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OPTIMIZATION OF PLURONIC LECITHIN ORGANOGEL OF TERBINAFINE HYDROCHLORIDE USING DESIGN OF EXPERIMENTS FOR THE TREATMENT OF FUNGAL DISEASES

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Keywords:

Terbinafine HCl, Pluronic lecithin organogels, Box-Behnken design, Microbial assay, Fungal skin diseases

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ABSTRACT: The research work focused on the optimization of Terbinafine HCl (TBH) pluronic lecithin organogels (PLOs) for increasing the penetration and efficacy of the drug. The factors affecting the PLOs such as viscosity and percentage drug release, were screened and optimized using the Box-Behnken design. The optimum formulation based on desirability (0.995) exhibited 3139.92 Cp for viscosity and 93.4561% for drug release. The optimized PLO was evaluated for drug content, pH, *in-vitro* drug release and *ex-vivo* studies. The percentage cumulative release of TBH permeated from optimized PLOs and marketed cream (MC) at the end of 12 h after the application was found to be 60.29 ± 0.69% and 19.35 ± 2.19% respectively. The comparatively higher permeability TBH in optimized PLOs could be due to increased solubility and diffusion coefficient of the drug. *In-vitro* microbial assay, studies showed that the PLO reduced the fungal burden of *Candida albicans* as compared to M.C (0.34 ± 0.08 mm) in a shorter duration of time with a larger zone of inhibition (0.58 ± 0.12 mm). Hence, it was concluded that TBH PLOs had better skin targeting ability and may serve as a promising carrier in the treatment of fungal skin diseases.

INTRODUCTION: Currently, the research is focusing its trends towards the noninvasive drug delivery system to overcome the painful strategies of drug delivery. Among all approaches, topical drug delivery gains the most attention due to its non-invasiveness, ease of use, larger surface area and, most important no risk of systemic adverse events and drug interactions [1]. In terms of the economic prospects, it increases patient compliance with reduction of the treatment cost.

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Topical drug delivery is designed for the potent drugs to ensure therapeutic efficacy with the correct physicochemical properties into the skin, and as penetration enhancers [2] to facilitate topical drug permeation. Invasive fungal infection is one of the common threats to human health in today's world. The developing and underdeveloped countries are more prone to fungal diseases [3].

About 40 million people are affected by a fungal infection in those countries. The researcher found that, the invasive diseases caused by *Candida spp*. and *Aspergillus* spp. is 30-50% prime among all fungal conditions [4]. Despite the advancement of diagnostics and therapeutic improved strategies, the overall mortality is still very poor. Terbinafine HCl (TBH) is most commonly prescribed topically as well as orally active allylamine antifungal drug

which prevents fungal ergosterol biosynthesis via specific and selective inhibition of fungal squale oxidase [5]. The research already proved that TBH is one of the best candidates for treating almost all forms of susceptible Candida infections in both immune-competent and immune compromised hosts. The safety profile and efficacy of the antifungal agent are important to decide oral or topical antifungal administration. Lower drug requirements and higher affectivity for many fungal or yeast skin infections are the main advantages of TBH. With the conventional tablet formulation, the drug is reported with gastrointestinal problems, hepatic problems, and sensory problems along with few adverse drug reactions possibly due to its extensive bio-distribution. So, delivery of TBH through the topical route is one of the best choices for the management of fungal infections [6]. However, the poor solubility of TBH limits its permeability and efficacy through the topical route. Many of the researchers tried to increase the permeability of TBH by liposome [7], SLN[8], NLC [9] nanoemulsion [10] and much more nanoformulation approach. However, these strategies are not economical and lack scale-up abilities.

Avramiotis *et al*., 2007 formulate PLO of bioactive compounds, and the result indicates that the PLOs used to incorporate model bioactive compounds with medical interests for the active molecules. The result also indicates that permeation was 35% after 24 h [11]. Wenqiang Ba *et al*., 2015 formulated sinomenine pluronic lecithin organogels system (PLO), and to evaluate the permeability of the optimized PLO *in-vitro* and *in-vivo*[12]. The results recommend that PLO can be used as an advantageous transdermal delivery vehicle to enhance the permeation and skin deposition of sinomenine.

PLO is the biphasic system facilitated to accumulate both hydrophilic and lipophilic drugs and form a reversed polymer –micelles in specific critical micelles concentration (CMC) [13]. The optimized CMC makes the PLO thermodynamically stable, and the ingredients use for the preparation of the reverse micelles are biocompatible and non-irritant. The visco-elastic gel has the advantage of rapid absorbance through the skin and produces localized action [14]. The thermoreversible property modifies the skin and acts as a matrix system for the various types of potential drugs [15]. The research article focused on the formulation of the TBH loaded PLO and optimized the formulation using the Box-Behnken method to provide better permeability and the skin deposition for the management of the fungal infection.

MATERIALS AND METHODS:

Materials: Terbinafine Hydrochloride, Pluronic F127, and Lecithin were purchased from Sigma Aldrich, Mumbai, Isopropyl Myristate (IPM) was purchased from S.D. Fine chemicals, Mumbai, Dialysis membrane (MWCO: 12 000–14 000) was purchased from Hi-media. All other reagents and solvents used were of reagent grade.

Preparation of TBH PLO System: PLOs were prepared, basically mixing the oil and aqueous phase. Generally, the first one is an aqueous phase (Pluronic gel) and the second one is the oil phase (lecithin phase). In cold temperatures, PLO gels are a liquid solution, and with increasing the temperature they changed their phase and became a gel [16].

For the preparation of lecithin phase first, a specific amount of soya lecithin was dissolved in IPM, which was used as dispersing, emulsifying, and stabilizing agent, and then the mixture needs to keep overnight for lecithin's complete dissolution. Sorbic acid (0.2-0.3% w/w) was added in the mixture as a preservative [17]. A weighed amount of Pluronic F-127 was dispersed in cold water to prepare the Pluronic gel. For the complete dissolution of Pluronic F-127, the mixture needs to keep overnight at 2-4 °C in a refrigerator. Then the drug was dissolved in ethanol and mixed with the aqueous phase. At last, in the final stage, 70% of aqueous phase needs to add slowly drop by drop in 30% of the oil phase with continuous stirring at 400 rpm by using a mechanical stirrer.

Optimization of PLOs:

Determination of Viscosity: A Brookfield digital viscometer (Brookfield Engineering Laboratories, Middleboro, MA) was used with Spindle no 7 to find out viscosities of PLO gels [18]. All tests were performed at room temperature (25 ºC). The viscosity of the TBH loaded PLO gels were evaluated.

***In-vitro* Permeation Studies using Synthetic Membrane:** Permeation studies of TBH loaded PLOs were performed using Franz diffusion cells with a diffusion area of 1.813 cm^2 [19]. The receptor chamber was filled with 27ml of Phosphate buffer (pH 7.4) solution and constantly stirred with a magnetic stirrer at 600 rpm. The water maintained a temperature of 32 ± 0.5 ºC. The known amounts (200 mg) of formulation were applied to the cellulose membrane surface. Four Franz diffusion cells were run simultaneously *i.e.* PLOs and blank. 2 ml of sample was withdrawn from each cell during the predetermined time intervals of 0, 0.50, 1, 3, 6, 9, 12 h respectively. The amount of penetrated drug in the collected samples was determined by the RP-UFLC method at 222 nm [20].

2.4. Experimental Design of Pluronic Lecithin Organogels using Box-Behnken Design: The critical independent variables [pluronic F127 (X1), lecithin (X2) and IPM (X3)] influencing the transdermal properties of the produced PLO were selected, and a three-factor, three-level Box–Behnken design (BBD) was developed. The viscosity (Y1) and drug release from the skin (Y2) in vitro were selected as responses. The coded and actual values of the variables are given in **Table 1**.

The most influencing factors effects, as well as the interactive and quadratic factor significance of these formulation parameters on the dependent variables, studied using Design-Expert® software (Version 8.0.7.1, M/s Stat-Ease, Minneapolis, USA) [21, 22]. Seventeen experiments with five center points were designed by the software (in order to allow the estimation of pure error) and experiments were run in random order. The nonlinear quadratic model generated by the design was:

$$Y = A0 + A1X1 + A2X2 + A3X3 + A4X1X2 + A5X2X3 + A6X1X3 + A7X1\ldots + A8X22 + \ldots A9X3\ldots..eq\ (1)$$

The Y and A0 – A9 represents the dependent variable and regression coefficients of the variables respectably. The interaction of the parameters computed from the observed experimental values of Y; while X1, X2, X3 represent the dependent variable with −1, 0, and +1 representation with analogous to low, middle, and high values shown in **Table 1**. The experimental matrix data produced by the software was shown in **Table 2**. BBD and contour plots were used to optimize the final PLO formulations [23, 24]. The responses (Y) for each experiment follow the eq (2), interactive eq (3) or quadratic model eq (4) carrying out multiple regression analysis. The F-test use to study the statistical significance.

$$Y = b0 + b1X1 + b2X2 + b3X3 \text{ linear eq (2)}$$

$$Y = b0 + b1X1 + b2X2 + b3X3 + \cdots b12X1\ X2 + b13\ X1\ X3 + \ldots b23X2X3 + \ldots b123\ X1X2\ X3 \ldots..eq(3)$$

$$Y = b0 + b1X1 + b2X2 + b3X3 + \cdots b11X12 + \cdots b22X22 + \cdots b33X32 + \cdots b12X1\ X2 + \ldots b23X2X3 \ldots + b13X1X3 + \ldots b123X1X2X3 \ldots..eq(4)$$

Seventeen runs of various combinations were generated and formulated using the data of selected variables X1, X2, and X3 at different levels. Mathematical modelling generated using Eq. (5) to get the second-order polynomial equation.

$$Y = b0 + b1X1 + b2\ X2 + b3X3 + b11X12 + b22X22 + b33X32 + b12X1\ X2 + b23X2\ X3 + b13X1\ X3 + b123X1\ X2\ X3 \ldots..eq\ (5)$$

TABLE 1: FACTOR LEVEL FOR BOX-BEHNKEN DESIGN FOR OPTIMIZATION OF PLOs

Independent variable		Dependent variable	
Actual value	**Coded value**	**Actual value**	**Coded value**
Amount of	A	20	-1
Pluronic F-127		27.50	0
(%)		35	+1
Amount of	B	2	-1
Soy lecithin		3	0
(%)		4	+1
Amount of	C	2	-1
IPM (%)		2.5	0
		3	+1

3D Response Surface Plots: The potential relationship between three variables studied by the 3D surface and 3D wireframe plots.

Desirability Criteria: The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value (Eq. 6). Desirability criteria use to optimize viscosity and drug release[25].

$$D = (d\text{Drug release} - d\text{Viscosity})\ ½\ldots..eq\ (6)$$

Where D is the total desirability, drug release and viscosity are individual desirabilities.

Evaluation of PLOs:

Organoleptic Properties: Physical examinations were carried out by inspecting visually of PLO gels color, appearance, odor, texture and phase separation.

Homogeneity Test: The prepared PLO gel pressed between the fingers to study the consistency and the presence of any coarse particles.

Wash Ability: To study the washability of the formulated gel, the formulation was rubbed on the backside of hand skin and wash with tap water after drying. The washable property of the gel was observed.

Determination of pH: For the determination of pH, 1 g of drug-loaded PLO gel was directly determined using a digital pH meter (Eutech Instruments, Mumbai, India). This study performed in triplicate and average pH values were reported.

Drug Content Determination: 1 g of PLO was taken and dissolved in methanol and sonicated for 1 h. The resulted solution was filtered with a 0.22µ filter to obtain a clear solution [26]. Then the drug content was analyzed at 222nm by the RP-UFLC method.

Gel Transition Temperature: The gel-sol-gel mechanism is the key to the PLO gel therapeutic efficacy [27]. The gel transition temperature is the significance parameter for gel efficacy. The gel transition temperature was studied using the vial contacting the magnetic beads and PLO in water bath maintains the temperature of 40 ºC. The constant heat flow was maintained with the agitation of 60 rpm.

The gelation temperature was measured to study the phase transition temperature.

Spreadability: Spreadability is another important parameter to study the uniform distribution of the gel through the skin for uniform drug release [28]. The spreadability was measured using the modified wooden block method and compare with the marketed formulation. A measured amount of the gel was placed on the fixed glass plate and a moveable glass plate is attached to the pan [29]. The gel was a sandwich between the two glass slides. Spreadability was determined using the following formula:

$$S = mX\ l/t$$

Where S is spreadability, m is weighing of the upper slide, l represents the length of glass slides and t is time is taken to separate the slides.

***Ex-vivo* Permeation Studies Using Rat Skin:** Permeation studies of optimized PLO and MC were performed using Franz diffusion cells with a diffusion area of 1.813 cm^2. The receptor chamber was filled with 27ml of phosphate buffer 7.4 solutions containing 0.5% of tween 80 and constantly stirred with a magnetic stirrer at 600 rpm. The water maintained a temperature of 32 ± 0.5 ºC. The known amount (200 mg) of the formulation was applied to the rat skin surface. Four Franz diffusion cells were run simultaneously *i.e.* optimized PLOs and, MC) and blank [30]. Two ml of the sample were withdrawn from each cell during the predetermined time intervals of 0.50, 1, 3, 6, 9, 12 h, respectively. The amount of terbinafine in the collected samples was determined by the RP-UFLC method at 222 nm.

Release Kinetics: The kinetic release model of zero order, first order, Higuchi, Pappas, and Hixson Crowell were generated using the *in-vitro* release of the formulation to study the best fit.

Microbiological Assay: The fungal stain (IMTECH Chandigarh) was grown in the nutrient agar medium for 18-24 h at 30 ºC. Every cell suspension containing the yield of $1\text{-}5.10^6$ organism/mL confirms with colony counter. A sterile hole bore was used to drug the 7 mm wells and the test and MC sample along with the pure drug in the equivalent weight of 2 mg was poured in a different well. After the incubation of 24 h at 35 ºC, the zones of inhibition were measured with the help of divider and scale and expressed in mm [31].

Acute Dermal Irritation Test: The test was performed on 20 weeks old albino rabbits, the fur was removed on the back of rabbit 24 h prior carefully to prevent abrading the skin. The test was done for 72 h. Test compound incorporated PLO was applied to an area of skin (5 cm x 5 cm) and untreated skin area served as the control. At the end of the contact time, the lasting preparation was removed using gauze and water without changing the prevailing response or integrity of the epidermis. The scoring system examined the presence of erythema and edema as per OECD 404 from 0 to 4 [32].

Statistical Analysis: Statistical analysis for all the descriptive statistics, values are given as mean ±

SD. Statistical significance was checked by the independent samples t-test. $p<0.05$ was considered statistically significant. Other levels of significance are as noted.

RESULTS:

Effect of the Lecithin Concentration on Viscosity and Drug Release: The lecithin concentration found to be the most significant factor in the drug release and the viscosity of the POLs. The research was clearly confirmed that the increase in the concentration of the lecithin delay the drug release and enhance the viscosity due to the formation of the matrix structure. So, the optimization of the lecithin concentration is the key to the desired therapeutic efficacy of the PLOs.

Effect of Pluronic Concentration on Viscosity and Drug Release: Pluronic F127 (PF127), the triblock water-soluble copolymer has the thermal gelling characteristics and affect the three-dimensional packing of micelles due to amphiphilic molecule interactions. The structural orientation of the pluronic concentration affects the viscosity of the PLOs and alters the drug release. The gel-sol transition of the formulation showed a liner drop with the increase of the PF127 concentration. And its significantly affect the viscosity of the formulation.

Effect of IPM on the Viscosity and Drug Release: Isopropyl myristate (IPM) is a commonly used penetration enhancer in topical and transdermal formulations. The percentage concentrations of the IPM synergistically enhance the drug release by enhancing the flux of the formulation and enhance the permeation. Thus, it can well be concluded that the amount of drug released, and flux show potential synergism when formulated with isopropyl myristate.

Box- Behnken Design: A total no of 17 experiments were performed and tabulated in **Table 2** to understand the effect of formulation variables on viscosity and drug release. The value of responses Y1 (viscosity) and Y2 (Drug release) ranges from 2200 to 3400 Cp and 85.4 to 95.4% respectively. The ratio of high and the low for responses Y1 and Y2 are 1.545 and 1.117 respectively. Therefore, the power transformation of values for viscosity and drug release was not required. Box-Cox plot provides a guideline for choosing the correct power transformation. Power transformation is required for responses if the ratio of maximum to minimum response is more than 10. If the ratio is less than 3 that means the transformation has less effect [30]. The study focused on the sequential model, sum of squares, and lack of fit tests and concludes the acceptability of the specific model to represent the maximum viscosity, drug release, and the results are shown in Table 3. The study recommends selecting the model for viscosity and drug release of Probability >F value of $P<0.0001$, low standard deviation, high R^2 and lower Predicted Residual Error Sum of Square (PRESS). ANOVA results indicate that the model was significant (Model Prob>*F* less than 0.05). The Model *F* value using Fischer's test for response *y*1 and *y*2 was 11.16 and 4.69 respectively, which implies model is significant and represented in **Table 4**. Lack of fit *F*-value for *y*1 and *y*2 were 3.78 and 0.033 respectively which implies Lack of fit was not significant relative to the pure error **Table 4**.

The multiple regression terms were also analyzed in **Table 5**. The coefficient of variation (C.V.) determines the precision of the experiment and its value for all responses was found to be low that indicates the deviation between actual and predicted values is low. It not only shows a high degree of accuracy but good reliability in conducting the experiments. The predicted *R*-squared values and Adjusted *R*-squared values for response *y*1 and *y*2 were 0.8329 and 0.8511 and 0.4364 and 0.4089 respectively which is in acceptable agreement and shows that the model has predicted the responses suitably. Adequate (Adeq.) precision for both responses *y*1, *y*2 was 10.610 and 7.135 respectively. The ratio of response to deviation was measured by the Adeq precision. The Suitable ratio should be greater than 4. Here in both cases, the ratio is greater than 4. To navigate the design space this model can be used. The final equation of coded factors for responses y1 and y2 is given in eq 7 and 8.

Viscosity = + 3000.00 + 262.50*A + 207.50*B + 70.00*C+125.00 * A * B - 50.00*A * C +10.00 *B*C - 142.50*A2-182.50*B2-157.50 *C2…..eq (7)

Drug release = + 90.54 + 2.78*A +1.25*B + 0.50*C…..eq (8)

The response of Y1, positive coefficient of A, B and C indicates that the higher the concentration of Pluronic F-127, lecithin, and IPM more the Viscosity. For response Y2, positive coefficients of A, B, and C indicate Drug release increase as the concentration of Pluronic F-127, lecithin and IPM increases. The theoretic data were compared with the experimental data of responses using predicted *vs.* actual response graph **Fig. 1** was found to be in close arrangement.

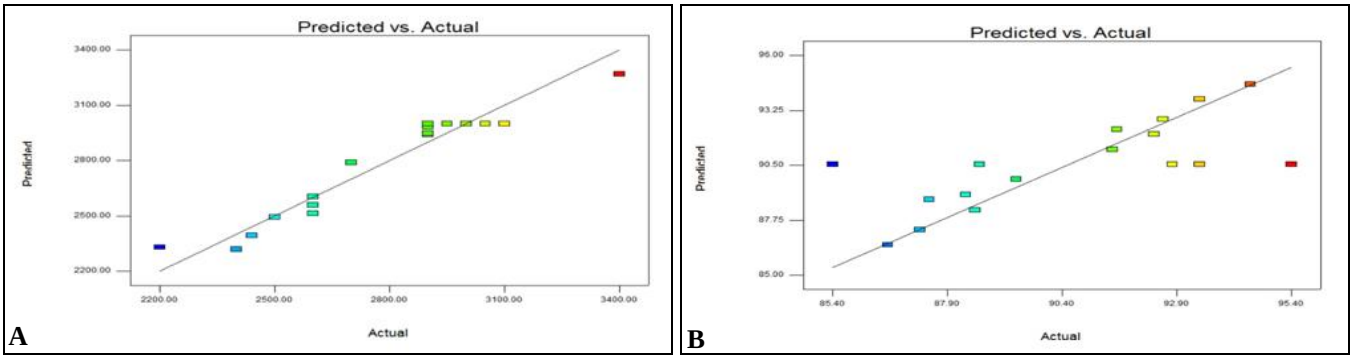

FIG. 1: LINEAR CORRELATION PLOTS REPRESENTING THE RELATIONSHIP BETWEEN PREDICTED AND ACTUAL VALUES FOR DIFFERENT RESPONSES A) VISCOSITY AND B) DRUG RELEASE

To find out the factors which affect the response perturbation graphs were plotted **Fig. 2**. For response *Y*1, factors A, B, and C show steep slope which indicates that concentration of pluronic, lecithin and IPM have a significant effect on the Viscosity of PLOs. For response *Y*2, factors B and C were slight bends, which indicate that lecithin and IPM have a significant effect on the drug release.

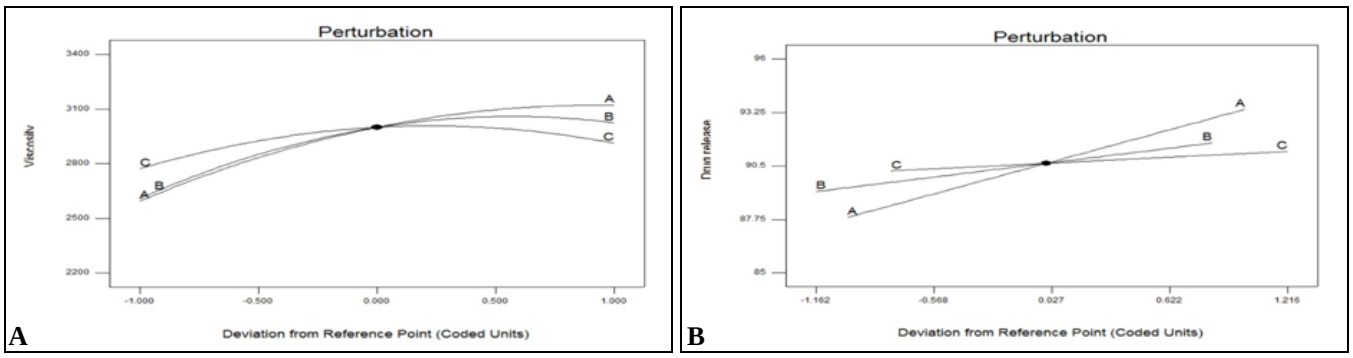

FIG. 2: PERTURBATION GRAPH REPRESENTING THE EFFECT OF AN INDIVIDUAL FACTOR ON A) VISCOSITY AND B) DRUG RELEASE RESPONSE. A, B, AND C REPRESENT PLURONIC, LECITHIN AND IPM RESPECTIVELY

TABLE 3: FIT SUMMARY OF THE MEASURED RESPONSES

Lack of fit tests										
Source	**Sum of square**		**df**		**F value**		**p-value (Prob>F)**		**Remarks**	
	Y_1	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**
Linear	45.76	4.93	9	9	9.76	0.033	0.0212	1.0000	-	Suggested
2FI	40.28	4.04	6	6	12.89	0.041	0.0136	0.9992	-	-
Quadratic	6.11	1.12	3	3	3.91	0.023	0.1103	0.9946	Suggested	-
Cubic	0.000	0.000	0	0	-	-	-	-	Aliased	Aliased
Pure Error	2.08	65.41	4	4	-	-	-	-	-	-

Model summary statistics												
Source	**Std. Deviation**		**R-squared**		**Adjusted R-squared**		**Predicted R-squared**		**PRESS**		**Remarks**	
	Y_1	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**	**Y_1**	**Y_2**
Linear	1.92	2.33	0.6418	0.5197	0.5591	0.4089	0.4375	0.4364	75.13	82.54	-	Suggested
2FI	2.06	2.64	0.6828	0.5258	0.4926	0.2412	0.1491	0.4000	113.65	87.87	-	-
Quadratic	1.08	3.08	0.9386	0.5457	0.8597	0.0385	0.2432	0.1792	101.09	120.20	Suggested	-
Cubic	0.72	4.04	0.9844	0.5533	0.9376	0.7866	-	-	-	+	Aliased	Aliased

TABLE 4: ANOVA FOR RESPONSE SURFACE QUADRATIC MODEL OF MEASURED RESPONSES

Source	Sum of squares		df		F- value		p-value (Prob>F)		Remarks	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Model	1.376E+006	76.11	9	3	11.16	4.69	0.0022	0.0197	Significant	Significant
A- Pluronic	5.513E+005	61.61	1	1	40.24	11.39	0.0004	0.0050	-	-
B-Lecithin	3.445E+005	12.50	1	1	25.14	2.31	0.0010	0.1525	-	-
C-IPM	39200.00	2.00	1	1	2.86	0.37	0.1346	0.5537	-	-
AB	62500.00	-	1	-	4.56	-	0.0701	-	-	-
AC	10000.00	-	1	-	0.73	-	0.4212	-	-	-
BC	400.00	-	1	-	0.029	-	0.8692	-	-	-
A^2	85500.00	-	1	-	6.24	-	0.0411	-	-	-
B^2	1.402E+005	-	1	-	10.24	-	0.0151	-	-	-
C^2	1.044E+005	-	1	-	7.62	-	0.0280	-	-	-
Residual	95900.00	70.34	7	13	-	-	-	-	-	-
Lack of Fit	70900.00	4.93	3	9	3.78	0.033	0.1157	1.0000	Not significant	Not significant
Pure Error	25000.00	65.41	4	4	-	-	-	-	-	-
Cor Total	1472E+006	146.4	16	16	-	-	-	-	-	-

TABLE 5: REGRESSION TERMS FOR VISCOSITY AND DRUG RELEASE

Response	C.V. %	Predicted R-squared	Adjusted R-squared	Adeq. precision
Viscosity	4.22	0.8329	0.4364	10.610
Drug release	2.57	0.8511	0.4089	7.135

Response Surface Plot:

Desirability Criteria: The optimum formulation obtained after the run based on desirability was found at 33.55, 3.27 and 2.84 level of X1, X2, and X3. The calculated desirability factor was 0.995, indicating the appropriateness of the designed factorial model. The results of the dependent variables given by the run were 3139.92 Cp for viscosity and 93.4561% for drug release **Fig. 3** and **4**. The predicted value of responses *Y1* and *Y2* were 3139.92 Cp and 90.25%. After the final formulation, the actual values of *Y1* and *Y2* were found 3013 Cp and 90.25% TBH loaded PLOs which is in close agreement to the predicted values **Table 6**.

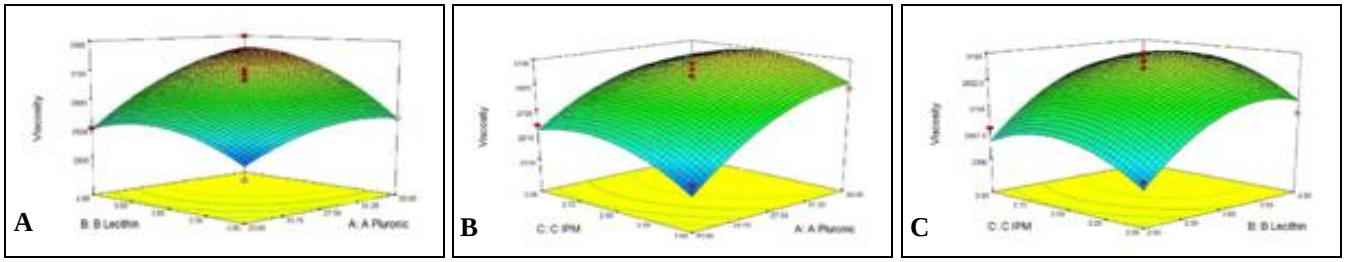

FIG. 3: 3D RESPONSE SURFACE PLOT SCREENING EFFECT OF INDEPENDENT VARIABLES A) PLURONIC CONCENTRATION (X1) AND LECITHIN CONCENTRATION (X2) B) PLURONIC CONCENTRATION (X1) AND IPM CONCENTRATION (X3) C) LECITHIN CONCENTRATION (X2) AND IPM CONCENTRATION (X3) ON VISCOSITY (Y3) (NOTE: REGION IN THE RED REPRESENTS MAXIMA AND REGION IN BLUE REPRESENTS MINIMA).

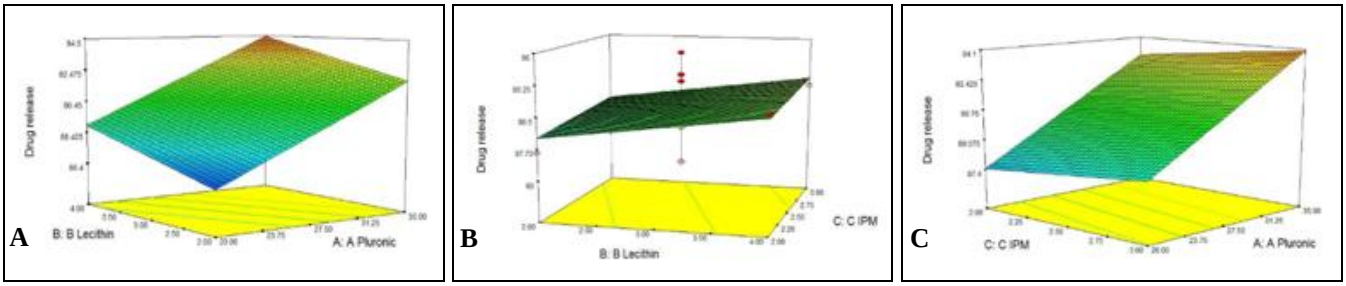

FIG. 4: 3D RESPONSE SURFACE PLOT SCREENING EFFECT OF INDEPENDENT VARIABLES A) PLURONIC CONCENTRATION (X1) AND LECITHIN CONCENTRATION (X2) B) LECITHIN CONCENTRATION (X2) AND IPM CONCENTRATION (X3) C) PLURONIC CONCENTRATION (X1) AND IPM CONCENTRATION (X3) ON DRUG RELEASE (Y3) (NOTE: REGION IN THE RED REPRESENTS MAXIMA AND REGION IN BLUE REPRESENTS MINIMA)

TABLE 6: PREDICTED AND ACTUAL RESPONSES FOR TERBINAFINE PLOs

Response	C.V. %	Predicted R-squared	Adjusted R-squared	Adeq. precision
Viscosity	4.22	0.8329	0.4364	10.610
Drug release	2.57	0.8511	0.4089	7.135

Determination of Viscosity: The viscosities for the 17 PLO gel formulations at 60 rpm were found in a range of 2200 Cps to 3400 Cps **Table 2**.

***In-vitro* Permeation Studies using Synthetic Membrane:** After the study for 12 h, the percentage drug release from the 17 PLO formulations was found in a range of 85.4-95.4% **Table 2**.

Evaluation of the PLOs:

Organoleptic Characteristics: The prepared formulations were found homogenous, odorless, off-white in color, greasy, and reluctant to wash and stable without any sign of phase separation.

pH: The pH of all the formulation was found within the range of 5.57-5.80, which similar to the normal skin pH. So, it can be concluded that the PLO gels are non-irritant to the skin.

Drug Content Determination: The drug content of optimized PLO formulation is 99.87%, which means the drug was dispersed homogeneously throughout the gels with good content uniformity.

Spreadability: The spreadability value indicates that the PLO gel formulation was easily spreadable, but the concentration of the lecithin and pluronic effect the spreadability of the formulation. The optimized formulation had the spreadability of 32. 67g.cm/S compare with the MC spreadability of 28.67g.cm/S.

The increased concentration of the polymer act as the resist of spreadability causes a decrease in the tendency of the spreadability.

Gel Transition Temperature: The gel transition temperature of PLO gels was found at 37.4-30.21 ºC. An increase in the concentration of the polymer increases the gel strength at a lower temperature.

TABLE 2: THE LAYOUT OF BOX-BEHNKEN DESIGN SHOWING THE VALUES OF DEPENDENT VARIABLES OF 17 FORMULATIONS

Run	Factor 1 A: Pluronic	Factor 2 B: Lecithin	Factor 3 C: IPM	Response 1 Viscosity (Cp)	Response 2 Drug release (%)
1	20.00 (-1)	2.00 (-1)	2.50 (0)	2207	86.6
2	35.00 (+1)	2.00 (-1)	2.50 (0)	26137	92.4
3	27.50 (0)	3.00 (0)	2.50 (0)	3059	93.4
4	20.00 (-1)	4.00 (+1)	2.50 (0)	2526	88.3
5	35.00 (+1)	4.00(+1)	2.50(0)	34018	94.5
6	27.50 (0)	3.00 (0)	2.50 (0)	3104	95.4
7	20.00 (-1)	3.00 (0)	2.00 (-1)	2426	87.3
8	35.00 (+1)	3.00 (0)	2.00(-1)	2913	92.6
9	27.50 (0)	3.00 (0)	2.50 (0)	2952	88.6
10	20.00 (-1)	3.00(0)	3.00 (+1)	2615	88.5
11	35.00 (+1)	3.00 (0)	3.00 (+1)	2904	93.4
12	27.50 (0)	3.00 (0)	2.50 (0)	2913	85.4
13	27.50 (0)	2.00 (-1)	2.00 (-1)	2445	87.5
14	27.50 (0)	4.00 (+1)	2.00 (-1)	2726	91.5
15	27.50 (0)	3.00 (0)	2.50 (0)	3007	92.8
16	27.50 (0)	2.00 (-1)	3.00 (+1)	2601	89.4
17	27.50 (0)	4.00 (+1)	3.00 (+1)	2938	91.6

Source	Sum of squares		df		F- value		p-value (Prob>F)		Remarks	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Mean versus Total	47006.43	1.394E+005	1	1	-	-	-	-	-	-
Linear versus Mean	87.73	76.11	3	3	7.76	4.69	0.0032	0.0197	-	Suggested
2FI versus Linear	5.48	0.89	3	3	0.43	0.043	0.7351	0.9875	-	-
Quadratic versus 2FI	34.16	2.91	3	3	9.72	0.10	0.0068	0.9562	Suggested	-
Cubic versus Quadratic	6.11	1.13	3	3	3.91	0.023	0.1103	0.9946	Aliased	Aliased
Residual	2.08	65.41	4	4	-	-	-	-	-	-
Total	47140.00	1.395E+005	17	17	-	-	-	-	-	-

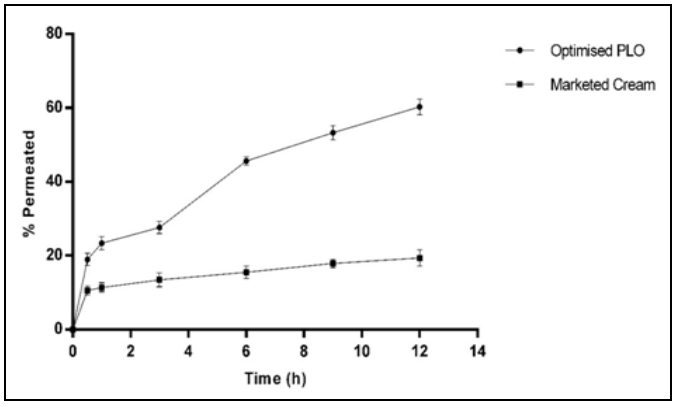

FIG. 5: COMPARATIVE *EX-VIVO* PERCENTAGE PERMEATION PROFILE OF TERBINAFINE HYDROCHLORIDE FROM OPTIMIZED PLO AND MARKETED FORMULATION THROUGH THE RAT SKIN AT pH 7.4. (PERMEATION TIME = 12 h, MEAN ± SD, n = 3)

***Ex-vivo* Permeation Studies using Rat Skin:** The permeation profile was obtained by placing PLOs and MC (each containing equivalent weight of 2 mg TBH) on the rat skin in the Franz diffusion cell **Fig. 5**. It was observed that TBH concentration steadily increased in the receptor chamber with an increase in time. The results also indicate that the permeation profile followed Fick's diffusion law. The % cumulative amount of TBH permeated from optimized PLOs and MC at the end of 12^{th} h after the application was found to be 60.29 ± 0.69 and 19.35 ± 2.19, respectively. The comparatively higher permeability of optimized PLOs could be due to the increased diffusion coefficient of the drug. The permeation was observed as a maximum for optimized PLO.

Release Kinetics: The *in-vitro* drug release data obtained from release studies were fitted into various kinetic models like zero order, first order, Higuchi, Hixson Crowell, Korsmeyer-Peppas, and represented in **Table 7**. The best linearity was obtained in Higuchi's plot for optimized PLO formulation indicating the release as a square root of the time-dependent process.

TABLE 7: REGRESSION VALUE FOR VARIOUS KINETIC MODELS

	A (%)	B (%)	C (%)	Viscosity (Cp)	Drug release (%)
Expected	33.55	3.27	2.84	3139.92	93.4561
Terbinafine PLOs (Predicted)	33.55	3.27	2.84	3013	90.25
Error (%)				4.21	3.55

Microbial Study: The microbial assay was performed using *C. albicans* strain. The maximum zone of inhibition for the PLO was found 0.58 ± 0.12 mm where the MC the zone of inhibition was 0.34 ± 0.08 mm shows in **Fig. 6**. The low viscosity of the PLO gel than MC, which helps in high penetration of TBH resulted in an increase in the inhibition zone. The result indicates that PLO could be more effective than the MC for the better management of the fungal infection.

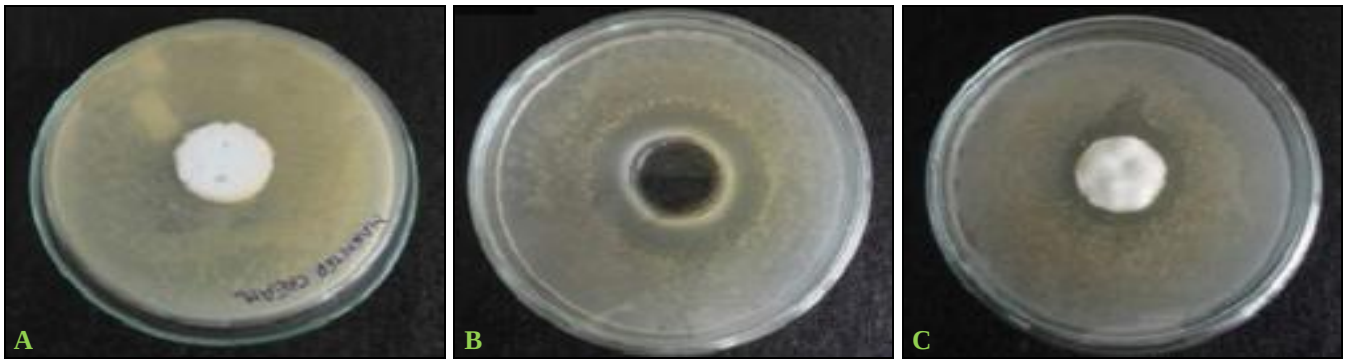

FIG. 6: RESULT OF FUNGAL (CANDIDA ALBICANS) GROWTH INHIBITION ON NUTRIENT AGAR MEDIUM BY AGAR DIFFUSION METHOD AT THE INCUBATION TIME OF 24 h AT 35 ºC. INHIBITION EFFECTIVENESS OF THE ANTIFUNGAL A) MARKETED CREAM (MC) B) PURE DRUG SOLUTION C) OPTIMIZED PLO OF TERBINAFINE HYDROCHLORIDE (CONCENTRATION USED: 2 mg EACH)

DISCUSSION: Gel-sol-gel phase transition of PLOs varies with the change of the temperature condition, which indicates that the optimization of the phase transition temperature is the critical factor for the PLO gels. The skin permeation study indicates the statically significant enhancement from the PLO compares to that of MC. The ingredients used for the preparation of the PLO are the key ingredient for the enhancement of the skin permeation, which alters the skin barrier function.

Lecithin and pluronic interact and disorganize the lipid layers of the stratum corneum and enhance the drug permeation through the skin. The permeation also depends on the various factors like the micellar structure of the PLO, which contains both the hydrophilic and the hydrophobic ingredients and enhances the diffusion through both the lipid layer and the distribution and slight disorganization of skin. Lecithin is a major component of the cell membrane itself, which may contribute to the good adhesiveness to the skin and good delivery of the drug. Additionally, the IPM acts as an emollient, moisturizer and penetration enhancer. The PLO gels showed a better zone of inhibition compares to the marketed formulation due to the controlled release as well as low viscosity of the formulation which enhances the penetration to facilitate in increasing the zone of inhibition. The pH of the PLO gel shows a significant effect as the low pH favor the inhibition of the fungal strain. The bioadhesive property of PLO made it a better candidate as a control release carrier to enhance the surface action of the TBH by enhancing drug concentration above its minimum inhibition concentration (MIC). The adhesive property due to the ionic interaction of the PLO and the bacteria cell wall propose to enhance the antimicrobial activity of the PLO gel formulation. The acute dermal irritation studies also revealed that the prepared formulation is nonirritant as per OECD 404 and can be used topically.

CONCLUSION: The present study was the attempt to increase the skin permeability of poorly water-soluble drug-like TBH using PLO as the carrier for the fungal skin disorder using DOE to optimize the formulation. The basic results showed that the viscosity and the drug release from the PLOs was the most critical factor for the release of drug from the gel formulation and the therapeutic efficacy of the formulation. The study effectively proved the enhancement of the permeation of the TBH from PLOs through the skin. As well as the kinetic studies indicate the sustain action of the gel formulation which was essential for the fungal disease. *In-vitro* drug release study shows the control action of the formulation for up to 12 h resulting in prolonging the active time for the drug in the topical treatment for the antifungal action. The result clearly indicates that the incorporation of TBH in the PLO gels certainly enhance the antifungal activity of the drug. Even the used ingredients act synergistically to enhancing the antifungal activity by 1.5-fold compared with MC formulation. These results support the hypothesis that TBH PLOs are the promising candidate for the topical drug delivery system to overcome the limitation of the existing marketed formulations for enhancing skin permeability.

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