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FORMULATION AND CHARACTERIZATION OF ECONAZOLE NITRATE LOADED TRANSFERSOMAL GEL FOR ANTIFUNGAL ACTIVITY

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Keywords:

Econazole nitrate, Transfersomes, Phospholipid, Edge activators, Antifungal activity

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ABSTRACT: The aim of the present work is to formulate and characterize Econazole nitrate (EN) loaded transfersomal gel for antifungal activity. EN is a broad-spectrum imidazole antifungal agent that belongs to BCS Class II. Due to poor solubility, EN is incompletely absorbed after oral administration and bioavailability vary among individuals. Topical treatment of fungal infections is usually preferred, but the barrier is to cross stratum corneum, so formulating the drug in Transfersomes (TFs) solved this problem. TFs are a highly adaptable, stress-responsive, complex aggregate, and self-regulating membrane. EN loaded transfersomes were prepared by a thin-film hydration method. The EN-loaded transfersomes were optimized by the three factors and two levels Box-Behnken design using Design-Expert software (version 12). Independent formulation variables such as concentrations of phospholipid and two edge activators were evaluated. The prepared TFs were evaluated with respect to particle size, % entrapment efficiency, and % drug release. The prepared EN loaded TFs particle size ranging from 0.28 to 0.71 µm, entrapment efficiency in between 31.5 to 75%, and drug release 80.01 to 95.9%. The optimized F12 TFs batch was formulated by incorporating it into a Carbopol-940 gel base. The EN-loaded transfersomal gel was further evaluated for antifungal activity against *Candida albicans*. The result showed that the antifungal activity of the EN-loaded TFs was significantly higher than the marketed product (Daktarin® Gel 2% w/w). Therefore EN loaded transfersomal gel has the ability to penetrate the skin, overcoming the stratum corneum barrier.

INTRODUCTION: Superficial fungal infections are common diseases of all ages and both sexes that occur in the skin, nails, and mucous membrane [1]. The high predominance of superficial infections shows that 20-25% of the world's population has skin mycoses, making these ones of the most usual forms of infection [2].

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Candidiasis is a primary or secondary infection caused by Candida species and has become more prevalent than *Escherichia coli* and *Pseudomonas*, *Aspergillus* species. Candidiasis is now the fourth most common fatal infection in the world [3, 4].

It may affect almost any skin surface on the body and most likely occur in warm, humid, wrinkled areas, including the armpits and the groin [5]. Econazole nitrate (EN) is a wide spectrum antifungal agent that has an imidazole group that is effective against *Candida albicans*. The EN interferes with the ergosterol synthesis by obstructing the enzyme Cytochrome P-450, which increase the permeability of cell that results in

leakage of cellular content and causes death of the fungus cell [6, 7]. Due to poor solubility, EN is incompletely absorbed after oral administration [8]. Topical treatment of fungal infections is usually preferred, which can maintain uniform plasma concentration; reduce dosing frequency associated with improved patient compliance and gastrointestinal action [9]. The Utmost challenge with the delivery of active across transdermal route is the barrier properties of the stratum corneum that limit the absorption of the majority of drugs [10]. Hence, it is necessary to design a drug delivery system for antifungal drugs which has the ability to overcome the barrier properties of the stratum corneum. Conventional formulations are given in higher doses to overcome this issue and compensate for low permeability [11].

Using the transdermal route of drug delivery along with novel approaches can help to solve these problems. Formulating the drug in Transfersomes (TFs) is one such approach. In recent year's use of lipid vesicles as the carrier for topical drugs has attracted great attention due to their ability to overcome the barrier properties of the skin. The word "Transfersomes" was introduced in 1991 by Gregor Cevc. Transfersome is a term registered as a trademark by a German Company IDEA AG. The name "Transfero" is derived from the Latin word "Transferee" and it indicates 'to carry across' and the Greek word "soma" for a 'body' [12]. TFs are ultra-deformable vesicles possessing an aqueous core surrounded by the complex lipid bilayer [13]. TFs overcome the skin penetration obstacle by pinching themselves along with the intracellular sealing lipid of the stratum corneum [14]. TFs act as penetration enhancers that disrupt the intercellular lipids from stratum corneum, which ultimately enlarges the pores of the skin and facilitate the molecular interaction and penetration of drugs across the skin [15, 16]. The objective of the present study was to formulate and characterize EN-loaded transfersomal gel to enhance skin permeability for antifungal activity and optimization using the Box-Behnken design approach. Candidiasis is used as model disease to evaluate the antifungal activity of the formulated EN-loaded transfersomal gel.

MATERIALS AND METHODS:

Materials: Econazole nitrate was received as a gift sample from Gufic Lifescience Pvt. Ltd. (Navsari, Gujrat, India). Soya-Lecithin, Tween 80 and Carbopol-940 were procured from Analab fine chemicals, Mumbai. Sodium cholate was obtained from Research-Lab Fine Chem Industries, Mumbai. Propylene glycol, Isopropyl alcohol and Triethanol-amine were provided by Merck Specialities Pvt. Ltd., Mumbai. Methanol and Chloroform were supplied by Hexon Laboratories Pvt. Ltd., Pune. All other chemicals and solvents were of analytical grade.

Methods:

Preliminary Studies:

Solubility Studies: Solubility studies of EN in various solvents and different edge activators were carried out. An excess amount of EN was added to a conical flask containing 5 ml of each solvent and edge activator until equilibrium was achieved. Samples were kept at 25 ^{o}C with constant shaking on an orbital shaker (Labline-SR. NO 213004) for 48 h. The resultant solution was filtered through 0.45 um membrane filter and diluted with a suitable solvent. The concentration of EN of samples was quantified by UV spectroscopy at 218 nm [17].

Drug-Excipient Compatibility Studies: A Fourier-transform infrared spectroscopy (Shimadzu IR affinity-1s, Japan) was used to study the drug-excipient compatibility studies. FTIR of the pure drug EN and a mixture of drug with excipients were taken. Infrared spectra were recorded. The peaks of the pure drug were compared with the physical mixture of drug and excipients [18].

Differential Scanning Calorimetry (DSC) Studies of Pure EN: Thermal analysis of pure EN was performed with Differential Scanning Calorimetry (METTLER-DSC823e). The DSC thermogram was obtained at a temperature ranging from 30 to 300 ^{o}C and a scanning rate 10 ^{o}C / min [19].

X-ray Diffraction (XRD): XRD analysis of EN was performed at Diya Lab, Mumbai (Shimadzu XRD-7000). The scanning range was 10^{o} to 80^{o} at angle 2Θ diffraction with a continuous scan speed of 6^{o} per minute. XRD pattern was measured with a current 30 mA and voltage of 40 kV.

Experimental Design: The Edge activators were screened in pre-optimization trials based on solubility studies Sodium cholate, and Tween 80

was selected for the formulation of EN loaded TFs. They are bilayer softening components, therefore, increases lipid bilayer flexibility and permeability. The EN-loaded transfersomes were further optimized by the three factors and two levels Box-Behnken design using Design-Expert software (version 12, Stat-Ease Inc and Minneapolis, MN, USA). Three independent formulation variables were evaluated: a) concentration of phospholipid b) concentration of sodium cholate c) concentration Tween 80. A three-factor, two levels Box-Behnken statistical experimental design of the Response Surface Methodology requires 17 runs, of which 12 represents the midpoint of each edge of the multidimensional cube while five are the replicates of the cube's center point.

The particle size (Y_1), % entrapment efficiency (Y_2), and % drug release (Y_3) were evaluated as the dependent variables. The one-way analysis variance (ANOVA) was applied to estimate the significance of the model ($P<0.05$) and individual response parameter.

TABLE 1: INDEPENDENT VARIABLES AND THEIR CORRESPONDING LEVELS FOR OPTIMIZATION STUDIES INCLUDE

Independent Variables		Levels	
		-1	+1
Concentration of Phospholipid (mg)	X_1	85	150
Concentration of Sodium cholate (mg)	X_2	30	50
Concentration of Tween 80 (mg)	X_3	10	50

TABLE 2: BOX-BEHNKEN DESIGN FOR FORMULATION OF EN LOADED TFs

Formulation no.	Factor 1 (X_1)	Factor2 (X_2)	Factor 3 (X_3)
1	0	0	0
2	0	0	0
3	0	0	0
4	0	-1	1
5	0	1	-1
6	-1	0	-1
7	1	0	-1
8	0	0	0
9	-1	1	0
10	0	0	0
11	0	1	1
12	-1	-1	0
13	1	-1	0
14	-1	0	1
15	0	-1	-1
16	1	1	0
17	1	0	1

Formulation of EN Loaded TFs: Econazole nitrate vesicles were prepared by the thin-film hydration method. To prepare a vesicle suspension EN, Soya lecithin edge activators (Sodium cholate, Tween 80) were taken in a clean, dry round bottom flask and dissolved in chloroform: methanol (2:1, v/v).

The organic solvent was evaporated in the rotary evaporator under vacuum at 55 °C (Popular India) until a thin film formed on the wall of the flask. The deposited lipid film was hydrated with 10 ml of phosphate buffer (pH 7.4) by rotation at 60 rpm for 30 min. The resulting vesicles were swollen for 2 h at room temperature to get large multi-lamellar vesicles (LMLVs). These LMLVs were sonicated using a bath sonicator (SS Biomedia, LX 300) for 30 min to obtain homogeneous suspension [20].

Preparation of EN Loaded Transfersomal Gel: 1.5 gm of Carbopol-940 was dispersed in 60 ml of distilled water. Then the mixture was stirred until thickening occurred. After complete dispersion, 10 ml of Propylene glycol was added slowly into the aqueous dispersion of Carbopol-940, and then other ingredients such as 5 ml of Triethanolamine were added. 10 ml of TFs dispersion was incorporated into Carbopol gel. Quantity sufficient of distilled water was added to make up the volume up to 100 gm of gel.

Characterization of EN Loaded TFs:

Particle Size Analysis: Particle size analysis was determined using a digital microscope. The morphological characterization of TFs vesicles, such as shape and surface features were projected by using a digital microscope. Pixel Pro software was used for particle size analysis. A drop of transfer of some dispersion was placed over the slide. The photomicrographs were taken at 10X resolution, and measurements were conducted in triplicate manner [21, 22].

% Entrapment Efficiency: Entrapment Efficiency of EN transfersomal vesicles was determined by centrifugation method. Transferosomal suspensions were ultra-centrifuged at 15,000 rpm and 10 °C for 30 min. After centrifugation, the supernatant solution was filtered through the membrane.

From filtered solution, 1 ml was diluted with the addition of 9 ml phosphate saline buffer (pH 7.4) and then the absorbance was measured using UV–Visible spectrophotometer (Shimadzu UV 1700, Japan) by measuring absorbance at 218 nm [23]. The % entrapment efficiency was calculated as below:

% Entrapment Efficiency = Total drug - Unentrapped drug / Total drug × 100

***In-vitro* Drug Release Studies:** The *in-vitro* drug release studies were performed using vertical Franz diffusion cell. An artificial cellophane membrane having a molecular weight of 12000 was mounted between the donor and receptor compartment.

Consequently, the donor compartment was filled with 1 ml of each formulation, and the receptor compartment was filled with phosphate buffer 7.4 up to the mark. The temperature in the Franz diffusion cell was maintained at 37± 0.5 ºC, and the receptor compartment was stirred continuously at 50 rpm using a magnetic stirrer. 1 ml sample was withdrawn from the receptor compartment and diluted up to 10 ml, and immediately replaced with an equal volume of fresh diffusion medium.

Finally, the concentration of EN in the sample was analyzed by using UV spectrophotometry at 218 nm. Similarly, after every one hour, the sample was withdrawn and subjected to UV analysis done in the triplicate manner, and mean ± SD was noted. The study was carried out for 4 h [24].

pH Measurement: The electrode was immersed in dispersion, and pH measurement of TFs was carried out using digital pH meter [25].

Determination of Particle Size and Zeta Potential: Particle size and Zeta potential of the prepared optimized EN loaded TFs batch was measured using particle analyzer (Horiba Scientific SZ-100), applying dynamic light scattering techniques. For particle size measurement, TFs dispersion was diluted with distilled water. Ultra-sonication was done to prevent agglomeration for 10 min. The measurements were performed in triplicate at 25 °C under a fixed scattering angle of 173^{o}and the mean ± SD calculated [26].

Morphological Characterization of TFs: The surface morphology (Scanning Electron Microscopy) of the optimized EN loaded TFs batch was identified by Carl Zeiss -Supra 5 at Diya labs. The sample was attached to an SEM-stub using double-sided adhesive tape. Sample coated with a thin layer of gold under vacuum. Sample stub kept in SEM chamber and operated at 10 kV. The sample was run, and images at different magnifications were captured.

DSC Studies of Optimized EN Loaded TFs: A thermal analysis of optimized EN loaded transfersonal formulation was performed. DSC was performed to observe any physicochemical interaction between drug and excipients.

Evaluation of EN Loaded Transfersomal Gel:

Physical Appearance: The prepared gel is checked visually to know its appearance and color.

Homogeneity: The prepared gel was tested for homogeneity by visual inspection after the gel has set in the container. The gel was tested for its appearance and presence of any aggregates.

Spreadability: The spreadability of gel formulation was determined by placing 1 gm of gel between horizontal plates for 1 min. Spread ability was measured by measuring the diameter of gel spread over 1 min [27].

pH Measurement: pH measurement of optimized ENloaded transfersomal gel was carried out using digital pH meter.

Viscosity: A Brookfield Programmable DV-II + Viscometer was used to measure the viscosity (in cps) of gel formulation. The spindle was rotated at 10 r/min and the sample was allowed to settle over 30 min at the temperature 25 °C before the measurements were taken [28].

***In-vitro* Drug Release:** The *In-vitro* drug release of the gel was performed by using a vertical Franz diffusion cell. The same procedure is followed, which was described earlier while carrying out In-vitro drug release of the TFs. The difference is only 1 gm of the gel was placed in the donor compartment, and study was carried out for 8 h.

The *In-vitro* Antifungal Activity: *In-vitro* anti-fungal activity was determined using the cup plate technique. The media was prepared by dissolving 10 gm of peptone, 20 gm of agar, and 40 gm of dextrose powder in per liter of distilled water and was sterilized using autoclave at 121 °C for 20 min. The plates were sterilized in a hot air oven at 160 °C for 60 min.

Candida albicans culture was introduced into the plate, and 20 ml of sterile sabouraud dextrose agar was poured into the plate. The plate was agitated carefully to allow for both an even distribution of the sabouraud dextrose agar and test organism in the plate. The plates were allowed to harden, and three cups, of each 6 mm in diameter, were bored in the medium.

The marketed product (Daktarin® Gel 2% w/w) was used as a standard. The standard, pure EN drug and optimized EN loaded transfersomal gel (test) were taken into cups. The plate was incubated for 48 h at 25 °C. The zones of inhibition were measured in mm after 48 h for the test, standard and pure EN drug [29, 30].

Stability Study: For stability study, TFs solution and optimized EN loaded transfersomal gel was stored at 25°C/60% RH for long-term stability, 40 °C / 75% RH for accelerated stability, and 5 ± 3 °C in refrigerator condition. Stability studies were carried out for three months [31, 32].

RESULT AND DISCUSSION:

Solubility Studies of EN in Different Solvents: Solubility of EN in different solvents was determined and tabulated in **Table 3**.

TABLE 3: SOLUBILITY OF EN IN DIFFERENT SOLVENTS

S. no.	Solvents	Solubility
1	Distilled water	Very Slightly Soluble
2	Ethanol	Slightly Soluble
3	Methanol	Soluble
4	Chloroform	Sparingly Soluble
5	Phosphate buffer 7.4	Sparingly Soluble

Screening of Edge Activators: The Solubility studies of EN in different edge activators were determined by the shake flask method. The edge activators having the highest solubility were selected for optimization and formulation of TFs.

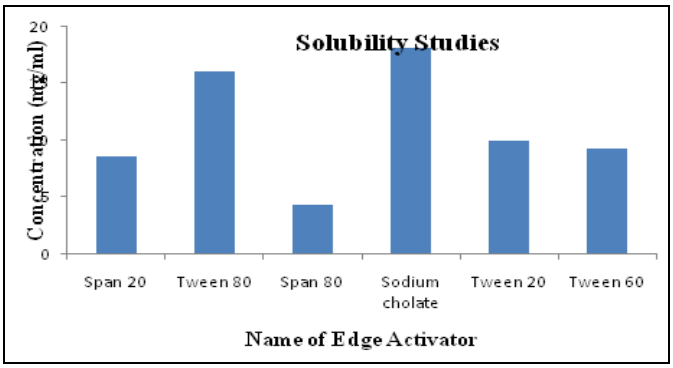

FIG. 1: SOLUBILITY STUDIES OF EN IN DIFFERENT EDGE ACTIVATORS

TFs are highly efficient edge activators based on ultra-flexible vesicles, so the selection of proper edge activators plays an important role in the formulation process.

EN was found to be more soluble in Tween 80 and Sodium cholate; hence they were selected for formulation (The type of edge activator and its concentration effects on the vesicle size, % entrapment efficiency and % drug release). Therefore the combination of two surfactants at different concentrations was selected for optimization and formulation of TFs.

Drug-Excipient Compatibility Studies: Drug-Excipient Compatibility studies were performed using Fourier Transform Infrared Spectroscopy (FTIR). The FTIR spectra showed the prominent peaks of the various bonds between the groups present in the EN chemical structure.

The prominent peaks of various groups are N-H stretching at 3174 cm^{-1}, C-Cl stretching at 787 cm-1, C-N stretching at 1330 cm^{-1}, C-H stretching for aromatic at 2964 cm^{-1} , C-O stretching for ether at 1089.58 cm^{-1}, -NO_2 stretching at 1480 cm^{-1}, -C-C-

bond at 1547 cm^{-1}. All the characteristics peaks of EN were present in all spectra at the respective wavelength when compared with standard frequencies of functional groups.

This confirmed that there was no chemical interaction between the EN and other excipients or confirmed that the EN is present in pure and unchanged form in the physical mixture of excipients.

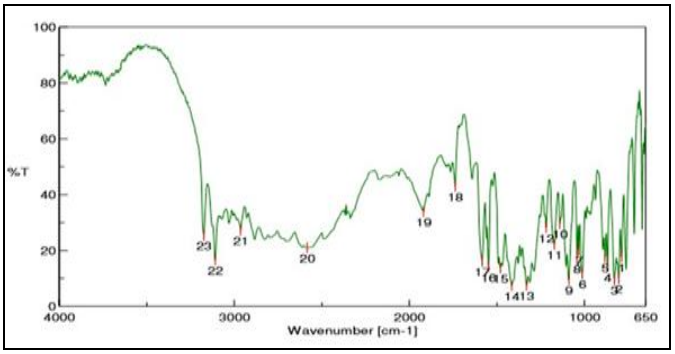

FIG. 2: FTIR SPECTRA OF PURE EN

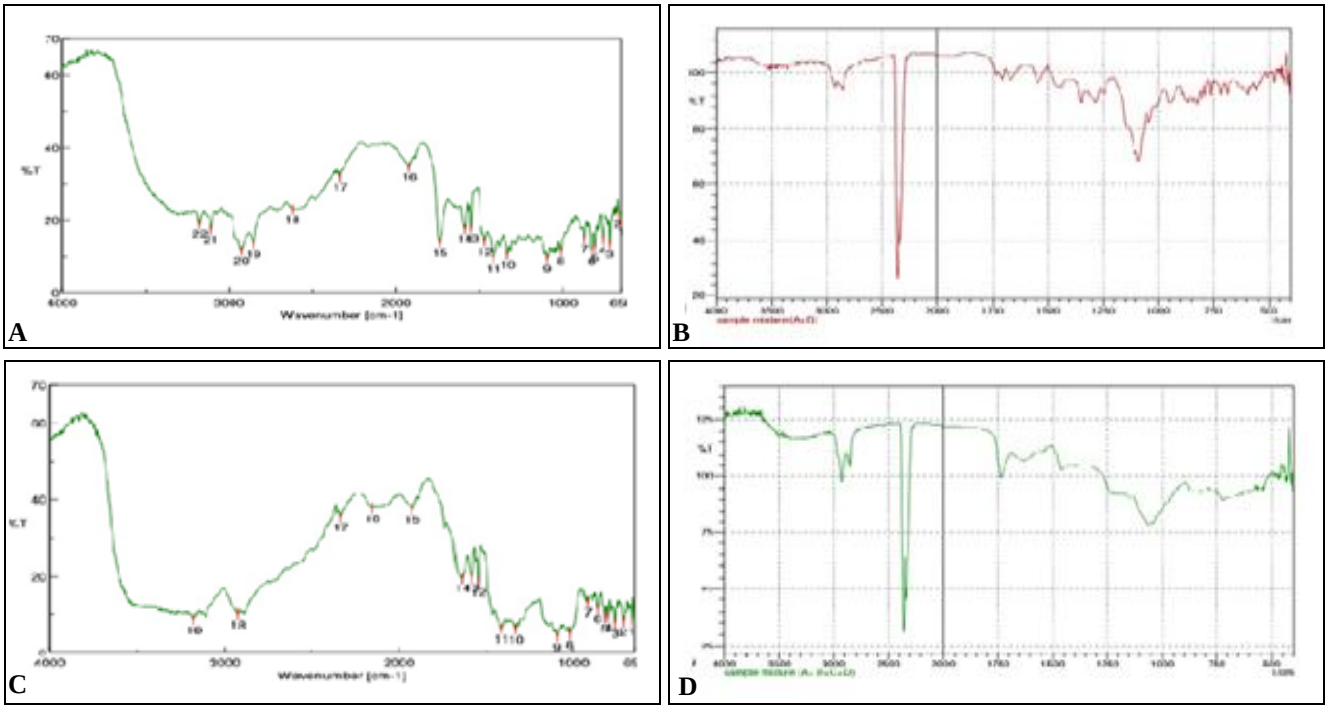

FIG. 3: FTIR SPECTRA (A) EN + SOYA LECITHIN (B) EN + SODIUM CHOLATE (C) EN + TWEEN 80 + (D) EN + PHYSICAL MIXTURE OF EXCIPIENTS

X-ray Diffraction Study: The results of XRD analysis are shown in **Fig. 4.** The XRD pattern of plain EN revealed that the presence of strong diffraction pattern peaks at 0.44800 °C (116 Counts), 0.39770 °C (113 Counts), and 0.37550 °C (95 Counts), indicating the crystalline nature of the EN.

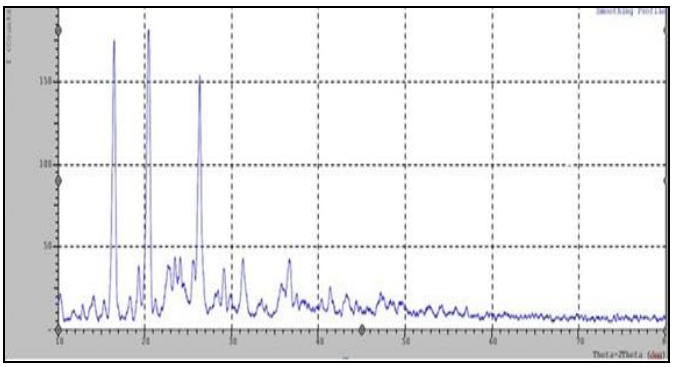

FIG. 4: XRD OF EN

Effect of Concentration of Phospholipid and Concentration of Edge Activators on Particle Size: Vesicle size is an important parameter governing transdermal skin permeation **Fig. 5.** illustrates 3D response surface plot for particle size. ANOVA test for observed data of vesicle size indicated that it followed the quadratic model. The following polynomial equation was projected by the model for particle size.

Y_1 (particle size) = + 0.4100-0.0125* A - 0.1074* B-0.1599* C - 0.0125* AB + 0.0075* AC 0.0178* BC + 0.0089* A^2 + 0.0486* B^2 + 0.03368* C^2

Where A is the concentration of phospholipid, B is the concentration of sodium cholate, and C is the concentration of Tween 80. It was seen that the particle size was significantly affected by variables A, B and C. Concentration of phospholipid had a

positive effect on the particle size. It was seen that the particle size increased with increasing the phospholipid concentration significantly with the previous findings. The largest particle size was observed in the concentration of positive one level of A, while the small particle size was observed in the negative one level of A. It might be due to the increase in phospholipid concentration; more phospholipids molecules will be distributed in the lipid bilayer, causing an increase in the TFs to mean diameter or due to the insufficient drug molecules for the complete degree of association with the phospholipids.

It was observed that the particle size decreased with increasing the sodium cholate concentration. It might be due to the higher concentration of edge activator allow better stabilization of the smaller lipid vesicles and thus prevent them from aggregation into larger droplets [33].

Here, it was seen that the concentration of Tween 80 had a positive effect on particle size with sodium cholate *i.e.,* Tween 80 on its negative level, then the particle size was increased while smaller particle size on its positive level.

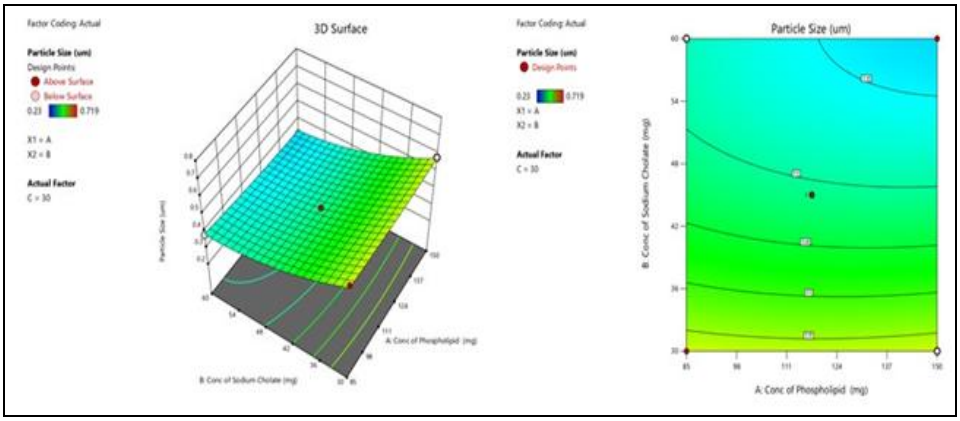

FIG. 5: 3D RESPONSE SURFACE PLOT AND CONTOUR PLOT FOR THE EFFECT OF CONCENTRATION OF PHOSPHOLIPID AND THE CONCENTRATION OF EDGE ACTIVATORS ON PARTICLE SIZE

Effect of Concentration of Phospholipids and Concentration of Edge Activators on % Entrapment Efficiency: The following polynomial equation was projected by the model for % Entrapment Efficiency.

Y_2 (% Entrapment efficiency) = + 57.33 + 4.19*A - 3.26*B - 3.26*C - 3.66*AB - 4.51*AC - 16.04*BC -5.64*A^2 + 10.15*B^2 - 13.03*C^2.

The model was found to be significant (F-value 75.95, p-value = < 0.0001). The predicted R-squared value is in reasonable agreement with the adjusted R-squared (0.8378 and 0.9768, respectively). The effect of different independent variables on the % entrapment efficiency is illustrated in **Fig. 6.**

The entrapment efficiency of all formulated TFs was found in the range 31.5 ± 0.17% to 75 ± 0.12%. The entrapment efficiency was found to be directly proportional to the concentration of phospholipid due to the phospholipids acts as solubilizing agents for highly lipophilic drugs.

It was observed that entrapment efficiency increases with an increase in sodium cholate concentration. The entrapment efficiency in TFs formulations increases due to the incorporation of the edge activators inside the structure of vesicles.

Another reason is sodium cholate is a salt of bile acids of a steroidal amphiphilic chemical structure that can form micelles consisting of 2-12 monomer units [34]. It was clearly seen that the Tween 80 had a positive effect on entrapment efficiency at a negative level of sodium cholate concentration.

For this reason increase in entrapment efficiency was seen only when Tween 80 or sodium cholate concentration was used in formulation on their opposite levels.

Effect of Concentration of Phospholipids and Concentration of Edge Activators on % Drug Release: ANOVA test for observed data of drug release indicates that the quadratic model was significant and fitting for the data.

The model was found to be significant (F-value 1027.61, p-value = < 0.0001). The polynomial equation in terms of coded factors is given as follows:

Drug release (Y_3) = + 89.86 -2.87*A + 1.24*B + 1.63*C - 0.1625*AB + 0.1500*AC + 0.6525*BC + 0.8425*A^2 + 0.8100*B^2 - 0.4175*C^2

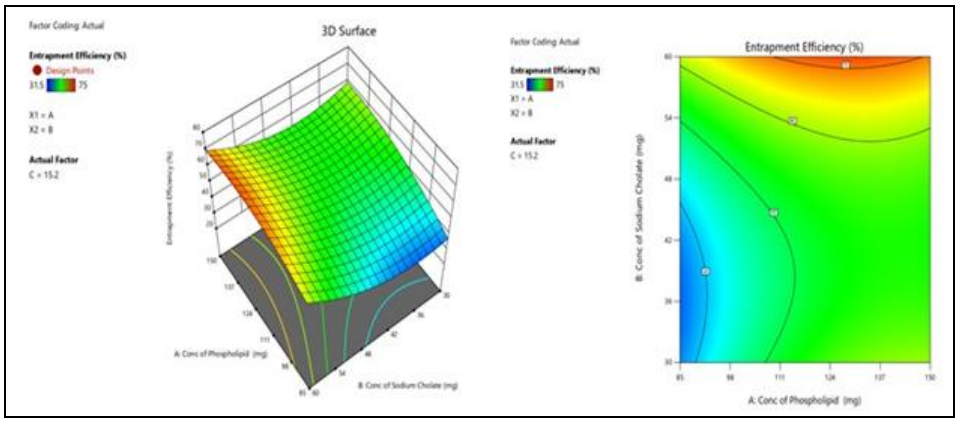

FIG. 6: 3D RESPONSE SURFACE PLOT AND CONTOUR PLOT FOR THE EFFECT OF CONCENTRATION OF PHOSPHOLIPID AND CONCENTRATION OF EDGE ACTIVATORS ON % ENTRAPMENT EFFICIENCY

The drug release was found to be in between 87.94 ± 0.26 to 95.9 ± 0.18% shown in **Fig. 8.** Results showed that the phospholipid concentration had an inverse effect on drug release. This may be due to the higher phospholipid concentration, then harder the vesicular structure, which tightly entraps drug molecule in the structure and hinders the drug release into the dissolution media [34]. It was observed that an increase in sodium cholate concentration then increases in the drug release. The sodium cholate concentration at a positive level (60 mg) showed 95.9 ± 0.18% drug releases. This is due to the increase in edge activator concentration, which increases the hydrophilicity of the vesicles, thereby promoting drug release into dissolution medium. It was seen that concentrations of edge activator had a synergistic effect on the % drug release.

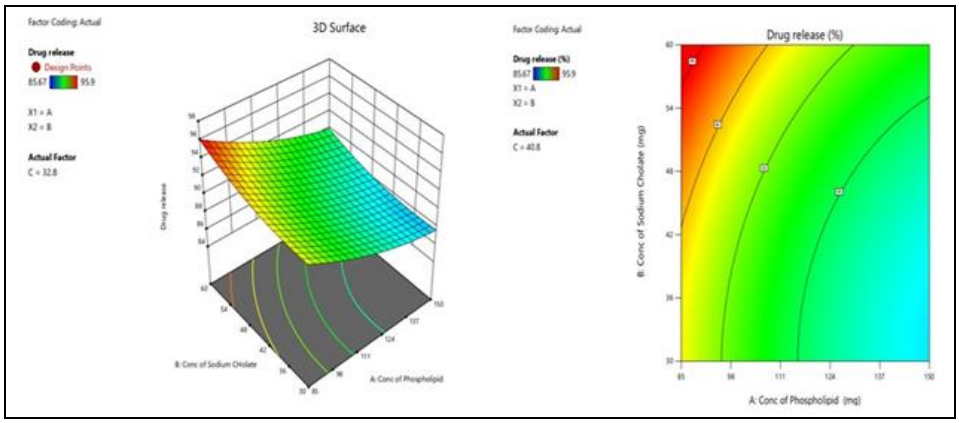

FIG. 7: 3D RESPONSE SURFACE PLOT AND CONTOUR PLOT FOR THE EFFECT OF CONCENTRATION OF PHOSPHOLIPID AND CONCENTRATION OF EDGE ACTIVATORS ON % DRUG RELEASE

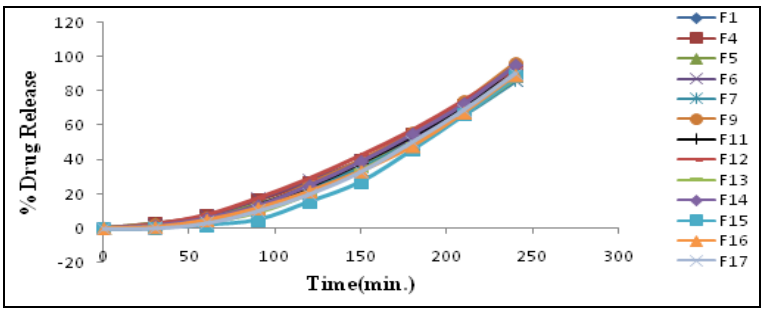

FIG. 8: *IN-VITRO* DRUG RELEASE PROFILE OF EN FROM FORMULATED TFS (BATCH F1 TO F17)

Optimization of EN Loaded TFs Using Design-Expert Software: Optimization of all EN loaded TFs formulations were done by evaluating the results of dependent variables (particle size, % entrapment efficiency, % drug release) in Design-Expert software. F12 batch of EN loaded TFs formulation was found to be optimized batch having the particle size of 0.59 ± 0.067µm; % entrapment efficiency 56.3 ± 0.1 % and % drug release 92.9 ± 0.65%.

pH of TFs: pH of EN loaded TFs formulation was found to be in between 3.67 ± 0.07 to 5.2 ± 0.02.

Particle Size and Zeta Potential: Zeta potential is an important physical parameter for predicting vesicle stability. The particle size and zeta potential of optimized EN-loaded TFs were found to be 0.656 ± 0.105 µm and -17.03 ± 2.32 mV, respectively, as shown in **Fig. 9.**

The EN-loaded TFs showed a negative zeta potential due to the negative charge of the sodium cholate edge activator. A phospholipid has a zwitterionic behavior which also contributed to the negative surface charge of the TFs [35].

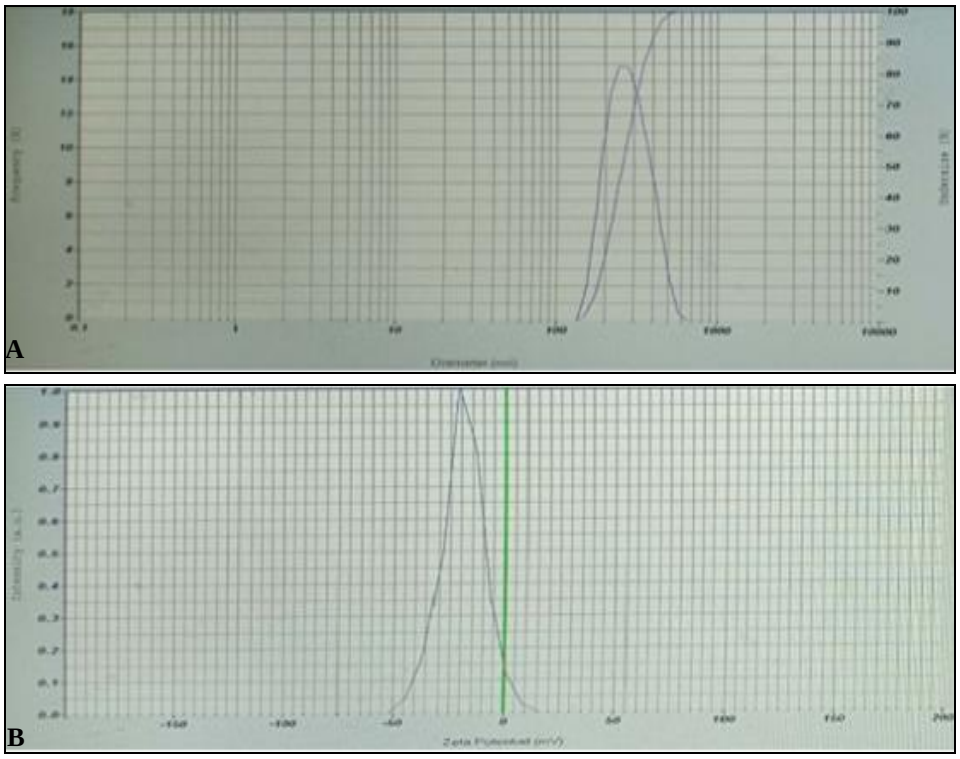

FIG. 9: (A) PARTICLE SIZE (B) ZETA POTENTIAL OF EN LOADED TFs

Surface Morphology of TFs: The Surface morphology characterization of the optimized EN loaded TFs was observed using Scanning Electron Microscopy (Carl Zeiss-Supra 5).

The images showed that the TFs vesicles were in a spherical shape and smooth surface, which indicates a uniform lipid layer. The SEM images of EN-loaded TFs vesicles are shown in **Fig. 10.**

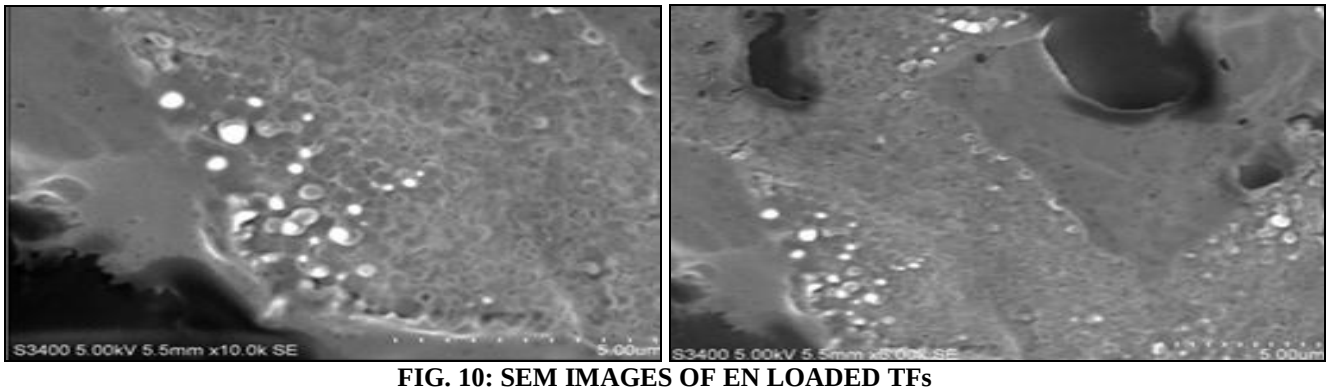

FIG. 10: SEM IMAGES OF EN LOADED TFs

DSC Study: DSC is one of the most broadly used calorimeter techniques to indicate solubility. DSC thermograms of EN and optimized EN loaded TFs are given below in **Fig. 11**, **12**. DSC thermogram of pure EN and EN loaded TFs showed an endothermic peak at 169.27 °C, 102.64 °C, respectively.

Endothermic peaks display the melting point of EN. The shifting of the endothermic peak of EN in EN-loaded TFs recommended the presence of the drug in a more soluble and amorphous form. The change in the melting point of EN could be due to the embedding of EN with excipients, and that resulted in a decrease in crystalline nature [36].

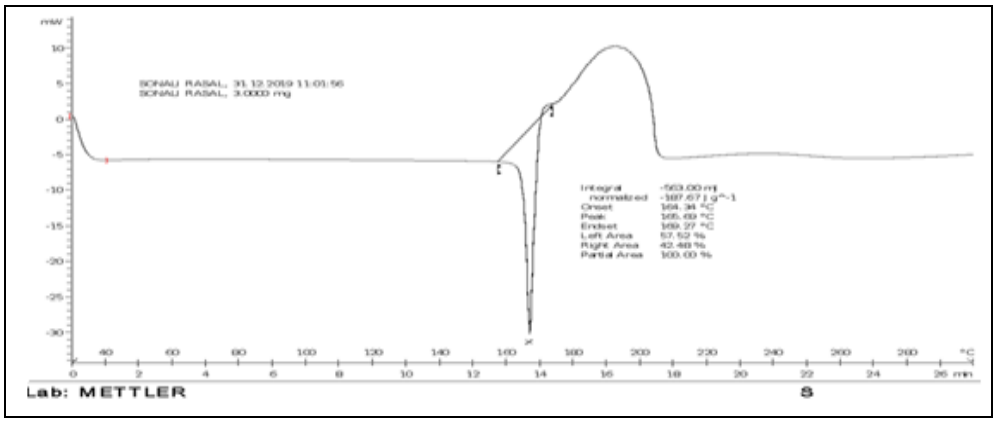

FIG. 11: DSC THERMOGRAM OF PLAIN EN

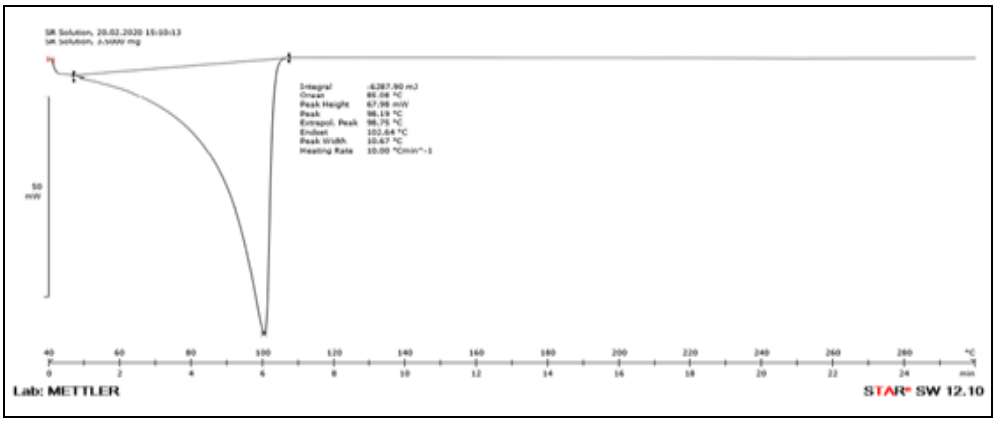

FIG. 12: DSC THERMOGRAM OF OPTIMIZED EN LOADED TFS

Evaluation Results of EN Loaded Transfersomal Gel:

Physical Appearance: The prepared optimized transfersomal gel checked visually for knowing its appearance and color. The gel has a smooth appearance and white in color.

pH: pH of optimized transfersomal gel formulation was found to be 4.67 ± 0.07, which is acceptable to avoid the risk of irritation upon application on the skin.

Spreadability: Optimized transfersomal gel formulation showed good spreadability with a diameter of was 4.1 ± 0.11 cm in 1 min

Viscosity: The viscosity of optimized transfersomal gel was found to be 2093 ± 40.82 cps.

***In-vitro* Drug Release of Transfersomal Gel:** The % drug release of EN loaded transfersomal gel was found to be 96.7 ± 0.7 % after 7 h.

***In-vitro* Antifungal Activity:** The EN loaded transfersomal gel was further evaluated for antifungal activity against *Candida albicans* by cup plate method. The results of the zone of inhibition of EN loaded transfersomal gel were compared with the marketed (Daktarin® Gel 2% w/w) product. The antifungal activity was measured in terms of the diameter of the zone of inhibition. These results demonstrated that the antifungal activity of the EN transfersomal gel was significantly higher than the marketed product. it was found that the antifungal activity of the EN transfersomal gel (18.46 ± 0.41 mm) was greater than that of Daktarin® Gel 2% w/w (15.03 ± 0.04

mm), which may be attributed to the high flexibility of TFs, facilitating its penetration through the cell walls of Candida albicans fungi, and the inhibition of ergosterol biosynthesis, which results in fungal cell membrane lysis and cell death [37].

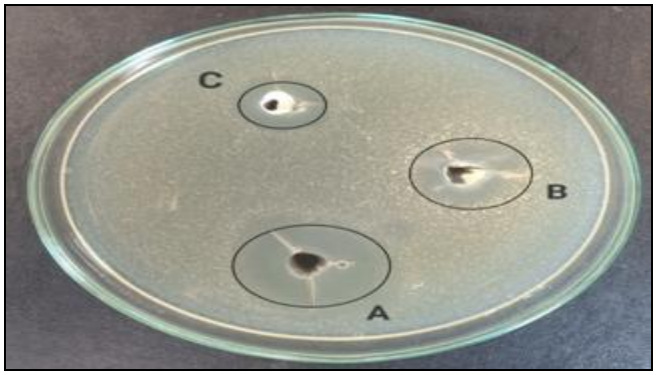

FIG. 13: ANTIFUNGAL ACTIVITY STUDY SHOWING THAT ZONE OF INHIBITION FOR (A) PURE EN DRUG (B) EN LOADED TRANSFERSOMAL GEL (C) MARKETED PRODUCT (DAKTARIN® GEL 2% w/w)

Stability Studies for EN TFs and EN Loaded Transfersomal Gel: The stability studies over different storage conditions of 5 ± 3 °C, 25 °C / 60% RH for optimized EN TFs, and 25°C /60%RH, 40 °C /75% RH for EN loaded transfersomal gel as per ICH guidelines were carried out. Stability studies were carried out for three months. Physical and chemical changes were checked for TFs and transfersomal gel for different time intervals (1 month, 2 months, and 3 months). The physical parameters were checked in terms of visual examination; whereas chemical changes were checked in terms of particle size, % entrapment efficiency, % drug release for TFs and spreadability, % drug release for transfersomal gel. The results showed that there was no significant change found in stability stored at 5 ± 3 °C, 25 °C / 60% RH, and 40 ºC / 75% RH after three months. The results are stated in bellow **Table 4, 5**.

TABLE 4: STABILITY STUDIES OF EN LOADED TFs

Months	Temperature Condition (°C)	Optimized EN loaded TFs		
		Particle Size (um)	% Entrapment Efficiency	% Drug Release
1	5 ± 3 °C	0.600±0.06	56.1±0.05	92.80±0.04
	25 °C / 60% RH	0.610±0.06	56.2±0.05	92.91±0.06
2	5 ± 3 °C	0.611±0.08	55.8±0.04	92.62±0.05
	25 °C / 60% RH	0.627±0.06	55.7±0.05	91.80±0.06
3	5 ± 3 °C	0.623±0.04	55.2±0.06	91.31±0.82
	25 °C / 60% RH	0.641±0.08	54.8±0.07	91.54±0.11

TABLE 5: STABILITY STUDIES OF EN LOADED TRANSFERSOMAL GEL

Months	Temperature Condition (°C)	EN loaded Transfersomal Gel	
		Spreadability (cm)	% Drug Release
1	25°C/60%RH	5.99±0.11	95.90±0.07
	40°C/75% RH	6.00±0.14	96.30±0.05
2	25°C/60% RH	5.78±0.09	94.89±0.08
	40°C/75% RH	5.81±0.12	96.02±0.04
3	25°C/60% RH	5.75±0.18	94.68±0.11
	40°C/75% RH	5.85±0.09	95.05±0.83

CONCLUSION: In this present work, EN loaded TFs were successfully formulated by the thin-film hydration method. The formulations were optimized by the three factors and two levels Box-Behnken design using Design-Expert software.

Results proved that particle size, % EE, % drug release were mainly affected by the concentration of phospholipid and concentrations of edge activators in the formulations. From this study, it was concluded that the Box-Behnken design had the ability to obtain an optimized formula of EN-loaded TFs, with small particle size, high %EE, and % drug release. F12 batch of EN loaded TFs formulation was found to be optimized batch having the particle size of 0.59 ± 0.067 µm; % entrapment efficiency 56.3 ± 0.1 % and % drug release 92.9 ± 0.65%.

EN Transfersomes may be used as alternative carriers for transdermal drug delivery system because EN loaded transfersomal gel has the ability to overcome the barrier properties of the skin and increase antifungal activity, as compared with the marketed product (Daktarin® Gel 2% w/w).

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