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A STATISTICAL APPROACH TO DEVELOPMENT OF SUSTAINED RELEASE PRONIOSOMAL MICONAZOLE NITRATE GEL

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Keywords:

Miconazole nitrate, Sustained release Proniosomal gel, Span 60, Cholesterol, 3^2 full factorial design

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ABSTRACT: Miconazole nitrate is a broad-spectrum antifungal agent, and it is used for the treatment of superficial fungal infections. It has low skin permeability. Therefore, the aim of this study was to prepare proniosomal 2% miconazole nitrate sustained release to a treat deep-seated fungal infections. The different batches of proniosomal 2% miconazole nitrate gel were prepared by the conservation phase separation method using different non-ionic surfactants and Cholesterol. Preliminary trial batches were formulated and evaluated for different evaluation parameters like pH, viscosity, % entrapment efficiency, % drug content, and *in-vitro* drug release study. A 3^2 full factorial design was used to check the effect of Span 60 (X_1) and Cholesterol (X_1) on % entrapment efficiency (EE) and % drug release at 20 h (Q_{20}). Multiple linear regression analysis, ANOVA, and graphical representation of the influence factor by 3D response surface plots were performed using Design Expert 9. Checkpoint batch was prepared to validate the evolved model. Optimized batch was found to be stable, and it showed release 94.76% in 24 h. It followed the non-fickian diffusion and showed flux 289 μg/cm^2/h in the *ex-vivo* study. SEM revealed that the noisome formed were spherical in shape. Therefore, proniosomal 2% miconazole nitrate gel has the ability to penetrate the skin and give the effect for a long time.

INTRODUCTION: Miconazole nitrate is a broad-spectrum antifungal agent of the imidazole group. It is primarily used for topical treatment for superficial mycoses. Miconazole nitrates have a poor skin penetration [1]. Therefore, deep-seated fungal infections can not be treated by conventional transdermal formulation. Penetration enhancement of transdermal formulation is mainly based on the usage of colloidal carriers.

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A number of vesicle systems such as liposomes, niosomes, ethosomes, emulsomes, and transfersomes have been developed [2, 3]. The colloidal carrier has distinct advantages over conventional drug delivery as it acts as drug-containing reservoirs, modification of the particle composition and surface can adjust the release rate to the target site [4].

The vesicular carrier, such as niosomes has distinct advantage over conventional dosage forms because these particles can act as a drug reservoir. Proniosomal gels are structurally similar to liposome and niosome having a bilayer; however, the materials used to prepare proniosomes to make them more stable and offer many more advantages over liposome and noisome.

Proniosomes reduce the physical stability problems of niosomes such as leaking, fusion, and aggregation. Proniosomes is providing additional convenience in transportation, distribution, storage, and dosing. It can entrap both lipophilic and hydrophilic drugs either in an aqueous layer or vesicular membrane. Proniosomes are and present low toxicity because of their nonionic nature [5, 6]. Therefore, our study focused on the development and evaluation of proniosomal is 2% miconazole nitrate gels use in the treatment of cutaneous diseases for a long time.

MATERIALS AND METHODS:

Materials: Miconazole nitrate was kindly supplied as gift samples by Mepro Pharmaceutical Ltd., Wadhwan, India. Cholesterol and Triethanol-amine were supplied as gift samples from Finar Chemicals, Ahmadabad, India. Span (20, 40, 60, 80) were purchased from Astron Chemicals, Ahmedabad, India. Carbopol 934 was purchased from Seva Fine Chemicals, Ahmadabad, India.

Methods:

Drug-Excipients Compatibility Study: Drug-Excipients interaction plays a vital role in achieving the stability of the drug in the dosage form. Fourier transform infrared spectroscopy (FT-IR) was used to study the physical and chemical interactions between drugs and excipients. FT-IR spectra of miconazole nitrate, span 60, and physical mixture of miconazole nitrate: span 60 was recorded using the KBr mixing method on FT-IR instrument. (FT-IR 1700, Shimadzu, Kyoto, Japan). Differential scanning calorimetry (DSC) method was also performed. The DSC results provided both qualitative and quantitative information about the physicochemical state of the drug present in formulations. (DSC TA-60). The analysis was performed at a rate of 10 °C per min from 50 °C to 350 °C under a nitrogen flow of 20 ml/min [7, 8].

Preparation of Proniosomal 2% Miconazole Nitrate Gel: Proniosomal 2% miconazole nitrate gel was prepared by using the Coacervation phase separation method. Different surfactant: cholesterol ratio was taken in a clean wide-mouthed glass vial which was containing 100 mg miconazole nitrate (2%) and 5 ml ethanol. To prevent the loss of solvent, the open end of the glass vial was covered with a lid. The mixture was warmed over a water bath at 60-70 °C for 5 min and add phosphate buffer 7.4. Then, this mixture was warmed in a water bath for 2 min and allowed to cool down at room temperature till the dispersion gets converted to proniosomal gel. Proniosomal gel was mixed with 1% carbopol gel in a 1:1 ratio. The gel was preserved in a glass container in the dark for characterization [9, 10].

TABLE 1: FORMULATION OF PRELIMINARY SCREENING OF PRONIOSOMAL 2% MICONAZOLE NITRATE GEL

Batch code	Span 20 (mg)	Span 40 (mg)	Span 60 (mg)	Span 80 (mg)	Cholesterol (mg)
T1	1600	-	-	-	200
T2	-	1600	-	-	200
T3	-	-	1600	-	200
T4	-	-	-	1600	200
T5	1800	-	-	-	200
T6	-	1800	-	-	200
T7	-	-	1800	-	200
T8	-	-	-	1800	200
T9	1800	-	-	-	400
T10	-	1800	-	-	400
T11	-	-	1800	-	400
T12	-	-	-	1800	400

Preliminary Screening of Proniosomal 2% Miconazole Nitrate Gel: Preliminary study of proniosomal miconazole nitrate gel was carried out to check the effect of various grades of span with different ratio of cholesterol. Each batch contained 2% Miconazole Nitrate and 5 ml ethanol. Briefly, in batch T1 to T4, span: cholesterol ratio was taken 8:1 and batch T5 to T8 was containing span: cholesterol ratio 9:1. Batch T9 to T12 contained span: cholesterol ratio 9:2 as shown in **Table 1** [11, 12].

Evaluation of Proniosomal 2% Miconazole Nitrate gel: The pH of the gel was determined by using pH meter. The viscosity of the formulated proniosomal gel was determined using Brookfield

viscometer. In optical microscopy method, small amounts of proniosomal gel are spread on a glass slide and examined for the structure and presence of the vesicles of insoluble drug crystals using an ordinary light microscope with varied magnification power 40X.

Entrapment Efficiency: Entrapment efficiency of proniosomal gel was evaluated by the dialysis method. In this, method the amount of entrapped drug can be obtained by subtracting the amount of free drug from total drug incorporated.

$$\text{Entrapment efficiency} = C_t - C_f / C_t \times 100$$

Where, C_t = total concentration of drug, C_f = concentration of free drug.

The drug content of proniosomal gel was determined by 1 gm gel dissolved in 100 ml phosphate buffer pH 7.4. From this 1 ml solution was withdrawn and diluted up to 10 ml phosphate buffer pH 7.4. Then, the absorbance was measured by U.V spectrophotometer at 272.5 nm [13].

***In-vitro* Release Studies:** An *in-vitro* release study of proniosomal 2% miconazole nitrate gel was performed using Franz-diffusion cell. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A 1 gm of proniosomal gel was placed on one side of the dialysis membrane and receptor medium containing 25 ml of phosphate buffer pH 7.4. The temperature of the receptor medium maintained at 37 ± 0.5 ºC and the medium was agitated at 100 rpm speed using a magnetic stirrer. Aliquots of 2 ml sample were withdrawn periodically and replaced with equal volume to maintain the volume constant of the receptor's phase. The collected samples were analyzed for the drug-containing at 272.5 nm absorbance using the UV spectrophotometer.

Vesicular Size: Vesicular size of the optimized formulation of proniosomal gel is to be evaluated by Scanning electron microscopy (SEM). For that, 0.2 gm of gel in a glass tube is diluted with 10 ml of phosphate buffer pH 7.4.

Zeta Potential: Zeta potential was done for determining the colloidal properties of the prepared proniosomal gel. The optimized formulation was diluted with phosphate buffer pH 7.4, and the proniosomal were converted to the niosomes, these niosomes were further used for the zeta potential determination.

Kinetic Modelling of Dissolution Data: In order to understand the kinetics and release mechanisms of proniosomal 2% miconazole nitrate gel, the result of *in-vitro* drug release study of formulation was fitted with various kinetic models like zero order, first order, Higuchi model and Korsmeyer Peppas model.

The linearity of the plots was obtained from the value of the regression coefficient (R). The model with the highest linearity (R-value approaches unity) was chosen as the Best-fit kinetic model [14, 15].

***Ex-vivo* Drug Permeation Study:** The permeation of drugs from proniosomal 2% miconazole nitrate gel was determined by using franz diffusion cell. The Wistar rat skin was mounted on the receptor compartment with a stratum corneum side facing upwards into donor compartment. The top of the diffusion cell was covered with paraffin paper. The donor compartment was filled with the proniosomal 2% miconazole nitrate gel. The receptor compartment was maintained at 37 ± 0.5 ºC and stirred by a magnetic bar at 600 rpm. Aliquots of 2 ml samples were withdrawn periodically and replaced with equal volume to maintain the volume constant of the receptor's phase. The collected samples were analyzed for the drug-containing at 272.5 nm absorbance using the UV spectrophotometer.

Microbial Antifungal Test: The microbiological activity of the optimized proniosomal gel was carried out using a cup plate technique. *Candida albicans* was used as an indicator strain. The culture medium selected for this purpose is nutrient Agar, and the final pH of the medium was kept at 5.6 ± 0.2 to retard the growth of unlike organisms. The medium was sterilized using an autoclave at 121 ºC for 20 min. The freshly prepared culture was used for inoculum preparation, which was prepared by suspending 1-2 colonies in tubes containing media and 10 ml of 0.9% w/v NaCl solution. The inoculum was spread over the surface of media after appropriate solidification proniosomal were applied with the help of borer.

The complete experiment was carried out in a sterile area. Finally, the plate was incubated at 37 ± 2 ºC for 24 h in the reverse position. The zone of inhibition was calculated in millimeter of diameter [16].

Skin Irritation Study: The study was conducted in accordance with the principles of Laboratory Animal Care and was approved by the Institutional Ethics Committee**.** The dorsal side of the rats was shaved 12 h before starting the experiments except in the control group. Wistar rats were divided into three groups (*n* = 3 per group) and were treated once daily over a period up to 24 h as the following groups. Group 1 consider was Control, Group 2 was applied with 0.8% v/v aqueous formalin solution, and Group 3 was treated with proniosomal 2% miconazole nitrate gel (CCPR/IAEC/29/ JAN2014).

The visual observation, such as scaling, lesion & erythema was carried out at a regular time interval. The symptom of erythema was graded as 3= severe, 2= moderate, 1= mild, and 0= absent. The scaling was graded as 1= present, 0=absent. On the 8th day, the application sites were evaluated visually for erythematic and edema.

Accelerated Stress Stability Study: Stability studies were done as per ICH guidelines. The optimized formulation was kept in amber color glass bottle and stored at 60 ± 0.5 ºC and 75 %RH for a period of two weeks. After the period of two weeks, the gel was tested for pH, Viscosity, Entrapment efficiency, drug content, and *in-vitro* release profile. The dissolution profile of products were compared using a f_2 which is calculated from the following formula,

$$f_2 = 50 \log [\{1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\}^{-0.5} x100]$$

Where log is the logarithm to the base 10, n is the number of time points, ∑ is a summation over all time points, R_t is the mean dissolution value of the reference profile at time t and T_t is the mean dissolution value of the test profile at the same time point. The USFDA draft guidance document contains more information on the similarity factor (f_2). The value of similarity factor (f_2) between 50 and 100 suggests that the two dissolution profiles are similar [17, 18].

Optimization of Variables using Full Factorial Design: A 3^2 randomized full factorial design was used in the present study. In this design, two independent factors were evaluated, each at three levels, and experimental trials were performed for all 9 possible combinations. The concentration of Span 60 (X_1) and concentration of Cholesterol (X_2) was chosen as independent variables in 3^2 full factorial design, while % Entrapment efficiency and % Cumulative drug release at 20 h (Q_{20}) were taken as dependent variables. Multiple linear regression analysis, ANOVA, and graphical representation of the influence of factor by contour plots were performed using Design Expert 9. The experimental runs and measured responses of 3^2 full factorial design batches of proniosomal 2% miconazole nitrate gel were depleted in **Table 2** [19].

RESULTS AND DISCUSSION:

Drug Excipients Compatibility Study: Fourier transform infrared spectroscopy (FT-IR) was used to study the physical and chemical interactions between drugs and excipients. FT-IR spectra of miconazole nitrate, span 60, and mixture of Miconazole nitrate and Span 60 **Fig. 1-3** were recorded using KBr mixing method on the FT-IR instrument. The drug exhibited peaks due to C-H, C-C, N-H, C-Cl, and C=C stretching. It was observed that there were no changes in main drug peaks in the IR spectra of the mixture and pure drug. The thermograph of pure miconazole nitrate showed an endothermic melting peak at 186.93º and a mixture peak was observed at 187.66º of miconazole nitrate. DSC study, show that there is no change in drug melting peak after the mixing of miconazole nitrate and Span 60. So we can conclude that drug and excipients are compatible which each other [20].

Evaluation of Preliminary Screening of Proniosomal 2% Miconazole Nitrate Gel: Preliminary trial batches were evaluated like pH, viscosity, % entrapment efficiency, % drug content, and *in-vitro* drug release at 24 h shown in **Table 2.** The result of preliminary trial batches of proniosomal gel showed pH variation range from 5.1 ± 0.08 to 6.8 ± 0.11. All the batches of proniosomal gel showed viscosity and % drug content in the uniform range from 1056 ± 1.04 to 2240 ± 2.18 cps and 93.09 ± 0.59 to 98.72 ± 0.46 respectively. In *in-vitro* drug release study of Batch

T1 to T4 shown fast release and leads to saturation level after a certain period of time as compared to T5 and T8. It may be because formulation T1, T2, T3, and T4 contain less amount of span. Batch T9 to T12 was contained span: cholesterol ratio 9:2, and it shows less release compare to T5 and T8. From the results of the preliminary study Span, 60 was found to be a better candidate, which forms have higher entrapment efficiency and satisfactory drug release. The ratio of span 60: cholesterol (9:1) was found to be the best among the other formulation in comparison to the different evaluation parameters.

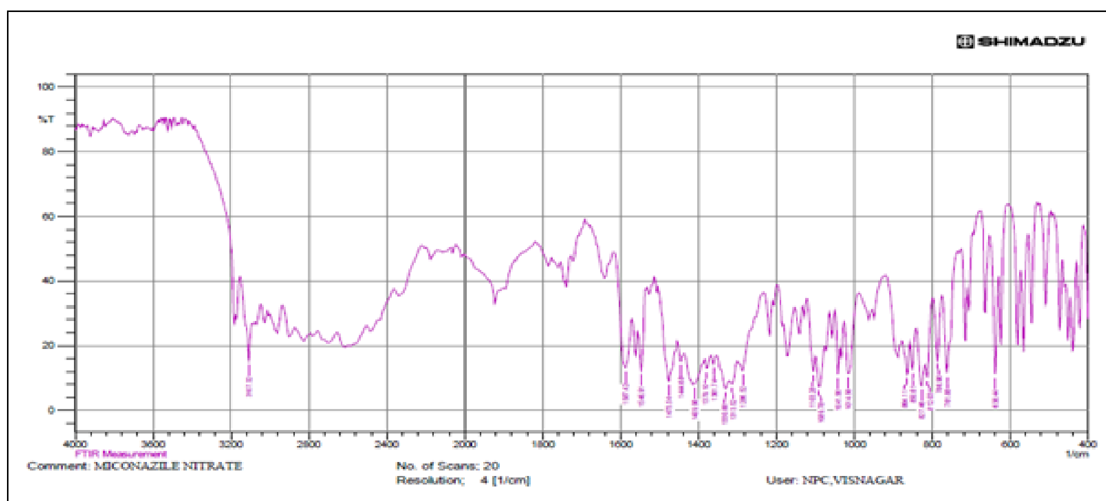

FIG. 1: IR SPECTRA OF MICONAZOLE NITRATE

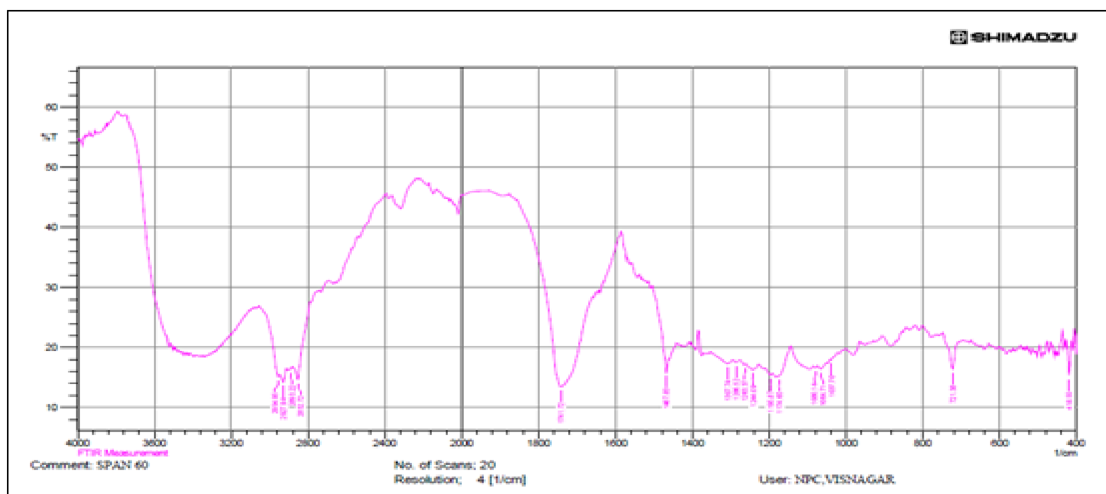

FIG .2: IR SPECTRA OF SPAN 60

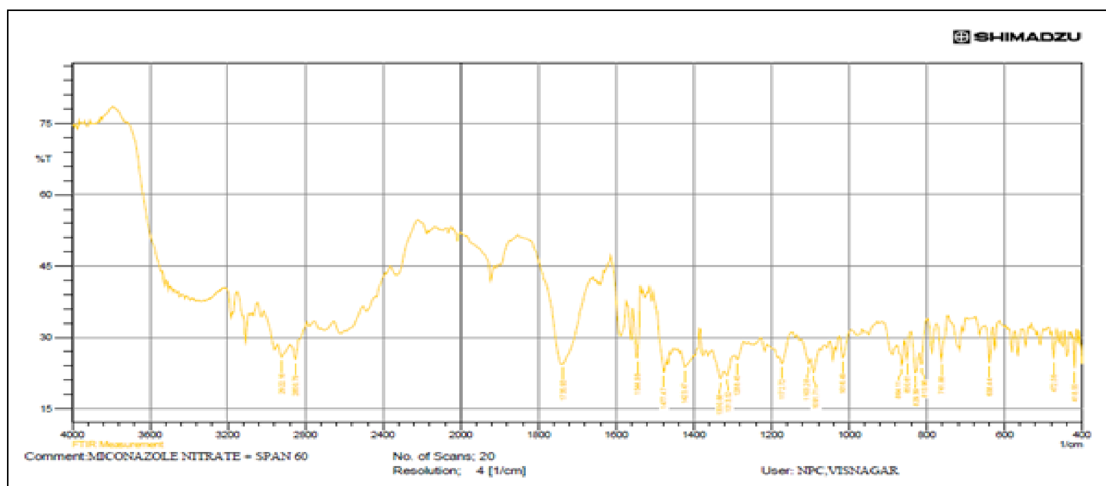

FIG. 3: IR SPECTRA OF MIXTURE OF MICONAZOLE NITRATE AND SPAN 60

TABLE 2: RUNS AND MEASURED RESPONSES OF 3^2 FACTORIAL DESIGN BATCHES

Batch Code	Span 60 (mg) (X_1)	Cholesterol (mg) (X_2)	% Entrapment Efficiency	% Cumulative drug release at 20 h (Q_{20} %)
F1	-1	-1	61.12±1.11	58.86±0.32
F2	0	-1	64.15±0.31	61.92±0.53
F3	1	-1	58.13±1.23	50.63±0.59
F4	-1	0	86.24±0.43	80.43±0.46
F5	0	0	89.31±0.73	86.55±0.38
F6	1	0	76.18±0.52	71.89±0.34
F7	-1	1	82.31±0.48	78.43±0.67
F8	0	1	85.37±0.54	77.49±0.53
F9	1	1	64.37±0.58	63.86±0.51
Factors and the levels in the design				
Independent variables	Low (-1)	Medium (0)	High (1)	
Span 60 (mg) (X_1)	1600	1800	2000	
Cholesterol (mg) (X_2)	100	200	300	

3^2 Full Factorial Design Model Evaluation: A statistical model incorporating interactive and polynomial terms was used to evaluate the responses:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

where Y is the dependent variable, b_o is the arithmetic mean response of the 9 runs, and any bi is the estimated coefficients for the related factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity. The interaction term "X_1X_2" shows how the response changes when the two factors change simultaneously. Evaluation data for proniosomal miconazole nitrate gel were presented in **Table 3**. The fitted equations relating the responses that are, % entrapment efficiency (%EE) and drug release at 20 h (Q_{20}) was to the transformed factor are shown in **Table 4**. The polynomial equations can be used to draw conclusions after considering the magnitude of co-efficient and the mathematical sign it carries (*i.e.*, positive or negative). The results of ANOVA suggested that calculated F values for % Entrapment Efficiency (%EE) and Drug release at 20 h (Q_{20}) are 37.44 and 75.36, respectively, shown in **Table 5.** Tabulated F value was found to be 9.013 at $\alpha = 0.05$. Calculated F values are greater than tabulated for all dependent variables; therefore, factors selected have shown significant effects. From the results of multiple regression analysis, it was found that all factors had a statistically significant influence on all dependent variables as $p < 0.05$ [21].

TABLE 3: EVALUATION OF PRELIMINARY SCREENING OF PRONIOSOMAL MICONAZOLE NITRATE GEL

Batch code	pH	Viscosity (cps)	% Drug content	% Entrapment efficiency	% Percentage drug release at 24 h
T1	5.2±0.05	1840±1.53	95.98±0.43	70.17±0.72	71.96±0.32
T2	6.3±0.14	1056±1.04	94.67±0.87	74.59±0.49	72.87±0.32
T3	5.7±0.04	2240±2.18	93.09±0.59	76.43±1.62	78.85±0.56
T4	5.1±0.08	1440±1.47	96.45±0.38	68.55±0.11	75.41±0.67
T5	6.8±0.11	1920±2.34	96.18±0.53	76.85± 0.69	88.21±0.64
T6	5.9±0.08	2160±1.21	98.60±0.61	82.46±0.58	88.93±0.35
T7	6.4±0.12	2204±2.06	98.72±0.46	88.15±0.52	93.51±0.74
T8	5.6±0.02	2081±2.13	95.28±1.11	87.87±0.83	86.85±0.52
T9	5.8±0.07	1248±1.87	94.45±0.43	87.53±0.87	86.43±0.28
T10	6.1±0.04	1680±1.61	93.72±0.51	84.11±1.12	81.74±0.76
T11	5.9±0.18	2160±2.01	97.36±1.02	85.60±0.68	72.87±0.37
T12	6.0±0.13	1360±1.74	95.87±0.76	83.76±0.93	83.76±0.67

TABLE 4: SUMMARY OF REGRESSION OUTPUT OF FACTORS FOR MEASURED RESPONSES

Responses	Model	Coefficient of regression parameters						
		b_0	b_1	b_2	b_{11}	b_{22}	b_{12}	R^2
% Entrapment efficiency	Full	89.39	8.11	-5.16	-3.74*	-14.67	-8.22	0.984
	Reduced	89.39	8.11	-5.16	-	-14.67	-8.22	
Drug release at 20 hrs (Q_{20})	Full	84.94	8.06	-5.22	-1.59*	-14.42	-7.97	0.992
	Reduced	84.94	8.06	-5.22	-	-14.42	-7.97	

* indicated the coefficient with p >0.05

TABLE 5: RESULTS OF THE ANOVA FOR DEPENDENT VARIABLES

% Entrapment Efficiency (%EE)					
Source of variation	DF	SS	MS	F	P
Regression	5	1175.81	235.16		
Residual	3	18.84	6.28		
Total	8	1194.65	149.33	37.44	0.0066
Drug release at 20 hrs (Q_{20})					
Source of variation	DF	SS	MS	F	P
Regression	5	1106.89	221.38		
Residual	3	8.81	2.94	75.36	0.0024
Total	8	1115.7	139.46		

Full and Reduced Model for % Entrapment Efficiency:

$Y_1 = 89.39 + (8.11 * X_1) – (5.16 * X_2) – (3.74 * X_1X_2) – (14.67 * X_1^2) – (8.22 * X_2^2)$

From the 3D response surface plot **Fig. 4** and the regression co-efficient values of factors, it was concluded that increase in the concentration of the surfactant from 1600 mg to 1800 mg showed a significant increase in the entrapment efficiency, but further increase in the concentration from 1800 mg to 2000 mg showed decreased in the entrapment efficiency. Initially, increasing the concentration of surfactants may increase the niosomes formed; therefore, the volume of hydrophobic domain increases, and entrapment efficiency increases. But further increasing the surfactant concentration resulted in decreased entrapment efficiency due to the formation of mixed micelles along with noisome vesicles. Due to the large size of micelle formed, which may lead to lower entrapment. There is a significant effect of cholesterol in the entrapment of drug in the vesicle. The entrapment efficiency was increased when the cholesterol amount was increased from 100 mg to 200 mg, but further increase in concentration showed decreased in the entrapment efficiency. Initially increase in the entrapment shows that cholesterol act as vesicular cement and prevent the gel to sol transition, thereby forming less leak vesicles.

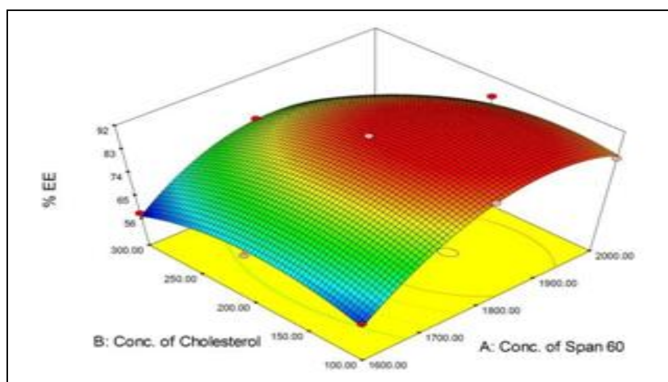

FIG. 4: RESPONSE SURFACE (3D) PLOT OF ENTRAPMENT EFFICIENCY

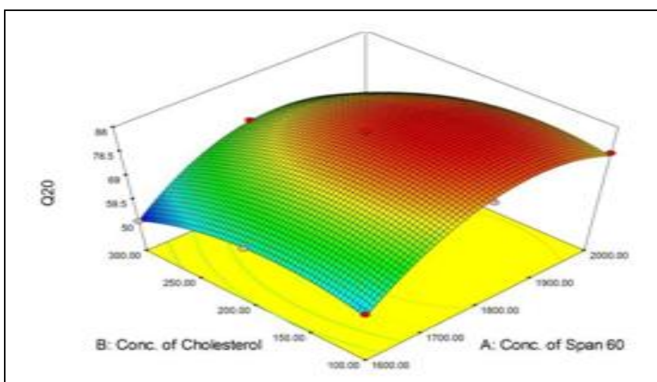

FIG. 5: RESPONSE SURFACE (3D) PLOT OF Q20

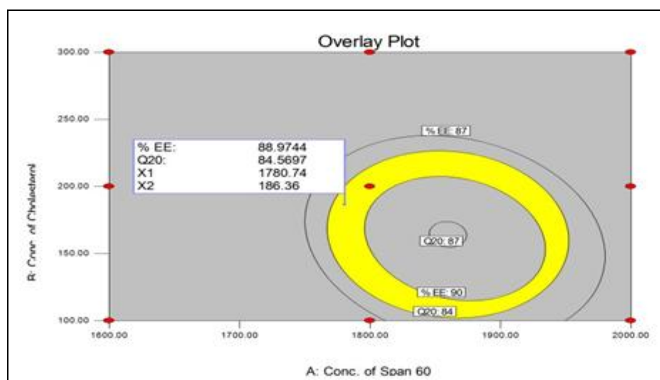

FIG. 6: OVERLAY PLOT OF ENTRAPMENT EFFICIENCY AND DRUG RELEASE AT 20 h

But when cholesterol amount was increased from 200 mg to 300 mg, entrapment was decreased, which may be quoted as cholesterol molecule will compete with the drug for the space in the bilayer and remove the drug from the bilayer. For % entrapment efficiency, the significance levels of the coefficients b_{11} were found to be P=0.058, so this term was omitted from the full model to generate the reduced model. The coefficients b_1, b_2, b_1^2, b_2^2 were found to be significant at $P < 0.05$; hence they were retained in the reduced model. The reduced model for the % entrapment efficiency,

$$\% \text{ EE} = 89.39 + (8.11 * X_1) - (5.16 * X_2) - (14.67 * X^2) - (8.22 * X_2^2)$$

Full and Reduced Model for % Drug Release at 20 h (Q_{20}):

$$Y_2 = 84.94 + (8.06 * X_1) - (5.22 * X_2) - (1.59 * X_1X_2) - (14.42 * X_1^2) - (7.97 * X_2^2)$$

From the 3D response surface plot **Fig. 5,** and the regression coefficient values of factors, it was concluded that increasing the surfactant concentration increased the drug release. Entrapment efficiency is the measure of the vesicle's ability to retain the drug in the vesicles, the slower the release profile. Indirectly entrapment of drugs within vesicles also affects the drug release. The general features of the release profile of the proteosome prepared using conventional surfactants revealed a significant increase in the percentage drug release with an increase in HLB value of surfactant. Vesicles formed, which will modifies the structural composition of stratum corneum and increase the thermodynamic activity of drug as well as skin vesicular partitioning. Thus both surfactant and cholesterol showed significant effect on drug release. For the % drug release at 20 h (Q_{20}), the significance levels of the coefficients b_{11} were found to be P= 0.165 so this term was omitted from the full model to generate a reduced model. The coefficients b_1, b_2, b_1^2, b_2^2 were found to be significant at $P < 0.05$; hence they were retained in the reduced model. The reduced model for % drug release at 20 h (Q_{20}),

$$Q_{20} = 84.94 + (8.06 * X_1) - (5.22 * X_2) - (14.42 * X^2) - (7.97 * X_2^2)$$

Formulation of Check Point Batch: To validate the evolved mathematical models (reduced models for % EE and Q_{20}), CP1 checkpoint batch was selected. The checkpoint was formulated by taking the quantities described in **Fig. 6.** As indicated in the flag the amount of span 60 taken was 1780 mg and amount of cholesterol was 186 mg in the proniosomal gel. Batch CP1 was prepared and evaluated. The observed and predicted values are shown in **Table 6.** Good correlation was found between observed and predicted values. Hence, it may be concluded that the evolved models may be used for the theoretical prediction of responses within the factor space.

TABLE 6: FORMULATION AND EVALUATION OF CHECKPOINT BATCH

Formulation of Check Point Batch		
Batch Code	X_1(mg)	X_2(mg)
CP1	1780	186
Evaluation of Check Point Batch and Comparison With Predicted Value		
Batch Code	Actual value	Predicted value
% Entrapment Efficiency (%EE)	88.97±0.52 %	88.41 %
Drug release at 20 h (Q_{20})	84.56±0.52 %	83.72 %

(n=6)

***In-vitro* Drug Release Study and Kinetic Modeling of Dissolution Data:** The drug release characteristics of the formulation were studied by using a semi-permeable membrane. The formulation F1-F3 has shown release of about 73.76, 91.38%, and 83.39% at 24 h, respectively. This is may be due to less amount of Cholesterol. The formulation F4-F6 has shown release of about 75.64%, 94.76%, and 89.87% at 24 h, respectively and the formulation F7-F9 has shown release of about 65.42%, 81.85%, and 70.84%, at 24 h, respectively. From **Fig. 7** it can be concluded that the drug release appeared to decrease more with an increasing amount of Cholesterol. The drug release data were fitted to the kinetic model, and the r^2 value was compared. The n value is used to characterize different release mechanisms. All Factorial batches showed n value between 0.5 and 1.0, so the drug released followed the non-fickian transport mechanism. The r^2 value of 0.994 was found to be maximum for zero-order kinetic for batch F5. Hence, batch F5 was selected as an optimum batch in the present study [22].

Vesicular Size and Morphology: The size of vesicles was determined by zetasizer. Size of the

vesicles of the optimized batch F5 was reported 6000.0 nm. The vesicular size and shape were also determined by scanning electron microscopy, and it was found 5 µm. Thus SEM revealed that the niosomes formed were spherical in shape, as shown in **Fig. 8.**

Zeta Potential Determination: The magnitude of zeta potential gives a potential stability of the colloidal dispersion. The zeta potential of the F5 batch was reported as -29.52 mV, and hence this indicates that the prepared formulation is stable.

***Ex-Vivo* Drug Permeation Study:** *Ex-vivo* skin permeation study of the batch F5 exhibited 89.60% of drug permeation in 24 h. Proniosomal miconazole nitrate gel showed the steady-state flux 289.7 µg/cm^2/h and diffusion coefficient 14.45×10^{-3}cm^2/h. These results indicated that the proniosomal gel enhanced the miconazole nitrate skin penetration and acted for a long time due to change in the barrier properties of the skin and in the vehicle-stratum corneum partition coefficient.

Microbial Assay: The outcome of microbiological activity studies showed that proniosome gel possesses a potential to inhibit the fungal growth of *Candida albicans* upto 24 h. The zone of inhibition of proniosomal 2% miconazole nitrate gel was recorded as 17 mm. The activity of miconazole nitrate may be attributed due to enhanced penetration of vesicles containing the miconazole nitrate through the fungal cell wall to inhibit ergosterol synthesis. Thus result indicated that the proniosomal formulation is a therapeutically promising candidate for the efficient transdermal delivery of miconazole nitrate.

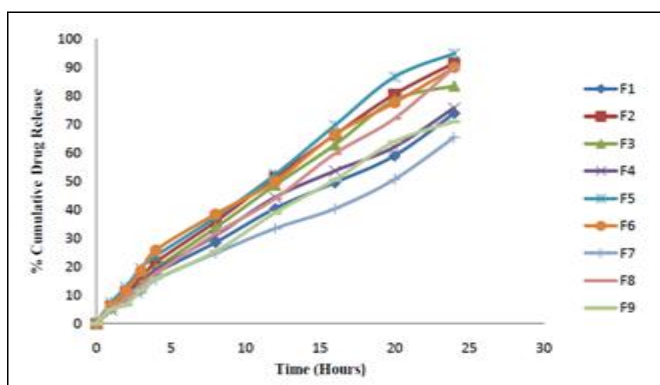

FIG. 7: DISSOLUTION PROFILE OF FACTORIAL BATCHES (F1-F9)

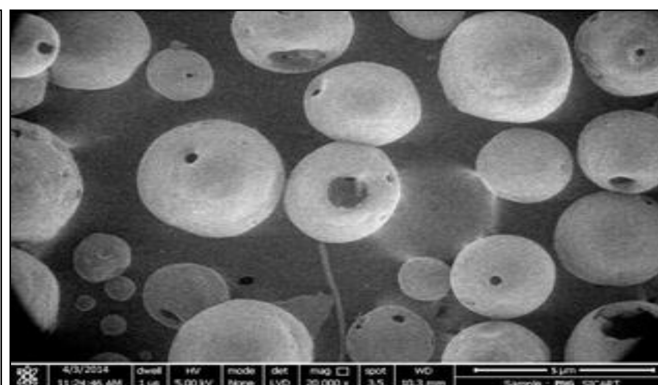

FIG. 8: SEM OF MICONAZOLE NITRATE PRONIOSOMAL GEL

Skin Irritation Study: The visual evaluation of the skin irritation study of drug control and batch F5 showed that the erythema and edema produced in the Group-III were considerably less as compared to the Group-II treated with a standard irritant, so it was concluded that proniosomal 2% miconazole produced very little or no irritation. According to Draize and Woodward, compounds producing scores of 2 or less are considered negative (no skin irritation). Hence, the developed gel was free of skin irritations [23].

Accelerated Stability Study: The results of the short term stress stability study showed that there was no major change in the formulation after two weeks. The value of similarity was 83.62%, indicating the good similarity of dissolution profiles before and after stability study. % Drug content after short term stress stability study was 97.59%, which shows that the drug did not degrade during the study. Also, Entrapment efficiency was found to be 88.65%, which indicates that the drug was retained within the vesicles.

CONCLUSION: Proniosomal 2% miconazole nitrate gel was prepared successfully by the conservation phase separation method. The prepared proniosomal gel exhibited a % entrapment efficiency of miconazole nitrate ranged from 61.12 ± 1.11% to 89.31 ± 0.73%, and vesicular size was in the ranged 5 to 6 µm. It was found that batch F5 which contains a mixture of span 60 and cholesterol. (9:1) is the most appropriate surfactant mixture for the preparation of proniosomal gel. It was found that the zeta potential of batch F5, which chosen as the best formula according to entrapment efficiency, particle size, and *in-vitro* release study, was found to be (−29.52 mV), that showed its

stability. It was maximum antifungal activity after 24 h, and *in-vitro* release studies proved that proniosomal gel considered being a successful topical drug delivery system and providing a sustained release of encapsulated drug. Proniosomal 2% miconazole nitrate gel can be further proved by the future in- vivo and bioequivalence studies. Furthermore, there was no significant change in Entrapment efficiency and dissolution profiles of miconazole proniosomal gel after the accelerated stability study.

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