to lie between 5.6- 6.8 that is within the acceptable range of compatibility in the skin. There was a slight difference in pH of formulations and this could be explained by a difference in composition especially with regard to ethanol content and phospholipid content. All the formulations were found to have pH values near pH of normal skin (pH~5.5), which means that none of the formulations should be irritating when applied. F6 demonstrated a superior pH value among the formulations indicating that it was compatible with the skin and could be used in more significant research Table 2.

Entrapment Efficiency

The EE% of all formulations ranged from 56.63% - 80.40%, indicating satisfactory incorporation of the drug within the vesicular system. The difference in entrapment efficiency could be explained by the difference in the concentration of phospholipids and ethanol, which have an effect on the formation of vesicles and drug encapsulation. F6 recorded the largest entrapment efficiency (80.40%), then F5 (76.07%), F4 (74.87%), indicating that the optimization of the composition increases the incorporation of the drug into the vesicular bi-layer. Formulations F 1, F 2, F 7 and F 8 were middlerange entrapment efficiency with F 3 entrapment at lowest (56.63) value. The findings show that the more phospholipids are added, the better the drug entrapment is as it is estimated to offer a larger bi-phases structure, whereas ethanol enhances the dissolution of the drugs in the vesicular system. In general, the drug loading of F6 was already higher, so it is the most adequate formulation concerning entrapment efficiency Table **3**.

Table 3: Entrapment Efficiency

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Formulation	Entrapment efficiency	Drug Content (%)
F1	60.98 %	91.25 ± 0.85
F2	65.17 %	93.40 ± 0.72
F3	56.63 %	92.10 ± 0.65
F4	74.87 %	96.35 ± 0.58
F5	76.07 %	97.80 ± 0.60
F6	80.40 %	99.12 ± 0.55
F7	62.93%	94.75 ± 0.68
F8	66.28%	95.60 ± 0.70
F9	68.28%	96.70 ± 0.70

Drug Content (%)

All ethosomal formulations were identified regarding their drug composition in order to have the same distribution of drug in the system. The similarity and good incorporation of the drug were seen in the trend of drug content values, which ranged between 91.25 and 99.12. The minor change in the drug content across various formulations can be explained by the change in the composition and conditions of processing. Drug content was within acceptable limits in all formulations and this implied that there was a low loss of drugs during preparation. F6 contained the highest drug content (99.12%), which was the highest drug content showing that the drug incorporation was effective and that the drug was evenly distributed throughout the vesicular system Table 3.

Ex Vivo Skin Permeation Study

The ex vivo permeation profile has shown an increase in the skin permeation of drug of the ethosomal formulation F6 in comparison with the commercial formulation. F6 had an approximate of ~88% drug permeation at 12h, as compared to the marketed product with about ~70% drug permeation which confirmed that ethosomes affect penetration Table **4,** Figure **3**.

Table 4: Result of Ex Vivo Permeation

Time (h)	F6 (%)	Marketed (%)
0	0.0 ± 0.0	0.0 ± 0.0
1	10.0 ± 0.8	6.0 ± 0.5
2	22.0 ± 1.0	15.0 ± 0.9
4	37.0 ± 1.2	26.0 ± 1.0
6	51.0 ± 1.3	39.0 ± 1.2
8	64.0 ± 1.5	50.0 ± 1.3
10	76.0 ± 1.4	60.0 ± 1.2
12	88.0 ± 1.2	70.0 ± 1.0

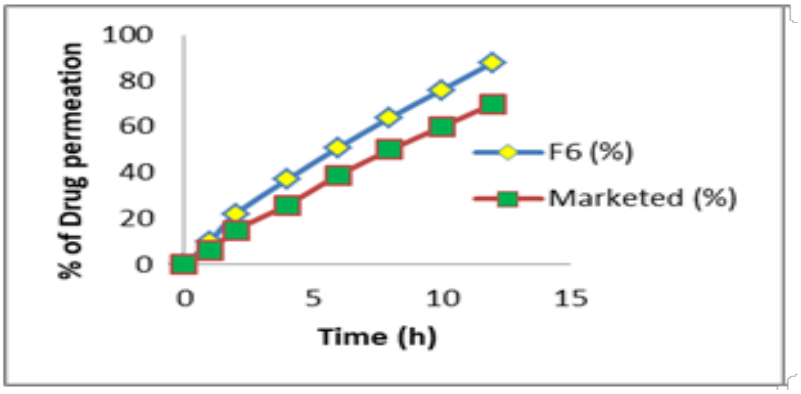

Figure 3: Result of ex-vivo skin permeation study of TH ethosome

In vitro drug diffusion studies

In-vitro diffusion of Terbinafine of ethosomal gel formulations (F1 to F9) in phosphate buffer (pH 5.5 - 6.5) at 37 suited to a frig at 37 ± 0.5°C was conducted by use of a Franz diffusion cell through cellophane membrane and using a UV-visible spectrophotometer. The diffusion of drugs was enhanced with the concentration of ethanol because the vesicle fluidity and permeation were increased. Formulations F1–F3 were less released (~70-77%), and F46 were better in diffusion. F6 exhibited the best release of the drug (~85 -88 or optimum release at 12 h) because the composition consisted of equal ethanol and phospholipid. More diffusion was not observed with increased ethanol (F7–F9) perhaps because of vesicle instability. Increased phospholipid concentration exceeding optimum also slowed down release as a result of high vesicle rigidity. All in all, formulation F6 was the selected optimized formulation, and it exhibited a equilibrium between the drug diffusion and vesicular stability Figure **4**

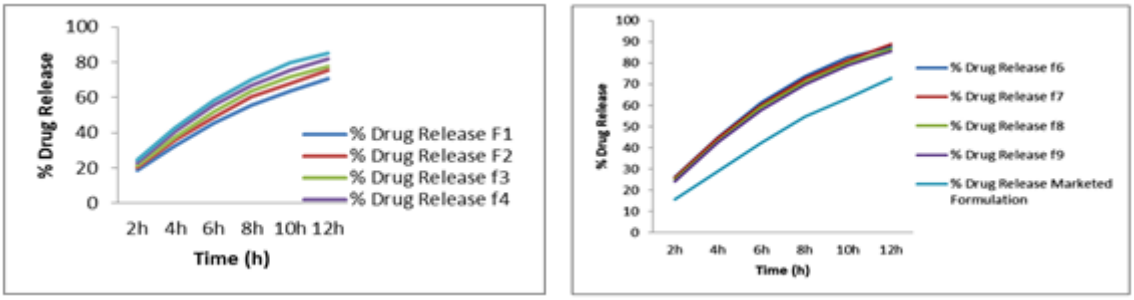

Figure 4: Comparison of in-vitro drug release profiles for the ethosomal gel formulations and marketed formulation

In-vitro Antifungal Activity

The efficacy of the antifungal Terbinafine-loaded ethosomal gel formulations (F1-F9) were compared against all *Candida albicans* and *Aspergillus niger* by the agar well diffusion method. These findings were reported as the zone of inhibition (ZOI, mm) and they are reported in Table **5**.

Table 5: Zone of Inhibition (mm) of Different Formulations

Formulation	Candida albicans (mm)	Aspergillus niger (mm)
Pure Drug	14.2 ± 0.5	13.5 ± 0.6
Marketed Formulation	16.8 ± 0.4	15.9 ± 0.5
F1	15.1 ± 0.6	14.4 ± 0.5
F2	16.0 ± 0.5	15.2 ± 0.6
F3	16.5 ± 0.4	15.8 ± 0.5
F4	17.2 ± 0.5	16.3 ± 0.4
F5	18.0 ± 0.6	17.1 ± 0.5
F6	19.3 ± 0.4	18.5 ± 0.4
F7	17.5 ± 0.5	16.6 ± 0.6
F8	18.2 ± 0.4	17.3 ± 0.5
F9	17.8 ± 0.6	16.9 ± 0.5

(A)

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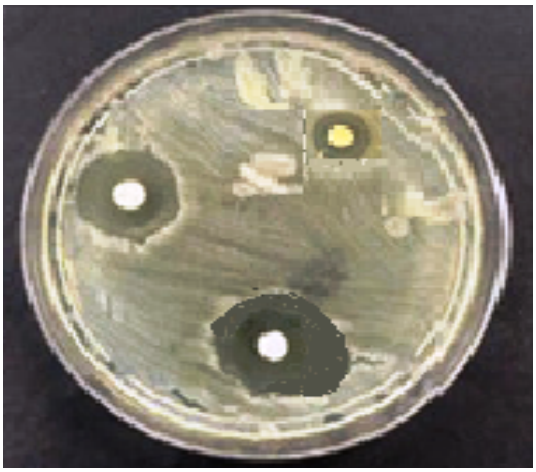

(B)

Figure 5: Antifungal activity of optimized formulation F6 against *A. niger (A) and C. albicans* (B) showing significant zone of inhibition.

Stability Studies

The stability tests found that the optimized formulation was relatively stable under refrigerated storage, with little variations in vesicle size, entrapment efficiency, and amount of drug in the formulation in a period of 3 months. Stored at ambient temperature, formulations, in contrast, had a negligible change in vesicle sizes, and reduced entrapment efficiency and drug content gradually. These modifications can be explained by vesicle aggregation and leakage of drugs to high temperatures. In general, the formulation was more stable at 4°C showing that refrigerated conditions are more favorable Table **6**.

Table 6: Result of stability data of optimized formulation (F6)

Time (Days)	Temperature (°C)	Vesicle Size (nm)	Entrapment Efficiency(%)	Drug Content (%)
0	Initial	252 ± 2.1	67.85 ± 1.24	99.12 ± 0.85
30	4°C	255 ± 2.6	66.92 ± 1.18	98.74 ± 0.72
60	4°C	258 ± 3.1	65.88 ± 1.35	98.12 ± 0.91
90	4°C	262 ± 2.9	64.95 ± 1.42	97.68 ± 0.88
30	25°C	260 ± 3.4	65.74 ± 1.56	97.92 ± 0.95
60	25°C	268 ± 3.8	63.82 ± 1.64	96.85 ± 1.02
90	25°C	276 ± 4.2	61.94 ± 1.72	95.76 ± 1.10

CONCLUSION

The current research was able to come up with Terbinafine impregnated ethosomal gels to improve the transdermal delivery. The preparations were found to have attractive vesicle properties such as small size of vesicles (approximately 248 nm), high entrapment efficiency (around 88 percent) and poor physicochemical stability. The gels were characterized by the acceptable pH (5.5-6.5), even distribution of drugs 98-100%, and appropriate rheological characteristics that showed their importance in application as a topical agent. Diffusion investigations in-vitro showed an enhanced drug release (~70-90%), and F6 released best (~85-88% in 12h). Antifungal analysis revealed increased activity of ethosomal formulations in comparison to pure drug (14 mm) and marketed product (16-17 mm) with F6 being the most activity (19 mm in *C. albicans* and 18 mm in *A. niger*). The results have indicated that the ethanol and phospholipid concentration significantly contributes towards the formulation performance. An ideal percentage (F6 25% ethanol and 2% lipid) caused optimality in vesicle flexibility, drug penetration and antifungal activity, but higher concentrations caused instability or low release. In general, this paper indicates that ethosomal gel has a high potential to be employed as an improved drug delivery system in addition to enhancing the pharmacological efficacy of Terbinafine. This formulated formula provides a potential substitute to standard topical therapy and could provide an improved response of the treatment of surface-level cases of fungi.

ABBREVIATIONS

TH – Terbinafine Hydrochloride, DLS – Dynamic Light Scattering, PDI – Polydispersity Index, SEM – Scanning Electron Microscopy, FTIR – Fourier Transform Infrared Spectroscopy, DSC – Differential Scanning Calorimetry, EE (%) – Entrapment Efficiency, PBS – Phosphate Buffer Solution, rpm – Revolutions per Minute, cP – Centipoise, nm – Nanometer, mV – Millivolt

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS
Mohini Patidar conceptualized the study, structured the content, and wrote the initial draft. Nitin Deshmukh contributed to methodology development, data analysis, and manuscript revision. Gagan Kukloria supervised the work and provided overall guidance throughout the study. Sujit Pillai assisted in experimental work, data collection, and preparation of figures and tables. Aman Sonane contributed to data analysis, interpretation, and literature support. Ruchita Raghuvanshi supervised the study, provided critical feedback, edited the manuscript, and ensured clarity and coherence. All authors read and approved the final manuscript.

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