



FORMULATION & EVALUATION OF EFINACONAZOLE TRANSUNGUAL DELIVERY SYSTEM IN THE MANAGEMENT OF ONYCHOMYCOSIS DISEASE

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ABSTRACT

Background: Onychomycosis is a fungal infection of the nail plate that is persistent in its nature and is known to have a poor response to drugs because of poor penetration of drugs through the bulky keratinized barrier. Ethosomal nanocarriers are phospholipid-based and have a high ethanol content to provide a superior membrane fluidization and deformability, thus becoming a potential solution to improving transungual drug delivery.

Aim: The purpose of this study was to design, urethrate and test an ethosomal-based Efinaconazole transungual delivery system to increase drug permeation, retention and antifungal activity as an effective means of onychomycosis treatment.

Methods: The ethosomes of efinaconazole were prepared by a thin-film hydration method and optimized with the help of a Box and Behnken Design taking into account the concentration of lecithin, ethanol, and cholesterol as independent variables. Dependent responses were a trapment efficiency, vesicle size and cumulative drug release. The optimized ethosomes were described by FTIR, DSC, XRD analysis, vesicle size and zeta potential, estimation of the drug content, viscosity and spreadability, in-vitro diffusion tests and antifungal activity against *Candida albicans*. The stability studies were performed in ICH accelerated conditions.

Results: Ethanol ethosolubility studies proved that Efinaconazole had maximum solubility in ethanol, and ethosolubility was appropriate. Optimization on BBD provided excellent fit of the model ($R^2 > 0.97$) with lecithin and ethanol having a significant effect on vesicle properties and drug release. Compared to the marketed formulations the optimized formulation had high entrapment efficiency, homogenous vesicle morphology, negative zeta potential, superior in-vitro permeation, and enhanced antifungal activity. The outcome of formulation was good.

Conclusion: Optimized ethosomal formulation demonstrated both high potential and high performance in terms of Efinaconazole permeation and antifungal effect, which indicates significant promise as a transungual delivery system to use as an agent that treats onychomycosis.

KEYWORDS: Efinaconazole, Ethosomes, Transungual Delivery, Onychomycosis, Antifungal Activity.

Introduction

Onychomycosis is a persistent progressive nail plate infection that is mostly caused by dermatophytes, yeasts, and non-dermatophyte molds. It has been documented as almost half of all nail disorders in the world and has a tremendous effect on the quality of life of patients due to pain, discomfort, and aesthetics[1-3]. Although there are systemic antifungal therapy available (terbinafine, itraconazole and fluconazole) the long term use is linked to hepatotoxicity, drug-drug interactions and noncompliance to therapy. Topical medications are safer but may not penetrate the nail bed to therapeutic drug levels because of the high density of the keratin structure of the nail which presents a stiff shell surrounding the nail that does not readily allow penetration of drugs. Efinaconazole is an innovative triazole based antifungal agent that has been proven to be a broad-spectrum agent against dermatophytes and *Candida* species[4-6]. It has a low affinity to bind keratin, high potency, and good tolerability thus making it a good candidate in the topical treatment of onychomycosis. Nevertheless, its clinical performance is still compromised with poor transungual permeation in the case of traditional gels or lacquer structures[7]. Therefore, the main issue when developing effective treatments of topical onychomycosis is to provide an effective diffusion of drugs through the nail plate. Another article was published by Touitou et al., who proposed ethosomes, soft and malleable lipid vesicles, which are characterized by high concentrations of ethanol. Phospholipids and ethanol synergy provide better deformability and membrane fluidity allowing the ethosomes to penetrate rigid and keratinized structures more efficiently than conventional liposomes[8]. Onychomycosis is the cause of 50% of nail infections that is generally caused by dermatophytes. Firstly, the infection was regarded as a cosmetic problem but because of the tenacious nature of onychomycosis and its relapses, these infections have attracted medical attention. The first line of therapy was the oral antifungal agents which were proven to be effective; nevertheless, they exhibited hepato-toxic side effects, alongside drug interactions[10].

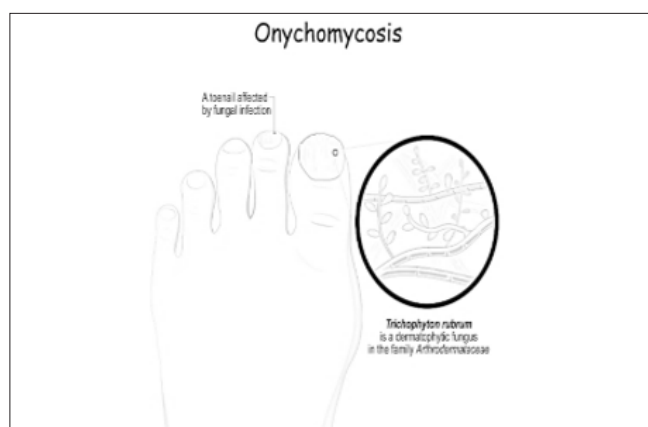


Figure 1. Onychomycosis disease

The use of ethosomes in transdermal and dermal delivery of drugs is widely studied; nevertheless, their usage in transungual delivery is relatively understudied. The colloidal-based drug delivery systems include nanoparticles, microemulsions, nanoemulsions, nanocapsules, nanovesicles, transferosomes, liposomes, and hydrogel systems[11-20]. Among all these drug delivery systems, the microemulsions based drug delivery has proven to be a thermodynamically stable and clinically beneficial system because of its versatility, biocompatibility, ability to penetrate a drug molecule to deep layers of nail unit due to unique hydration properties of microemulsion ingredients as well as the longer shelf life of formulation. Considering the physicochemical characteristics of Efinaconazole, low water solubility, lipophilicity, and good ethanol solubility, ethosomes can be regarded as a good carrier to increase its penetration into the nail plate. Moreover, optimization of formulation variables must be done systematically to optimize entrapment efficiency, vesicle stability, and drug release properties[21-30]. The current study is aimed at developing and optimizing Efinaconazole-carrying ethosomes based on Box-Behnken design (BBD), which is statistically robust. Formulation variables are assessed to determine their effect on the vesicle size, entrapment efficiency and cumulative drug release and then, the physicochemical, structural and biological characterization is done in detail. This work will combine the carrier design using nanotechnology with principles of transungual delivery to create a better, patient-friendly option in effective treatment of onychomycosis[31-38].

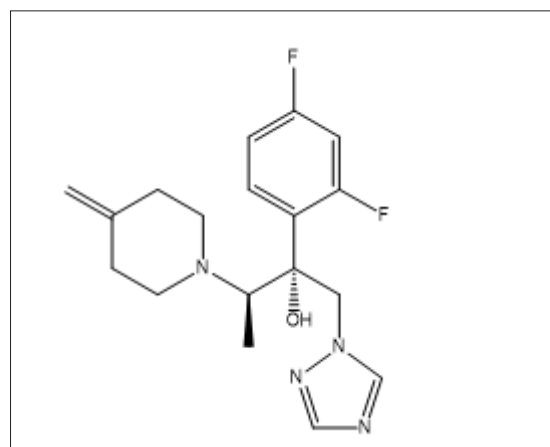


Figure 2. Structure of Efinaconazole

Materials and Methods

Efinaconazole was received as a gift sample provided by a certified pharmaceutical manufacturer, whereas soya lecithin, cholesterol, Carbopol 934, ethanol, methanol, n-octanol, and phosphate buffer (pH 6.8) were obtained by reputed suppliers; all chemicals were of analytical grade, and deionized water was utilized in the whole course of the study[13]. To determine the solvents that give the highest solubility of Efinaconazole, excess Efinaconazole

was added to different solvents, aqueous phase, methanol, ethanol, phosphate buffer pH 6.8, and n-octanol, then the mixtures were shaken at 37 °C (37.0 ± 0.5 °C) for 24 h, followed by filtration, dilution, and spectrophotometric analysis to determine the best solvent to use in the formulation design[14]. The Box-Behnken design was a three-factor, three-level design that was implemented with Design-Expert software to optimize the ethosomal formulation variables, which are lecithin (A), ethanol (B), and cholesterol (C) as independent variables and entrapment efficiency, vesicle size, and cumulative drug release as dependent responses under seventeen experimental runs followed by model fitting through ANOVA and the production of response surface and contour plots[15]. Efinaconazole-loaded ethosomes were made through the process of thin-film hydration, whereby the lecithin, cholesterol and the drug were dissolved using ethanol, dried to create a lipid film and hydrated using phosphate buffer (pH 6.8), and then sonicated to form homogeneous nanosized vesicles[15]. Dynamic light scattering was used to characterize the vesicle size, PDI, and zeta potential; ultracentrifugation was used to determine the entrapment efficiency; and FTIR, DSC, and XRD were used to determine the drug and excipient compatibility; the morphology was examined using microscopy or TEM. To prepare the gel, carbopol 934 was first moistened overnight and then triethanolamine was added to the optimized ethosomal suspension with continuous mixing and then pH was adjusted with triethanolamine. The gel obtained was judged in terms of appearance, clarity, pH, viscosity, spreadability, drug content and washability. Diffusion into phosphate buffer (pH 6.8) and diffusion experiments were carried out in a Franz diffusion cell at 37 degrees Celsius at 37 °C and samples were withdrawn at specific times to calculate cumulative drug release. Antifungal action was determined using agar diffusion test against *Candida albicans* and the zoning was compared to the marketed formulations. The stability was studied at ICH accelerated conditions (40 °C ± 2 °C/75 per cent ± 5 per cent RH) at the periodical intervals during 90 days and the following physical characteristics, pH, viscosity, drug content and particle size were observed.

Result and Discussion

Solubility Studies

To determine the most appropriate dispersion medium to use in the formulation of ethosomal, the solubility of Efinaconazole in various solvents was tested. As indicated in Table 1, Efinaconazole had extremely low solubility in the aqueous phase ($0.9435 \pm 0.126\%$), confirming it was hydrophobic and showing the restriction of the use of topical systems based on water. It dissolved in n-octanol (8.734 ± 0.032 percent) that indicated the lipophilic nature of the drug but was significantly higher in methanol (27.629 ± 0.287 percent) and phosphate buffer pH 6.8 (28.672 ± 0.021 percent). Nevertheless, ethanol had the

best solubility (49.182 ± 0.756) and thus it was the most effective solubilizer of the solvents being tested. This trend is well depicted in the graphical representation in Fig. 3 the degree of solubility increases sharply with the increase in ethanol concentration. This improved solubility is of great significance since ethanol is not only a major solvent but a major structural constituent of ethosomes. Increased solubility promotes a greater incorporation of the drug into vesicular core and consequently, an increased entrapment efficiency and uniformity in vesicle formation. This is in line with the already known behavior of lipophilic antifungal agents which show increased encapsulation when present in ethanol-based lipid carriers.

Table 1: Solubility expression system of different solvents

S. No .	Solvents	% solubility
1	Aq. Phase	0.9435 ± 0.126
2	PBS pH6.8	28.672 ± 0.021
3	n-octanol	8.734 ± 0.032
4	Methanol	27.629 ± 0.287
5	Ethanol	49.182 ± 0.756

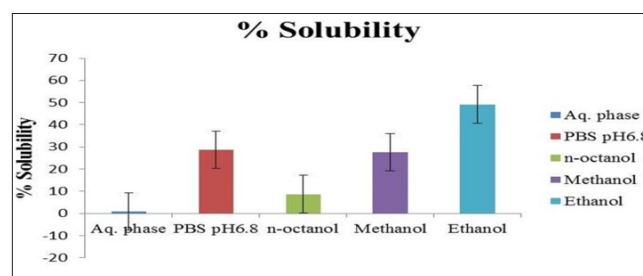


Figure 3. Graphical Representation of Solubility Expression System

The results of the solubility thus justified the choice of the formulation of ethosomes based on ethanol to be further developed. Enhanced solubility in ethanol is positively associated with rises in entrapment efficiency (EE), falls in vesicle size, and rise in cumulative drug release (CDR), each of which leads to an improved transungual penetration performance of Efinaconazole.

Experimental design for Formulation of Efinaconazole Solution

The preparation of ethosomes with Efinaconazole was done through the thin-film dispersion method and optimized statistically with a Box-Behnken Design (BBD) with 3 factors and 3 levels. The concentration of lecithin (A), ethanol (B), and cholesterol (C) were investigated on the efficacy of the independent variables on the entrapment efficiency (EE), vesicle size, and percent cumulative drug release (CDR). The model outputs showed that the formulation variables showed high correlations to the measured responses. The goodness-of-fit of the quadratic model was very good to the BBD optimization data and R

2 values of 0.9904 of EE, 0.9838 of vesicle size and 0.9892 of CDR showed that over 98% of the variation in responses was explained by the model. This confirms the existence of curved response surfaces, which justifies the fact that the second-order polynomial model is used and not a simple linear approximation.

Figure 4, above shows that the size of the vesicles grew proportionate to the concentration of lecithin (A) and cholesterol (C). This is not surprising as an addition of phospholipids leads to the formation of thicker bilayers and multilamellar vesicles whilst higher levels of cholesterol may add rigidity and structural density. On the contrary, the concentration of ethanol (B) had a contrary effect: ethanol was inclined to decrease the size of vesicles because it possesses bilayer-fluidizing and charge-modifying characteristics. Ethanol interferes with the lipid molecule

packing to form smaller and more deformable vesicles, which are typical of ethosomal systems. There was a high dependence of entrapment efficiency and CDR on the level of ethanol and lecithin. Increase in lecithin increased drugs accommodation in the bilayers, maximizing EE and ethanol maximized drugs solubility and bi-layer permeability, leading to an increase in CDR. Nonetheless, high cholesterol decreased the entrapment efficiency, probably due to competition of cholesterol with drug molecules in the bilayer space that decreases the amount of drug that can be incorporated. Predicted and actual plotting as well as residual analysis confirmed that the quadratic model was an accurate representation of the experimental data. The desirability functional forecasted an ideal formulation blend, which compromised high EE, small vesicle size, and improved drug release.

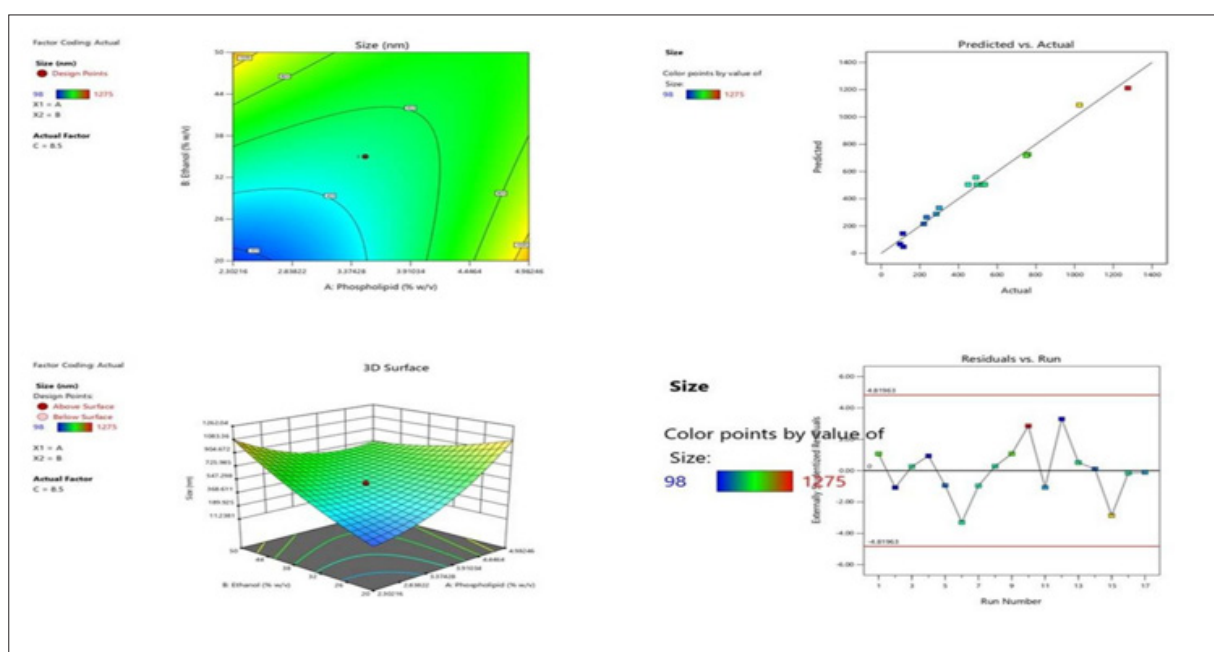


Figure 4. Contour plots and response surface plots showing the interactive effects Point desirability as suggested by Design-Expert® software A & B Particles Size, C & D Predicted vs. Actual Runs and Residuals vs., Runs

Table 2: Dependent variables: BBD Experimental Design and Response

F cod e	X1 (mg)	X2 (mg)	X3 (ml)	Y1 (%)	Y2 (%)	Y3 (nm)	PDI	Zeta
1	5	35	10	65.51±3.5	750±9.2	78.56±3.2	0.45 ±0.05	-27.8±1.01
2	2	20	8.5	71.72±2.8	112±5.4	90.23±2.4	0.56 ±0.01	-26.4±0.62
4	3.5	20	10	88.89±4.1	98±8.2	96.49±1.4	0.37 ±0.01	-35.8±0.22
5	3.5	50	7	50.82±3.2	235±3.5	69.41±5.4	0.41 ±0.02	-33.4±0.62
6	3.5	50	10	55.69±5.8	490±8.2	60.28±6.2	0.80 ±0.07	-31.9±1.64
7	3.5	35	8.5	75.93±9.1	450±9.1	68.72±3.5	0.58 ±0.01	-38.7±1.06
8	3.5	35	8.5	73.84±6.4	521±9.6	70.62±7.1	0.41 ±0.02	-30.9±0.62
9	5	50	8.5	46.19±7.2	759±5.8	70.27±8.2	0.47 ±0.01	-33.5±0.92
10	2	50	8.5	45.76±5.8	1275±7.9	68.81±6.1	0.80 ±0.07	-29.7±1.08
11	2	35	7	59.79±4.6	300±8.7	70.34±5.4	0.92 ±0.10	-28.7±0.62

12	3.5	20	7	82.38±6.2	115±8.9	65.24±3.8	0.41 ±0.02	-26.4±1.64
13	3.5	35	8.5	74.49±6.4	535±9.2	68.34±9.4	0.58 ±0.01	-25.6±1.13
14	2	35	10	51.61±7.2	220±9.6	66.13±7.5	0.82 ±0.04	-34.1±0.72
15	5	20	8.5	72.38±7.9	1025±7.6	88.69±4.9	0.80 ±0.07	-30.4±1.44
16	3.5	35	8.5	73.49±8.4	495±6.4	69.76±2.9	0.47 ±0.01	-32.3±1.61
17	5	35	7	50.25±3.5	285±8.3	48.98±9.7	0.41 ±0.02	-29.1±0.62

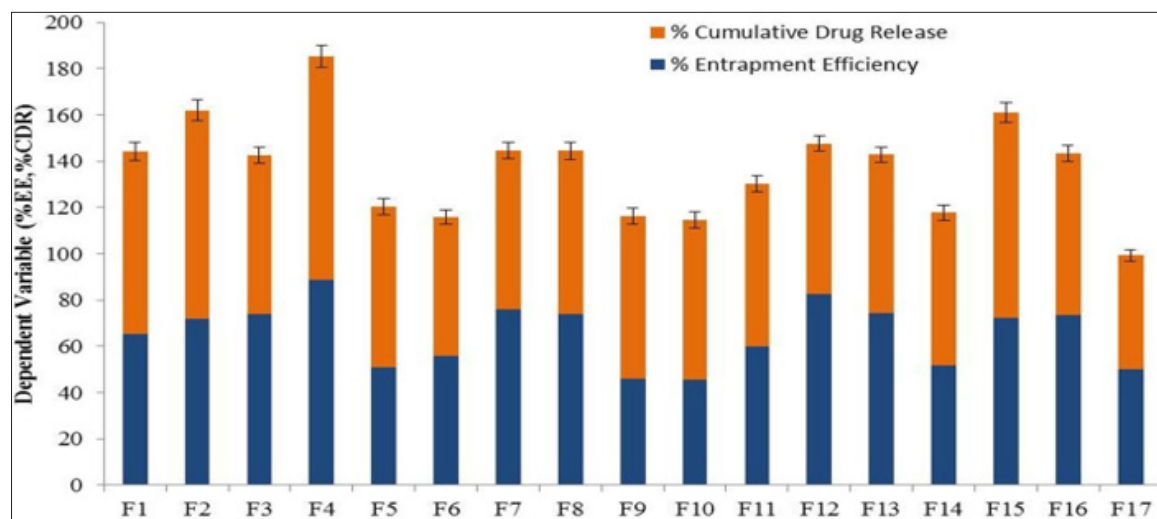


Figure 5. Comparisons of % CDR and %EE

A summary of the Box-Behnken experimental runs was presented in Table 2, and it can be observed that the change in phospholipid (X1), ethanol (X2), and cholesterol (X3) had a significant effect on entrapment efficiency (Y1), vesicle size (Y2), and cumulative drug release (Y3). Increased ethanol tended to decrease the size of vesicles, whereas increased phospholipid caused an increase in the size of vesicles and drug entrapment. The most favorable combination of the formulation variable showed the highest overall performance with high EE (88.89%), small vesicle size (98 nm), and high CDR (96.49%), suggesting the optimal balance of the formulation variables. These observations are further supported by Figure 5, that clearly illustrates that the formulations with a moderate phospholipid and ethanol concentration had better EE and CDR values than those with extreme concentration levels, which validated the model-identified optimal ethosomal formulation range.

Figure 6, demonstrates an effect on the entrapment efficiency (EE%) by the phospholipid (A) and ethanol (B) concentrations. Based on contour and 3D surface plot, it is observed that EE rises to middle values of phospholipid and ethanol and peaks to optimum values towards the optimum area. High or low levels of one or both factors result in very high or very low EE and this is the confirmation of the non-linear interaction between the variables. The predicted

vs. actual plot indicates a strong linear correlation which shows that the model is predictable. The Table 2 Residual vs. Run plot shows random distribution of residues within control limits, which proves that the model is accurate and there is no systematic error. All in all, Figure 6.4 justifies the optimum combination of A and B to reach the maximum EE%.

Figure 7, shows the effects of concentration of phospholipid (A) and ethanol (B) on cumulative drug release (CDR%) of Efinaconazole ethosomes. The figure of the contour and the 3D plot depict that the optimum level of ethanol leads to a rise in CDR as a result of the increased bilayer fluidization, and an overabundance of phospholipid decreases release by creating thicker vesicles. The predicted and actual plot shows excellent linear agreement and the distributional one of the residuals is random, which establishes the high model validity and the prediction of CDR behavior is accurate.

The comparison of the size of particles and zeta potential of various formulations as presented in Fig. 8 indicates that the optimized formulation has the least vesicles size and the most negative zeta potential. This implies high-quality nanoscale uniformity and electrostatic stability, which is necessary in long-term dispersion stability. The bigger vesicle size was related with the greater level of phospholipid and less amount of ethanol.

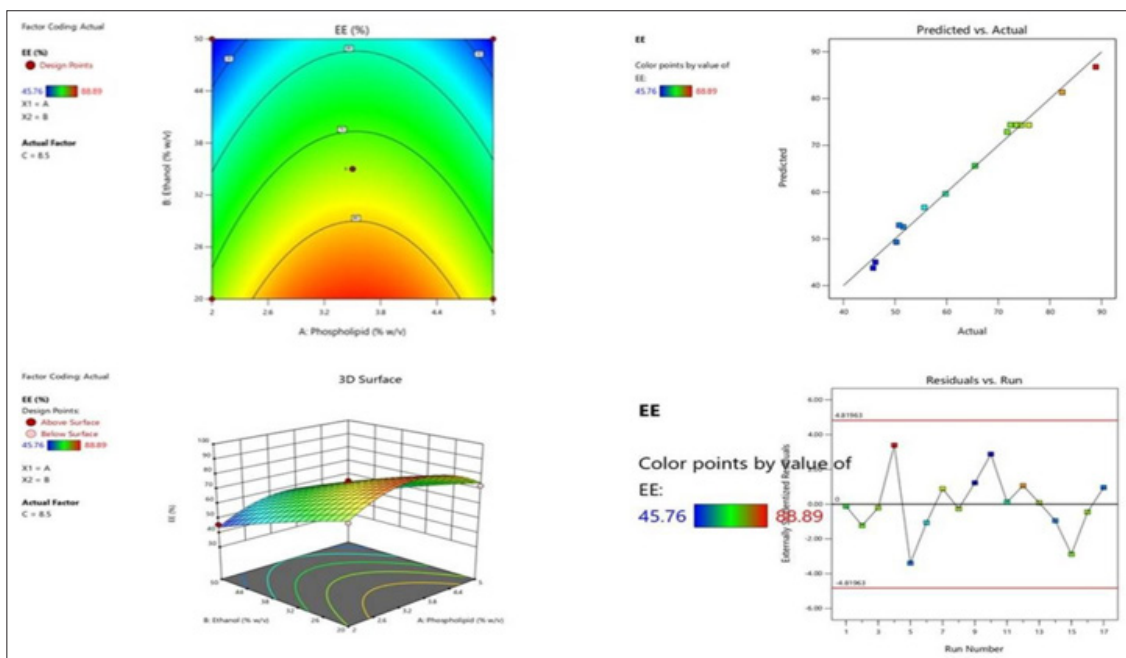


Figure 6. Contour plots and response surface plots showing the interactive effects Point desirability as suggested by Design-Expert® software A & B entrapment efficiency %, C & D Predicted vs Actual Runs and Residuals vs, Runs

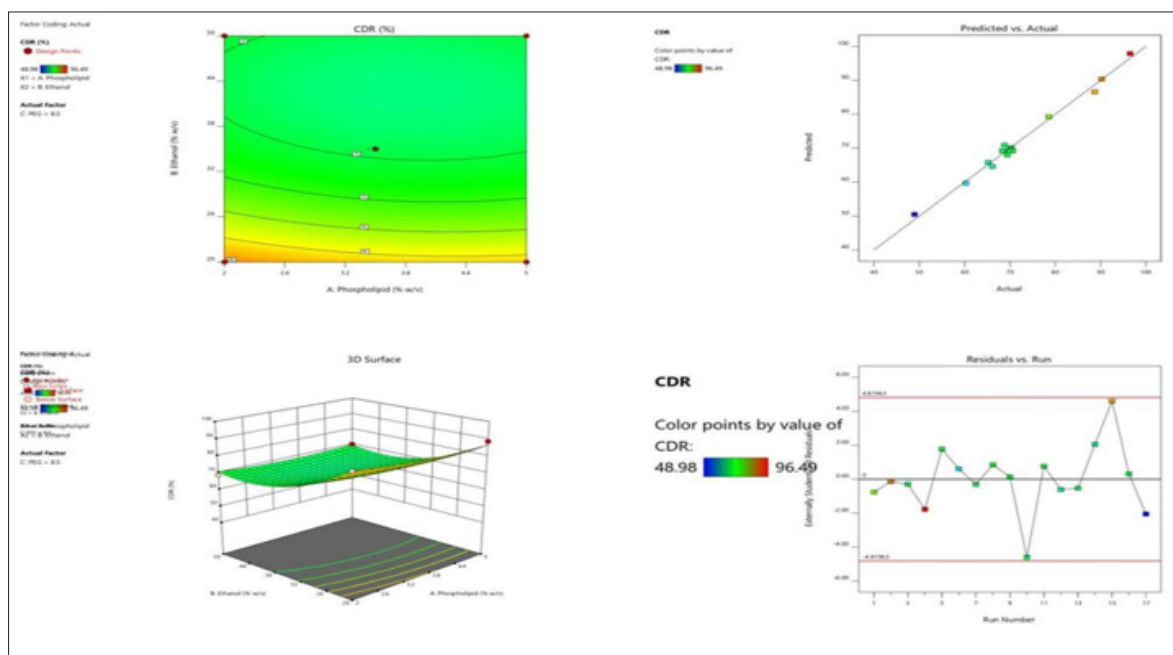


Figure 7. Contour plots and response surface plots showing the interactive effects Point desirability as suggested by Design-Expert® software A & B % CDR, C & D Predicted vs Actual Runs and Residuals vs, Runs

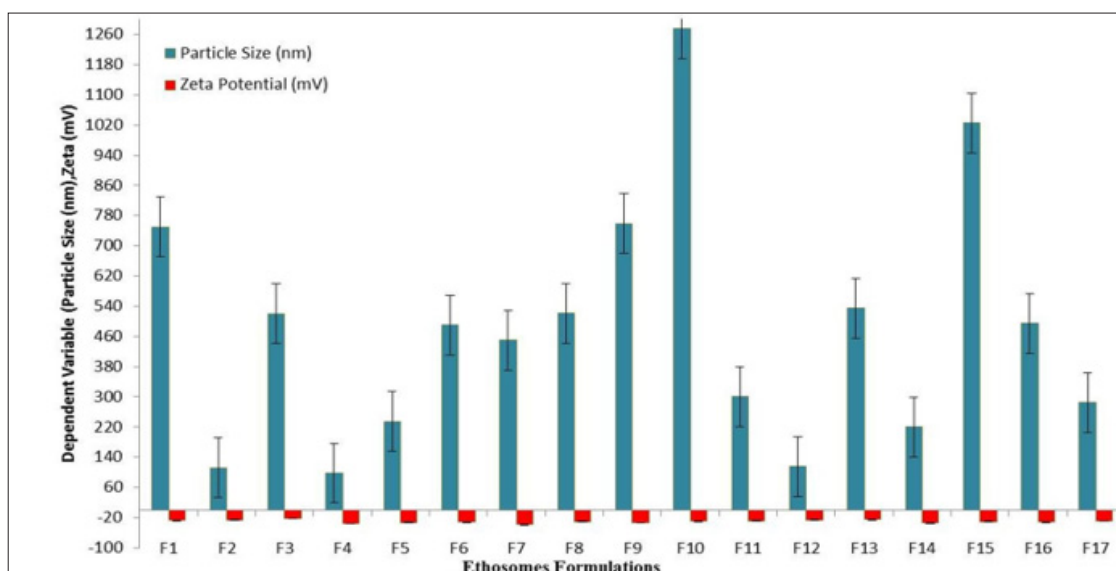


Figure 8. Comparison of Particle Size and Zeta Potential

Table 3: Analysis of variance in RSM and regression analysis predicted second-order polynomial model for entrapment efficiency

Model	Y1 (% EE)			Y2 (Cumulative % DR)			Y3 (Vesicle size)		
	R ²	Adjusted R ²	Predicted R ²	R ²	Adjusted R ²	Predicted R ²	R ²	Adjusted R ²	Predicted R ²
Linear	0.61	0.528	0.333	0.45	0.328	-	0.23	0.059	-
	69	5	1	43	4	0.125	57	3	0.595
						9			9
2FI	0.66	0.464	-	0.79	0.665	-	0.59	0.344	-
	54	7	0.157	11	8	0.005	06	9	1.086
			4			2			9
Quadratic	0.99	0.978	0.865	0.98	0.975	0.852	0.98	0.963	0.779
	04	1	7	92	4	3	38	1	7
p value	≤0.0001			≤0.0001			≤0.0001		

Table 4: ANOVA analysis of Dependent parameters

Parameter	Source	DF	Sum of squares	Mean of squares	F Value	p Value
% EE	Model	9	2817.42	313.05	80.54	< 0.0001
	Residual	7	27.21	3.89		
	Lack of fit	3	23.52	7.84	8.52	0.0328
	Pure error	4	3.68	0.9208		
% Cumulative DR	Model	9	2042.15	226.91	71.45	< 0.0001
	Residual	7	22.23	3.18		
	Lack of fit	3	18.72	6.24	7.10	0.0443
	Pure error	4	3.52	0.8788		
Vesicle size	Model	9	1.672E+06	1.858E+05	47.35	< 0.0001
	Residual	7	27472.55	3924.65		
	Lack of fit	3	22969.75	7656.58	6.80	0.0475
	Pure error	4	4502.80	1125.70		

Table 3, indicates that the quadratic model had the highest fit of all responses (percentage of EE, percentage of CDR, and size of vesicle) with R² values exceeding 0.98 which reflected a great predictability and low error. There was poor fit with linear and 2FI models, which proved the need of a second-order model to explain the non-linear associations among variables of formulation.

Table 4, denotes that the three responses, EE, CDR and vesicle size, were all significant changing under the three formulation factors chosen ($p < 0.0001$). The high F-values affirm significant model importance whereas the small errors in the residual indicate the accuracy and repeatability of the experimental data.

FTIR Analysis

The lack of chemical interactions between Efinaconazole and the formulation excipients is confirmed by Fig. 9. The ethosomal formulation retained all characteristic peaks of the pure drug, which is an indication of compatibility and stability within the lipid matrix.

DSC Studies

Figure 10, demonstrates that the intensity and the extension of the Efinaconazole endothermic peak in the formulation are reduced, which is the evidence of amorphization partially and the achieving of the effective integration of the drug in the lipid bilayers. This change in structure helps in better entrapment as well as dissolution.

XRD Studies

In Figure, 11, reduced or dispersed crystal peaks are observed

in the ethosomal formulation and not pure Efinaconazole, and thus indicate a decrease in crystallinity. This amorphous conversion can make the substance more soluble and adds to better drug release.

Zeta Potential

Figure 12, depicts the optimized formulation, and the zeta potential of the formulation was approximately -35 mV, and this value indicates high repulsion due to the electrostatic forces between the particles and aggregation is prevented thus ensuring close physical stability of the nanosystem.

Physical Evaluation

The developed ethosomal gel possessed satisfactory organoleptic properties where it was white in color, had characteristic odor, transparent in appearance and no phase separation was observed and it was also occlusive and it could be washed easily. The pH of the gel base and the newly prepared optimized formulation (F4) was tested as 7.1 and 8.0 respectively which showed that the gel base was suitable to be used topically without any irritation to the skin. The Brookfield viscometer was used to measure the viscosity of the formulation, and it was established that the viscosity of the formulation was 40, 200 cps, which showed there was sufficient consistency to apply the formulation uniformly. Moreover, the spreadability of the Efinaconazole ethosomal gel was measured at 12.88 g/cm², which shows that the formulation spreads easily with a little force applied to it, thus increasing the acceptability of the patients and ease of use.

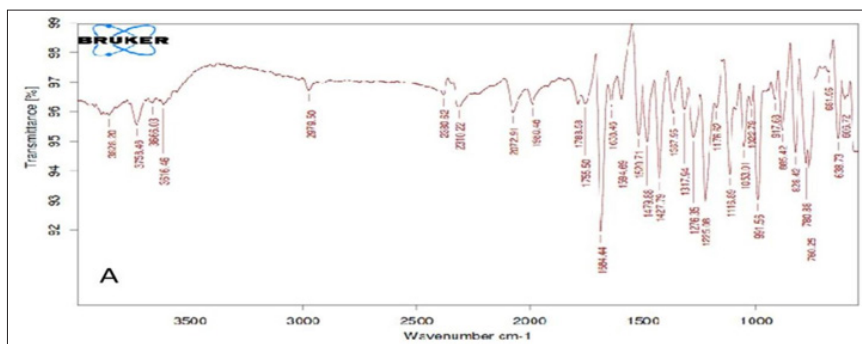


Figure 9. FTIR Spectral Studies of A). Efinaconazole

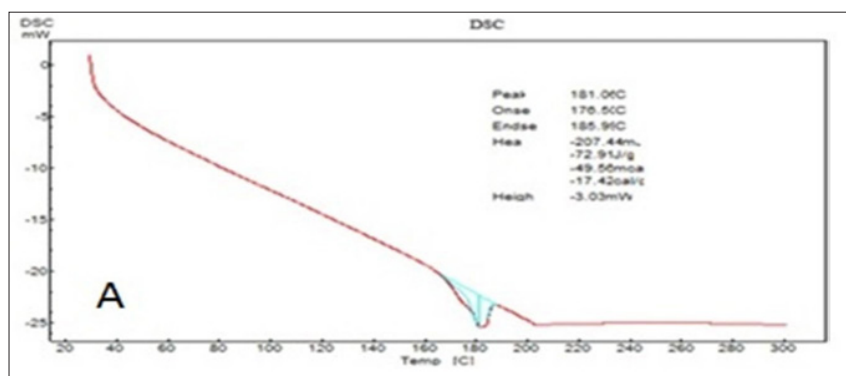


Figure 10. DSC Studies of Efinaconazole

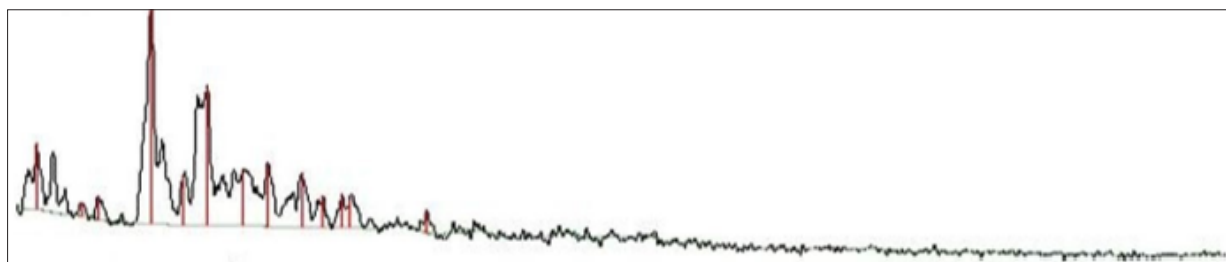


Figure 11. XRD Studies of Efinaconazole

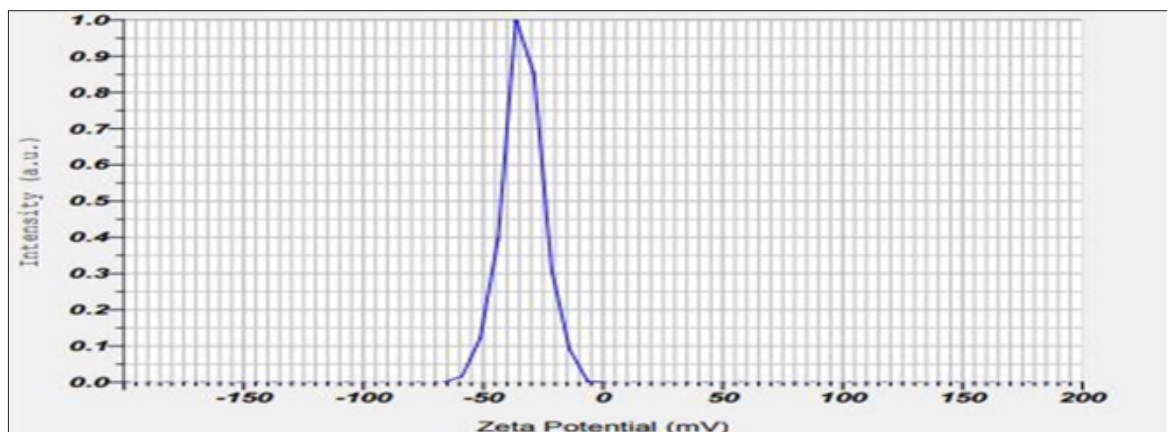


Figure 12. Zeta potential of Solution

Table 5: Evaluation of Efinaconazole solution formulations

Formulation	Drug content (%)	pH	Viscosity (cP)	Spread ability (g.cm/sec)
G1	92.57±0.21	7.8±0.01	1.18±1.24	9.68±1.31
G2	99.82±0.02	6.8±0.03	1.97±2.31	12.84±2.43
G3	95.43±0.14	8.2±0.01	2.61±1.06	6.57±1.27
G4	98.54±0.37	7.6±0.02	2.98±1.08	7.64±0.98
G5	94.21±0.28	7.8±0.01	9.34±1.17	6.82±1.34
G6	96.56±0.11	8.1±0.02	15.46±1.27	5.37±1.29
G7	92.54±0.13	7.4±0.01	18.43±0.29	7.81±1.17

Physical Evaluation i.e., it showed best result for spread ability

Table 5, illustrates that gel formulations exhibit acceptable drug content (92 to 99 percent), pH (6.8 to 8.2) and topical application viscosity. The spreadability was quite different with G2 indicating the best spreadability which means that it has the best consistency and can be accepted by the user.

Figure 13, compares the relative pH, viscosity, drug content and spreadability among formulations. The optimized formulation exhibits optimal pH (7 -8), the appropriate viscosity to be used in topical application and increased spreadability, which validate good user acceptability and applying ease.

In vitro Diffusion Studies

To determine the amount of drug released at various time intervals, an in vitro investigation of release was conducted. It was observed through graphic representation that the

release rates differed among all the formulations tested. The drug release was initially high but as time went it became even higher than at the beginning. The test formulation had a significant cumulative release of Efinaconazole in comparison with the gel formulation, as seen towards the graph.

Table 6 indicates that optimized formulation (F4) exhibited the best cumulative drug release of almost 100 percent at 7 -8 hours. The optimized ethosomal composition was proved to be successful with the formulations with a balanced ethanol and phospholipid always showing a better permeation.

Figure 14, shows all the release profiles of BBD formulations. Optimized formulation exhibits a fast and slow release, which is greater than 98% at 7-8 hours. Recipes using moderate ethanol and lecithin performed optimally, which validates their contribution to increasing the fluidity of membranes and diffusion of drugs.

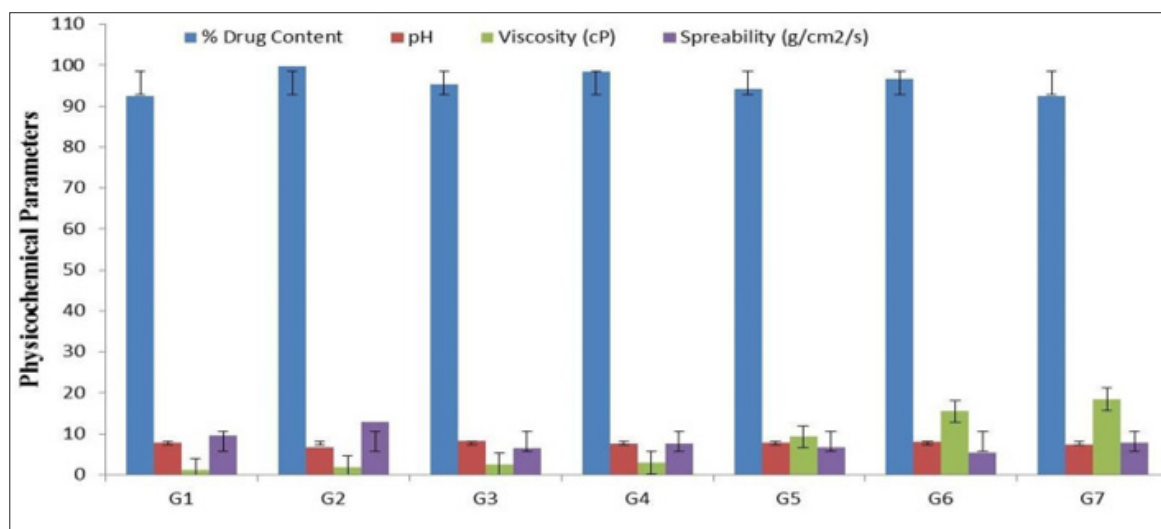


Figure 13. Graphical representation of the physical evaluation of the Efinaconazole Solution formulation

Table 6: In vitro diffusion studies of Formulation

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
0hr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1hr	12.03	23.95	13.88	20.12	14.25	11.25	14.25	20.62	13.88	11.83	23.92	12.05	15.22	22.15	16.26	15.25	2.621
2hr	25.96	33.86	26.87	39.52	20.17	21.83	20.17	35.89	26.81	26.89	32.27	20.95	22.14	39.51	22.85	22.86	3.854
3hr	32.15	50.45	32.95	55.44	32.94	30.45	32.94	51.61	32.98	34.58	44.49	35.15	43.87	57.86	33.62	31.42	4.717
4hr	43.96	62.85	43.72	70.62	46.27	42.85	40.27	67.62	43.77	41.75	60.92	46.88	50.45	62.87	43.77	46.76	5.545
5hr	52.18	74.94	56.91	89.14	58.86	55.16	53.86	75.89	56.95	52.85	70.99	56.21	58.98	72.96	50.42	52.77	7.213
6hr	60.79	83.89	67.82	99.57	69.15	66.12	61.15	82.33	67.82	60.76	82.47	62.14	70.92	89.14	58.26	66.72	8.824
7hr	75.16	98.78	81.95		82.65	79.18	77.88	97.16	81.95	77.44	96.75	68.92	78.56	98.15	65.42	79.18	9.726
8hr	88.93		91.28		98.22	83.67	87.52		93.15	89.58		71.45	88.23		79.66	84.43	

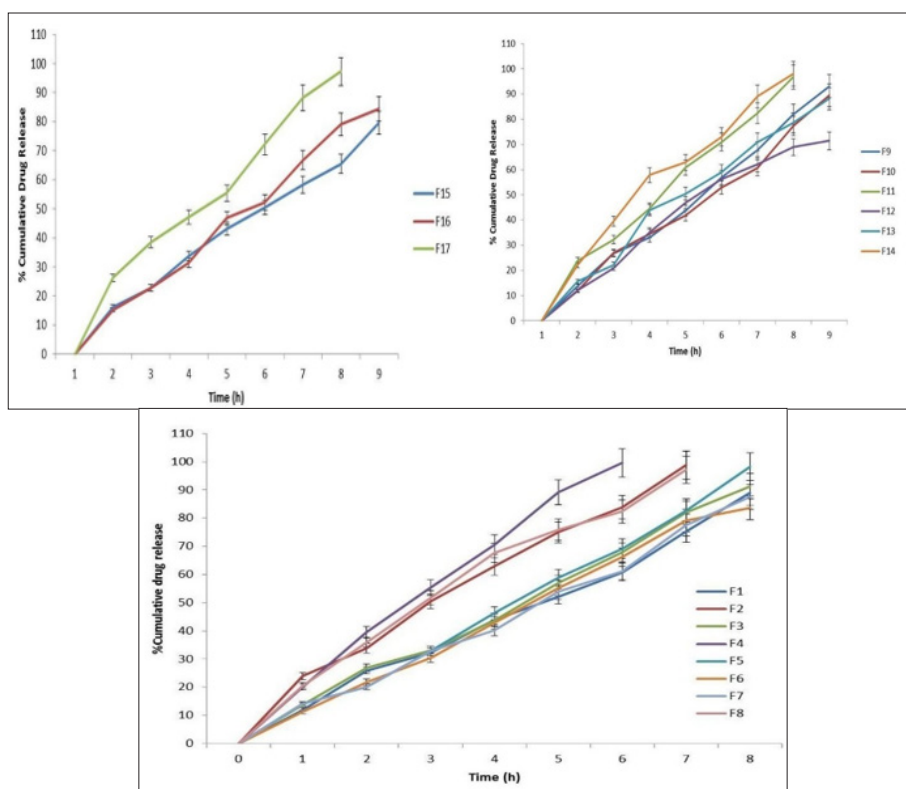


Figure 14. In vitro release studies of F1-F17

Table 7: In vitro diffusion studies of Formulation

Time	G1	G2	G3	G4	G5	G6	G7
0hr	0	0	0	0	0	0	0
1hr	19.98	25.67	15.91	20.92	19.42	22.54	19.22
2hr	27.89	40.53	25.46	32.18	28.98	32.36	28.98
4hr	49.91	58.98	37.27	55.98	50.65	52.87	44.45
6hr	68.98	72.65	58.19	68.54	61.51	65.49	62.15
8hr	76.87	89.91	67.22	79.76	75.87	71.67	75.41
10hr	85.97	99.35	76.41	89.97	83.62	80.52	86.79
12hr	95.46		88.62	96.48	92.16	89.22	98.26

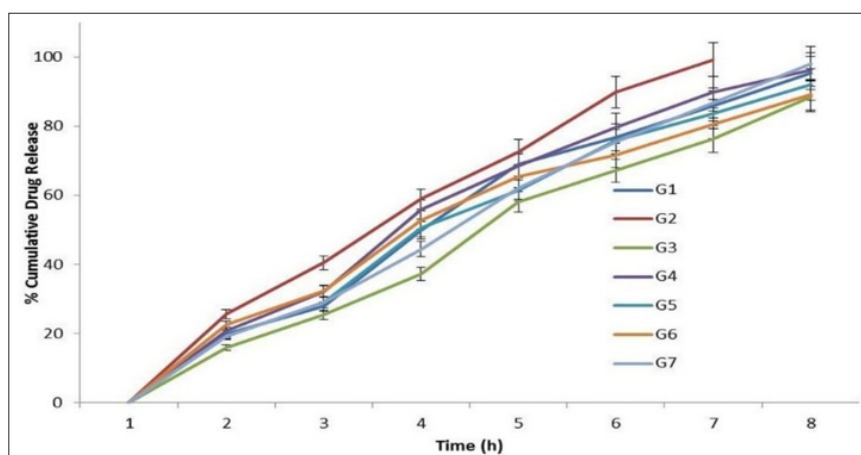


Figure 15. Represents the profiles of gel formulations release, which shows that G2 released the drug the highest, then G7, then G4. Recipes with the best viscosity and ethanol concentration exhibited better diffusion and it was found that gel microstructure has a significant effect on the release kinetics

Table 8: Comparison of Ex Vivo Studies

Time (hrs)	D+W	Ethosomal suspension	Ethosomal gel
0hr	0	0	0
1hr	22.14	39.21	20.35
2hr	29.65	54.76	35.43
3hr	37.45	62.61	48.98
4hr	44.22	78.93	55.41
5hr	51.87	89.45	67.76
6hr	57.42	99.56	74.22
8hr	63.65		89.47
10hr	72.18		99.51
12hr	75.86		
24hr	89.43		
28hr	99.42		

Table 7, indicates that the gel formulation G2 can be used since it can be retarded to release maximum (99.35) after 10-12 hours, compared to other formulations. This implies that G2 had the best viscosity and diffusion characteristics that could be used in sustained transungual delivery.

Figure 15, represents the profiles of gel formulations release, which shows that G2 released the drug the highest, then G7, then G4. Recipes with the best viscosity and ethanol concentration exhibited better diffusion and it was found that gel microstructure has a significant effect on the release kinetics.

Ex-vivo Permeation Studies

Comparison of Ex-Vivo permeation studies among aqueous solution of pure drug.

Table 8, indicates that the ethosomal suspension had the greatest permeation than pure drug and ethosomal gel. Ethosomes greatly improved the drug permeation into the nail and these results demonstrated that ethosomes had a superior deformability and ethanol-mediated permeation promotion.

Antifungal Studies

Figure 16, represents the relative in-vitro drug release of optimised ethosomal formulation and the marketed formulation. The optimized ethosomal gel shows a lot faster and cumulative drug release during the study period. The increased release kinetics may be explained by high level of ethanol and high deformability of the vesicles that facilitates the diffusion of drugs faster through the hydrated membrane. Conversely, the released formulation of the marketed one is more gradual and slow, suggesting low penetration efficiency. The sharp rise and diffusion exhibited in the ethosomal system at the first release and the subsequent

sustained one has validated its capacity to sustain therapeutic concentration during prolonged periods. Altogether, Fig. 16 confirms the superiority of the ethosomal formulation to be used to increase the effectiveness of transungual delivery to prove its higher efficiency in using compared to traditional products. Agar diffusion method was used in evaluating the antifungal activity of the Formulation against *Candida albicans* ATCC 14057 and the results showed that Efinaconazole Solution had an inhibition zone of 25.03mm 1.11mm. No notable differences ($p=0.05$) were observed in the free drug activities. Also, the inhibition zones reported no visible effect on the antifungal action of the prepared Efinaconazole Solution. Interestingly, formulations in which there were no drugs or the diluents used in the study did not have any inhibition site.

In vitro Antifungal Activity of Efinaconazole Solution

The antifungal efficacy of the Formulation against *Candida albicans* ATCC 14057 was assessed using the agar diffusion method, revealing inhibition zones of 25.03 ± 1.11 mm for Efinaconazole Solution. No significant differences ($p \leq 0.05$) were noted in the activities of the free drug. Additionally, the inhibition zones indicated no discernible impact on the antifungal activity of the prepared Efinaconazole Solution. Notably, formulations lacking the drug or the study's utilized diluents did not exhibit any inhibition zones.

Stability Studies

Table 9 indicates that the ethosomal formulation was also stable after three months with the only slight changes being in the percent EE and zeta potential. The zeta potential was low enough (between -28 and -35 mV), which means that the colloidal stability was preserved at refrigerated and room temperature.

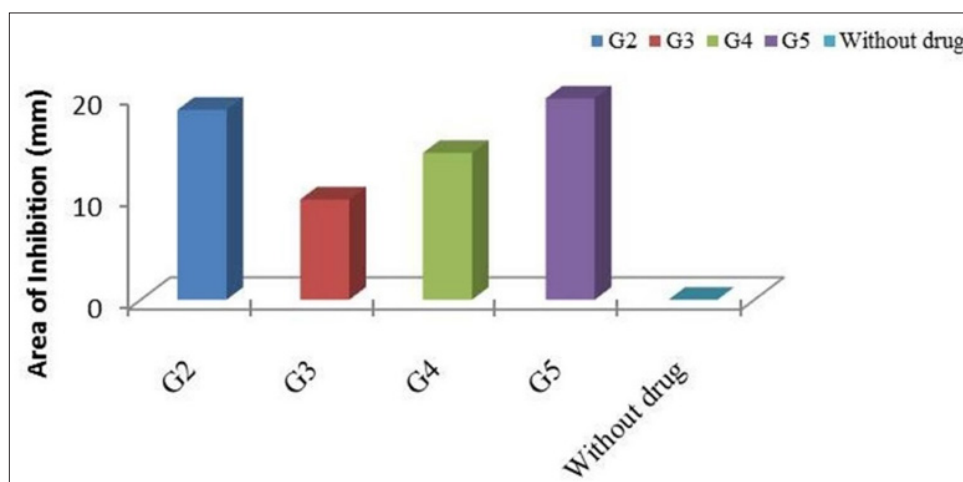


Figure 16. Anti-fungal studies of Efinaconazole formulation

Table 9: Results for stability studies of Efinaconazole Solution

Months	Temperature	%EE	Zeta value(mv)
1 st Month	Refrigeration	96.25±1.3	-35.6
2 nd Month	temperature	92.70±0.6	-32.1
3 rd Month	(4±2°C)	86.65±3.4	-28.7
1 st Month	Room	96.25±1.6	-35.6
2 nd Month	temperature	89.91±2.9	-30.8
3 rd Month	(27±2°C)	80.87±3.5	-29.2

The findings of the current research show clearly that ethosomal-based delivery of Efinaconazole greatly increases its transungual permeation, drug loading and antifungal activity than the traditional formulations. The Box-Behnken optimization has shown that the concentrations of ethanol and phospholipids are the key variables that define the size of the vesicles, entrapment efficiency, and the cumulative release of drugs, whereas, cholesterol is used to regulate the rigidity of the membrane. The optimized F4 formulation had desirable nanoscale characteristics such as small vesicle size (=98 nm), high entrapment (=88.89%), and excellent drugs release (=96%), all of which play roles in enhancing penetration through the dense structure of the nail of different density. The compatibility of the drugs and partial amorphization in the vesicular matrix was confirmed by FTIR, DSC, and XRD analyses, and it is assumed that these results prove increased solubility and diffusion. The gel system that was made of the optimized ethosomes presented an ideal pH, viscosity and spreadability to be applied topically. The in-vitro and ex-vivo tests verified that the ethosomes possessed a better permeation capacity and that the antifungal tests showed better performance against *Candida albicans* than the marketed drugs. Taken together, these results suggest that ethosomes provide a safer, more convenient, and efficient system of transungual delivery of Efinaconazole as a solution to multiple constraints of current topical treatment and offer better therapeutic success in treating onychomycosis.

Conclusion

This paper was able to formulate and optimize an ethosomal delivery of Efinaconazole through a Box-Behnken statistical design. The optimized formulation exhibited better physicochemical features, such as high entrapment efficiency, less vesicles size and good stability. The compatibility studies provided were able to confirm the lack of drug-excipient interaction, but the thermal and structural analysis revealed partial amorphization of the products, which is favorable to the increased solubility. Diffusion in-vitro, ex-vivo permeation and antifungal experiments conclusively demonstrated that the ethosomal formulation performed better than the traditional gels as well as marketed products. These results confirm that ethosomes can be used as an effective, stable and efficient transungual drug delivery system that has the potential of greatly improving therapy of onychomycosis.

Summary

The study was meant to address the shortcomings of the traditional Efinaconazole preparations by establishing an ethosomal nanocarrier platform that would best suit transungual administration. BBD was used to optimize ethosomal vesicles which had good properties including high entrapment efficiency, nanoscale size vesicles, negative zeta potential, and improved drug release. The drug stability and its integration into the lipid matrix was confirmed through comprehensive characterization (FTIR, DSC, XRD). The

ethosomal gel had the right pH, viscosity, spreadability and drug content to be used topically. The ethosomes were proved to perform better in in-vitro, ex-vivo and antifungal studies than the commercially available formulations. The research has been able to develop an optimized ethosomal system that can deliver Efinaconazole effectively through the nail plate which gives it better antifungal effect and better therapeutic potential.

Future Scope

Based on the potential potential of this study, future research can be conducted on in-vivo clinical trials to confirm the therapeutic efficacy and safety of ethosomal Efinaconazole gel in human beings with onychomycosis. The real-time stability experiments on a long-term basis should be performed to determine commercial feasibility. Additional penetration enhancers or a combination of ethosomes and the use of microneedle-based delivery can help to improve transungual penetration even more. This ethosomal system can also be applied to other antifungal drugs that are poorly soluble or reused to provide specific dermatological therapy needing greater penetration of the drug through nail or skin barriers. The transition of this system to industrial manufacturing and commercialization will also be assisted by scale-up studies and standardization of the formulation.

Conflict of Interest

None

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