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Development and Optimization of Microemulsion Formulation using Box-Behnken Design for Enhanced Transdermal Delivery of Lornoxicam

Muhammad NAEEM [1]*, Nisar UR RAHMAN [2], Jawad A KHAN [1], Ayesha SEHTI [1], & Zarqa NAWAZ [3]

[1] *Faculty of Pharmacy and alternative medicine, the Islamia University of Bahawalpur, Bahawalpur- Pakistan*
[2] *Department of Pharmacy, COMSATS Institute of Information and Technology, Abbottabad- Pakistan*
[3] *Department of Chemistry, the Islamia University of Bahawalpur, Bahawalpur- Pakistan*

***SUMMARY*. The purpose of present study is to optimize microemulsion of poorly water soluble NSAID, lornoxicam. The microemulsion was optimized using three factor, three level Box Behnken statistical design. Independent variables were oil, surfactant and cosurfactant mixture (Smix) and water, while dependent variables were cumulative quantity permeated through rabbit skin, flux and lag time. Microemulsion was prepared using almond oil, Tween20 (surfactant) and dimethylsulfoxide (DMSO) (co-surfactant) and water. The ranges of pH, conductivity, droplet size, polydispersity index and viscosities was found to be 4-5, 102-205 μsiemens/cm, 50-90 nm, 0.120-0.350 and 50-430 cp, respectively, of all Box-Behnken derived formulations. *In vitro* release studies on Franz cell showed the optimized formulation consisted of 20% almond oil, 54% Smix (Tween20 and DMSO in 3:1) and 27% water. The cumulative quantity permeated, flux and lag time of the optimum formulation was found to be 8503 μg, 229 μg/cm2 h and 0.41 h. The results indicated that microemulsion containing DMSO as permeation enhancer is promising vehicle for enhanced transdermal delivery of Lornoxicam.**

INTRODUCTION

Limited joint mobility throughout the body, inflammation and pain are caused by rheumatic arthritis (RA) and is syndrome of hundreds of diseases. The ratio of osteoarthritis and RA are almost 21 million adults in USA and more than 400,000 people in UK, respectively [1]. The direct and indirect medical treatment cost of arthritis is $51.1 and $35.1, respectively [2].

Non-steroidal anti-inflammatory drugs (NSAIDs) which have both beneficial (formation of prostaglandins from arachidonic acid, through inhibition of the enzyme COX) and potential risks (gastrointestinal, liver and cardio-renal toxicity) are used to relieve the symptoms of RA [3,4]. NSAID lornoxicam (BCS class II drug) that belongs to class of oxicam, with less gastric toxicity is used for the treatment of RA. It has pKa of 4.7 [5-9].

The provision of medicine through oral and injection is substituted with transdermal route of administration to prevent pre-systemic and systemic toxicity respectively. Skin acts as reservoir for sustain action and provides convenience and non-invasiveness. But stratum corneum act as barrier to deliver the drug viable to the target site. So the nano-carriers with small droplet size and different chemistry are to be used to reduce the barrier of skin and increased permeability and produce local and systemic effects [10].

Microemulsion is a transparent, thermodynamically stable with droplet size of 10 to 100nm. It is of two types water in oil and oil in water and; unable to coalesce [11,12]. It comprised of two phases such as oil and aqueous phase which are usually stabilized with the mixture of surfactant and co-surfactant. Physicochemical characteristics of microemulsions are stability studies, optical isotropy, low viscosity and transparency [13]. It is used for dermal and transdermal drug delivery system [14-17]. The advantages of microemulsion has various mechanisms for dermal and transdermal drug delivery system [18,19]. First, the maximum amount of drug can be solubilized to the microemulsion; second, drug partitioned to the skin due to enhance thermodynamic activity; third, permeation enhancers in microemulsion are used for decreasing the diffu-

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* Author to whom correspondences should be addressed. *E-mail:* naeem_pk4@yahoo.com

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sional barrier of the stratum corneum and increase the rate of permeation. Microemulsion is used for incorporation of many drugs for dermal and transdermal study. Lornoxicam is not used until now. In the present study, oil in water microemulsion of Lornoxicam was formulated after screening of oils, surfactants and co-surfactant. The components of microemulsion and their ranges were selected after constructing ternary phase diagrams. Seventeen microemulsion formulations were obtained using Box Behnken statistical design and characterized for cumulative quantity permeated through rabbit skin, flux and lag time.

MATERIAL AND METHODS

Materials

Lornoxicam (Hilton Pharmaceutical, Karachi), Oleic acid (OA), Tween 80, Propylene Glycol (PG), Ethanol and Isopropyl Alcohol (IPA) were purchased from Merck (Germany). Isopropyl Myristate (IPM) was obtained from Panreac Quimica (Europe). Tween 20 was purchased from Fisher Scientific (UK). Water was purified by distillation using distillation apparatus (IRMECO, Germany) and then membrane filtered. All other chemicals and solvents were of HPLC or analytical grade and used as received without further purification.

Screening of oils for microemulsions

The solubility of lornoxicam in oils such as sesame oil, almond oil, soybean oil, olive oil, sunflower oil, eucalyptus oil, oleic acid, nutmeg oil, peanut oil, coconut oil and isopropyl myrisatate, surfactants such as Tween 20 and Tween 80 and co-surfactants such as DMSO, ethanol and IPA were determined. Individual oils, surfactants or co-surfactants (6 mL) were used for solubility of excess quantity of drug and stirred for 72 h at 26 °C. The resulted suspension was centrifuged for 15 min at 5000 rpm. Membrane filter (0.45 µm) was used for filtration of supernatant. The clear drug solution was diluted and analyzed 376 nm using UV spectrophotometer. The oils, surfactants and co-surfactants having maximum solubility of drug were then selected for making microemulsion containing 0.250% lornoxicam.

Pseudoternary phase diagrams and formulation of microemulsion of lornoxicam

Almond oil (oil), tween 20 (surfactant), DMSO (co-surfactant) were used for formulation of microemulsion using water titration method. Pseudoternary phase diagrams were used for the selection of microemulsion area. The surfactant to co-surfactant (Smix) weight ratio varied as 1:0, 1:1, 2:1 and 3:1. The weight ratios of specific Smix and oil was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (w/w) for the construction of ternary phase diagram. A clear and transparent mixture of Smix and oil was obtained with the help of drop wise addition of water. Pseudoternary diagram was completed using different component concentrations and on the basis of such results optimized concentrations of oil, Smix and water were found. The microemulsion was formulated by dissolving lornoxicam into mixture of oil, Smix and water. The stability of microemulsion was studied at high and low temperatures. Drug content and phase separation was also found. The control formulation was prepared by dissolving sufficient quantity of Lornoxicam into phosphate buffer solution (PBS) 7.4.

Experiment design

The ranges of oil, Smix and water were selected from ternary diagrams. These values were manipulated using Design expert (Version 7.1) and 17 formulations of microemulsion were obtained. The independent variables were oil, Smix and water and the dependent variables were cumulative quantity permeated, flux and lag time. This design expert is used to explore quadratic response surface and constructing second order polynomial models using 3 factor 3 level Box Behnken Design. It has fewer runs than central composite design. This design expert is discriminated with set of points lying at center point of the multi-dimensional cube and the midpoint of each edge [20]. The computer generated non-linear quadratic model is given as $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_{21} + b_{22}X_{22} + b_{33}X_{23}$, where the measured response associated with each factor level combination is represented by Y; an intercept is b_0; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y; and independent variables are represented with X_1, X_2 and X_3. The interaction and quadratic terms are represented by X_1, X_2 and X_2 (i = 1, 2 or 3), respectively [21,22].

Analysis of check point and optimization model validation

Design Expert software was used for the statistical validation of polynomial equations using ANOVA. Statistically significant coefficients and R^2 values were evaluated on the basis of mod-

els. Optimum parameters were found by conducting various feasibility and grid searches. Design Expert software was used to provide various 3D response surface graphs. The experimental model and polynomial equations were validated with intensive grid search over the whole experiment region using ten optimum checkpoint formulations. Various response properties of optimized checkpoint formulation were evaluated. The percentage prediction error was calculated quantitatively comparing resultant experimental values of the responses and predicted values.

Characterization of Microemulsion

Nano Zetasizer (Malvern instruments, UK) was used for the measurement of droplet size and polydispersity index at 25 °C. Conductometer WTW Cond 197i (Weilhein, Germany) was used for the determination of electrical conductivity while pH meter (WTW inolab, Germany), was used for the determination of pH at 25 °C.

Rheological measurements

Brookfield Programmable DVIII + Digital Rheometer (Brookfield Engineering Laboratories Inc., MA, USA) was used for measuring rheological properties of microemulsion. A controlled stress rheometer with the cone (24 mm) and plate geometry was used for rheological measurements. The viscosity was measured using torque sweep within range of 10 to 110%. All the measurements were performed at 25 °C in triplicate. The equilibrium time before every measurement was 5 min and the sample volume used was approximately 0.5 mL. Rheocalc 32 software (Brookfield Engineering Laboratories Inc., USA) was used for performing calculation of rheological properties. The data was analyzed using "Power Law" [30] as expressed in Eq. [**1**].

$$\tau = KDn \qquad [1]$$

where τ is shear stress, K is gel index (GI) or consistency index, D is shear rate, and n is flow index. Rheocalc 32 software was used to automatically apply the model to generated data and the value of GI was recorded.

In Vitro skin permeation studies

Skins were obtained from the dorsal region of rabbit after removing hair with razor [23]. Heat separation was used to remove epidermis and scalpel was used to remove subcutaneous fat and checked for skin barrier integrity [24,25]. Diffusion cells of Vertical Franz type (PermeGear, Bethlehem, PA) were used with diffusional surface area of 1.767 cm^2. The receptor compartment contained the phosphate buffer solution (PBS) with capacity of 12 mL at 7.4 pH [26,27]. The skin was placed between the donor and receptor compartments of the cell [28]. Water bath and a peristaltic pump were used to maintain the temperature of the receptor solution at 37 °C. Teflon-coated magnet bar was used for stirring. The concentration of test formulation was 4.0 g and applied to donor compartment. After 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, and 24 h, 1 mL of the sample was removed from receptor compartment for UV determination and immediately replaced with an equal quantity of fresh PBS. There was no interference of the components. These dose conditions are infinite [29].

The cumulative quantity of Lornoxicam permeated was plotted as function of time through excised rabbit skin. The permeation release rate of Lornoxicam was calculated from the slope and intercept of the straight line at steady state.

Stability Tests

The studied formulations were centrifuged at 10,000 rpm for 15 min to check physical stability. These formulations were also evaluated for visual clarity and phase separation and UV assay of lornoxicam at 40 °C for 6 months.

Skin Irritation Study

The studied microemulsions were applied on gause dressing (1 × 1 cm^2) and then placed to the inner arms of 10 human volunteers in the age range of 21-27 years. Stretch adhesive tape was used for fixing the microemulsion at inner arm. The reading was taken after 8 h.

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to measure the skin permeation release rate by statistical data. For the study of skin irritation, statistical paired sample t-test was used at the level of P = 0.05. These tests were performed by SPSS 12.0 software.

RESULTS AND DISCUSSION

Microemulsion component selection

Components of microemulsion (oils, surfactants and co-surfactant) were selected on the basis of solubility. The solubility of Lornoxicam in different oils, surfactants and co-surfactants were determined and shown in Table 1. The almond oil was selected on the basis of its high miscibility in DMSO while Tween20 was selected due to high solubility of Lornoxicam.

Construction of pseudo-ternary diagrams

Almond oil (oil), Tween20 (surfactant) and

Components		Solubility (mg/mL) Mean ± SD
Oils	Sesame oil	0.03 ± 0.001
	Almond oil	0.035 ± 0.005
	Soybean oil	0.041 ± 0.009
	Olive oil	0.01 ± 0.001
	Sunflower oil	0.048 ± 0.008
	Eucalyptus oil	0.395 ± 0.010
	Oleic acid	0.125 ± 0.002
	Nutmeg oil	1.2 ± 0.02
	Peanut oil	0.052 ± 0.006
	Coconut oil	0.027 ± 0.003
	Isopropyl myrisatate	0.060 ± 0.002
Surfactant	Tween 20	4.9 ± 0.20
	Tween 80	3.3 ± 0.10
Co-Surfactant	DMSO	7 ± 0.10
	Ethanol	0.083 ± 0.02
	Isopropyl alcohol	0.075 ± 0.015
	Propylene glycol	1.241 ± 0.13
Water	Water	0.023 ± 0.013

Table 1 Solubilty of Lornoxicam in various components of microemulsion (n = 3).

DMSO (Co-surfactant) were used for making microemulsion. Various weight ratios of Tween20 and DMSO were used for the construction of pseudo-ternary phase diagrams and shown in Fig. 1. Phase diagrams were distinguished with transparent microemulsion region and remaining turbid and conventional emulsions region based on visual observation. From Fig. 1, it is apparent that the increased weight ratios of Tween20 and DMSO (3:1) resulted in increased isotropic microemulsion region.

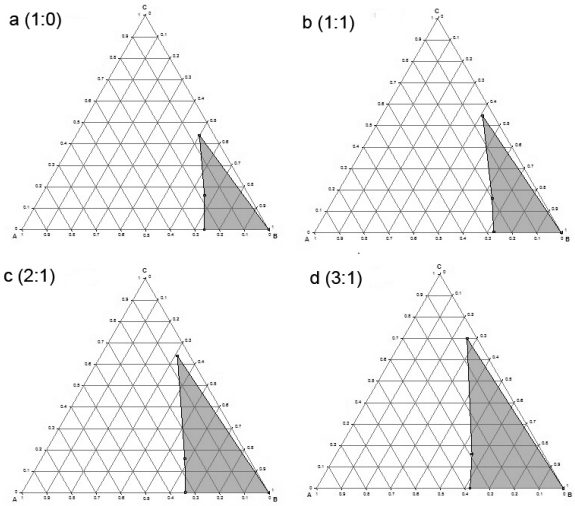

Figure 1. Pseudoternary phase diagrams of microemulsion composed of oil phase, surfactant (Tween 20) and cosurfactant (DMSO) and water; (a) Smix (mixture of surfactant and cosurfactant) 1:0, (b) Smix 1:1, (c) Smix 2:1, and (d) Smix 3:1.

Preparation and Characterization of Microemulsion

Microemulsions were selected from the 3:1 phase diagram. The ranges of pH, conductivity, droplet size and polydispersity index was found to be 4-5, 102-205 µsiemens/cm, 50-90 nm and 0.120-0.350, respectively of all Box-Behnken derived formulations and shown in Table 2. pH showed mild irritation. Conductivity showed oil in water microemulsion. Polydispersity index showed the narrow size distribution of all microemulsions [18]. The pH, conductivity, droplet size and polydispersity index of optimized for-

Formulations	pH	Conductivity	Droplet size	Polydispersity index
ME1	4.5	198 ± 2.0	70.5 ± 2.1	0.221 ± 0.020
ME2	4.5	198 ± 2.0	70.5 ± 2.1	0.221 ± 0.020
ME3	4.2	205 ± 1.3	80.9 ± 3.2	0.292 ± 0.090
ME4	4.3	102 ± 3.2	82.5 ± 3.5	0.341 ± 0.040
ME5	4.7	204 ± 4.4	54.7 ± 4.1	0.301 ± 0.070
ME6	4.5	198 ± 2.0	70.5 ± 2.1	0.221 ± 0.020
ME7	4.6	120 ± 5.1	60.5 ± 2.5	0.198 ± 0.015
ME8	4	190 ± 2.3	80.5 ± 3.5	0.187 ± 0.025
ME9	5	130 ± 1.9	55.5 ± 2.2	0.18 ± 0.030
ME10	4.1	200 ± 3.5	52.5 ± 2.5	0.21 ± 0.050
ME11	4.5	198 ± 2.0	70.5 ± 2.1	0.221 ± 0.02
ME12	4.4	201 ± 1.5	59.8 ± 3.4	0.14 ± 0.045
ME13	4.5	198 ± 2.0	70.5 ± 2.1	0.221 ± 0.02
ME14	4.8	186 ± 2.6	50 ± 4.4	0.165 ± 0.033
ME15	4.6	140 ± 1.2	81.1 ± 4.6	0.35 ± 0.030
ME16	4.3	160 ± 1.9	83.1 ± 3.9	0.155 ± 0.025
ME17	4.9	175 ± 1.7	90 ± 4.9	0.12 ± 0.039

Table 2. Physicochemical parameters of microemulsion formulations (mean ± S.D., n =3).

Formulation	Oil (X_1) g	Smix (X_2) g	Water (X_3) mL	Cumulative Quantity permeated (Y_1)	Flux (Y_2)	Lag time (Y_3)	Gel Index (GI)
ME1	0	0	0	7983	203	0.48	63.5
ME2	0	0	0	7983	203	0.48	102.5
ME3	+1	0	+1	7573	185	0.42	112.6
ME4	+1	0	-1	7518	184	0.84	77.8
ME5	-1	0	+1	8503	229	0.41	12.4
ME6	0	0	0	7983	203	0.48	49.1
ME7	0	-1	-1	7901	203	0.59	20.7
ME8	+1	-1	0	7546	184	0.67	19.2
ME9	-1	0	-1	8448	229	0.56	16.4
ME10	0	-1	+1	8038	203	0.46	17.4
ME11	0	0	0	7983	203	0.48	19.6
ME12	0	+1	+1	7847	205	1.02	18.5
ME13	0	0	0	7983	203	0.48	12.4
ME14	-1	-1	0	8421	228	0.74	2.0
ME15	-1	+1	0	8366	203	0.85	1.3
ME16	0	+1	-1	7874	202	0.88	1.4
ME17	+1	+1	0	7491	183	1.17	4.5

	Low (-1)	Medium (0)	High (+1)
X_1= Oil	6.45	13.23	20
X_2= Smix	57.35	60.67	64
X_3= Water	21	28.24	35.48

Table 3. Variables and observed responses in Box Behnken design for Lornoxicam microemulsion formulations.

mulation ME5 was found to be 4.7, 204 µsiemens/cm, 54.7 nm, and 0.301, respectively.

Rheological measurements

Flow properties of microemulsion were determined using Viscometric CP-40 spindle. Exponential function was used to describe increase in shear rate with decrease in viscosity and power law was used to analyze data 30. Table 3 was used to represent gel index (GI) value for different formulations. The value of GI was found to be in the range of 2.1 to 165.7.

Ex vivo skin permeation experiments

Fig. 2 shows permeation of Lornoxicam from various formulations using rabbit skin. Cumulative quantity permeated, flux and lag time of all formulations is given in Table 3. The permeation was found to be zero order ($R^2 > 0.974$). The values of cumulative amount permeated, flux and lag time was found to be in the range of 7491 to 8503 µg, 183-229µg/cm².h and 0.41-1.17, respectively. The variation in composition of microemulsion affected the permeation of lornoxicam. The diffusion barrier capacity of stratum corneum was reduced using surfactant and co-surfactant which act as permeation enhancers [31].

The DMSO selected in the present work also act as an effective penetration enhancer [32]. The

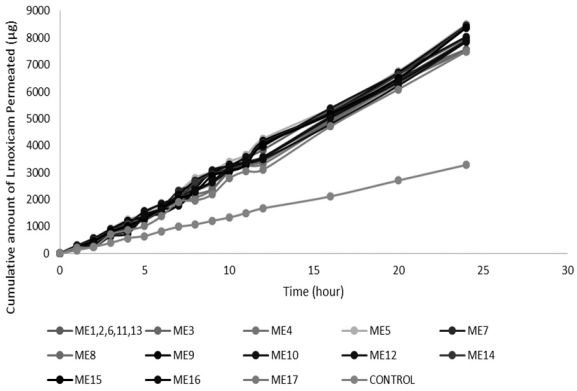

Figure 2. *In vitro* permeation profile of Lornoxicam from microemulsion and control.

cumulative quantity permeated, flux and lag time of optimized microemulsion formulation was found to be of 8503 µg, 229 µg/cm².h, and 0.41 h, respectively. The permeation, flux and lag time of Lornoxicam control solution was found to be of 3281 µg, 75 µg/cm².h, and 0.07 h, respectively. The microemulsion resulted in enhanced transdermal delivery. The permeation was found to be 2.6 times greater than control. It is also noticed that increased concentration of surfactant resulted in decrease permeation and flux and increase lag time. The higher concentration of surfactant resulted in decrease thermodynamic activity of Lornoxicam in microemul-

sion. The increase and decrease in GI resulted in decrease permeation of drug and increase concentration of oil respectively and this was reported in earlier studies [33,34]. The viscosity of microemulsion played an important role in controlling the permeation of Lornoxicam into the receptor compartment as microemulsion act as diffusional barrier due to its property of double layer [35]. The interfacial tension of microemulsion containing surfactant was reduced due to higher concentration of co-surfactant and resulted in the formation of dynamic and flexible layer [36,37]. The drug has to pass through the interfacial film between the water and oil. The diffusion and partitioning of stratum corneum seemed to increase due to its thermodynamic process. However, the permeation and flux was found to be reduced due to increase solubility of Lornxicam into the surfactant and oil phase.

Formulation optimization by experimental design

Box Behnken statistical design was used for the construction of 17 formulations which were checked for their responses. Fig. 3 is used to contour plot and 3D response surface plots which were drawn from Design Expert software. Pseudo ternary phase diagrams were used for the selection of oil, water and co-surfactant ranges. The ranges of oil, water and surfactant mixture was found to be optimum 6.45-20%, 21-35.48% and 57.35-64%, respectively. It was observed that there was a strong relationship between permeation of drug dermally and stratum corneum hydration effect. The release and transdermal permeation of drug across the skin was carried out by thermodynamic activity which was a significant driving force [38]. The cumulative quantity permeated (Y_1), flux (Y_2) and lag time was found to be in the range of 7491-8503, 183-229, and 0.41-1.17, respectively. The responses Y_1 and Y_2 of formulation ME5 and ME9 were higher with less lag time.

The responses, Y_1 and Y_2 were found to be significantly higher (Y_1, 8038-8503; Y_2, 203-229 µg/cm².h) only when 6.45 or 13.23% (w/w) concentration level was used for oil and 57.35 or 60.67% (w/w) concentration level used for Smix. The lag time (Y_3) was found to be ranging from 0.41-0.56 h at low to high levels of Smix. The responses of these formulations ranged from a low drug penetration of 7491 µg (ME17, high level of oil, Smix, and medium level of water) to a higher penetration of 8503 µg (ME5, low level of oil, high level of water, and medium level of Smix). For estimation of quantitative effects of the different combination of factors and factor levels on cumulative quantity permeated, flux and lag time, the response surface models were calculated with coded values of factor levels. The model described could be represented by Eqs. [**2-4**]:

$$Y_1 \text{ (Permeation)} = 7983 - 451X_1 - 41X_2 + 27X_3 + 2X_1X_2 - 7X_1X_3 - 40X_2X_3 + 34X^2_1 - 61X^2_2 - 6X^2_3 \quad [\mathbf{2}]$$

$$Y_2 \text{ (Flux)} = 203 - 19X_1 - 3X_2 + 0.64X_3 \quad [\mathbf{3}]$$

$$Y_3 \text{ (Lag time)} = 0.48 + 0.06X_1 + 0.018X_2 - 0.069X_3 + 0.97X_1X_2 - 0.069X_1X_3 + 0.07X_2X_3 + 0.10X^2_1 + 0.28X^2_2 - 0.19X^2_3 \quad [\mathbf{4}]$$

Fitting of data to the model

Y_1 and Y_2 were found to be significantly higher for formulation ME5. Design Expert 7.1.5 was used to fit the responses of all 17 formulations to the first order, second order and quadratic models. Quadratic model was found to be best fit model and the regression equation generated for each response and the comparative values of R^2, standard deviation and % coefficient of variation are shown in Table 4. It is observed that independent factor X_3 and X_2 has positive effect on the responses Y_1 and Y_2 respectively. The effects of the independent variables on response were shown in three dimensional response surface plots (Figure 3d–f) and optimized formulation was selected.

Data Analysis

The predicted, experimental and %age prediction error values of checkpoint formulations are shown in Table 5. The independent variables produce second order polynomial equation by multiple regression equation. The R^2 value was found to be 0.9982. The reasonable agreement of predicted R^2 (0.9714) with respect to adjusted R^2 which was found to be 0.9959. The signal to noise was measured with adequate precision. In Table 4, 64 represent the adequate signal which is greater than required ratios (4). Cumulative quantity permeated values for different formulations have wide variations from lowest to highest. The negative value showed unfavorable effect on cumulative quantity permeated while positive val-

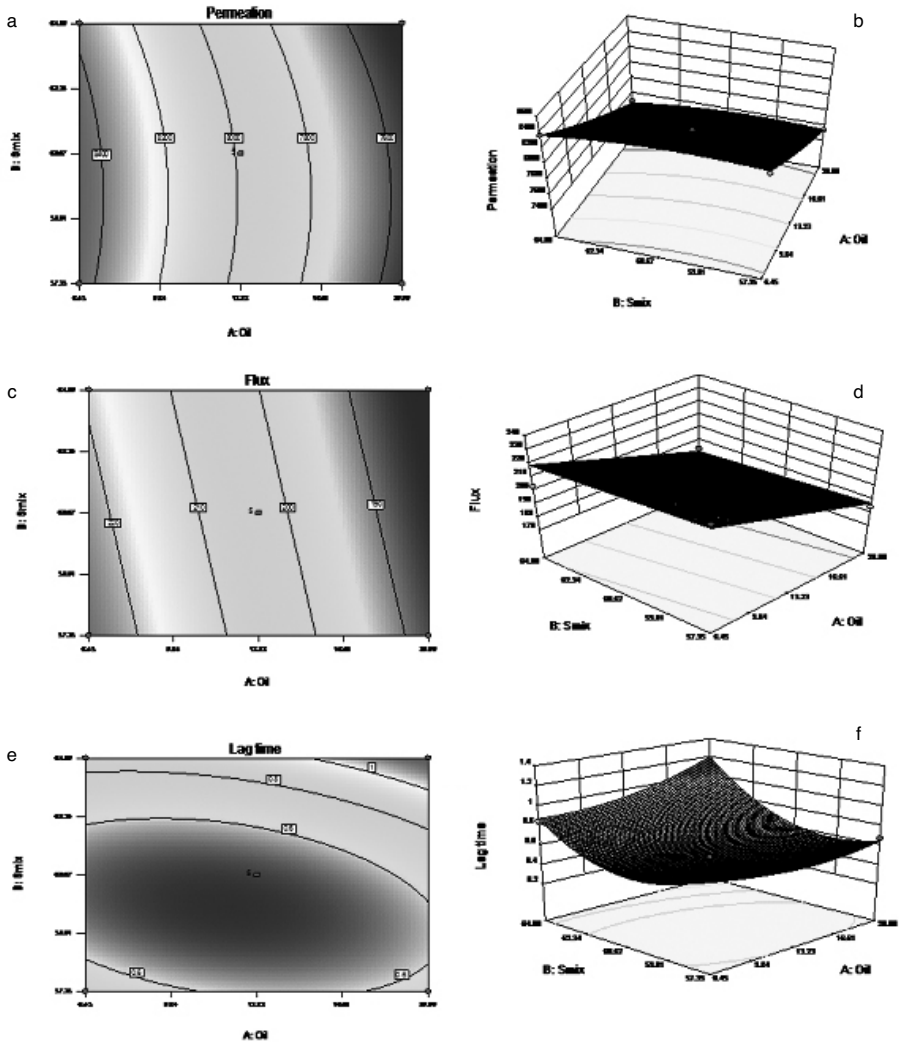

Figure 3. Contour plots showing effect of oil (X_1) and Smix (X_2); (**a-f**) on responses cumulative amount permeated (Y_1), flux (Y_2) and lag time (Y_3).

Response	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	%CV
Y1	0.9982	0.9959	0.9714	64	20.7	0.26
Y2	0.8834	0.8565	0.7561	16	5.51	2.71
Y3	0.9445	0.8731	0.1117	12	0.081	12.5

Table 4. Summary of result of regression analysis for responses Y_1, Y_2 and Y_3 for fitting to quadratic model.

ue showed favorable effect on cumulative quantity permeated. The equation [**3**] represents value of R^2 equal to 0.8834 that indicate good fit, 16 was the value of signal to noise ratio, that indicate adequate signals. The R^2 value for equation [**4**] was found to be 0.9445 that indicates good fit. The 12 was adequate precision that indicate an adequate signal.

Contour plots and response surface analysis

Two and three dimensional contour plots were used to study the interaction effects of factors on the response at once. The third factor was kept at constant level, in all Figure 3 (a-). The three variables show the non-linear relationship, although factor X_1 shows nearly linear relationship with X_2 and X_3 in the form of straight lines up to Smix medium level. Curvilinear or non-linear are found at higher concentration. Curvilinear relationship is found for X_2 and X_3 at all levels of two variables on the response Y_2. The cumulative quantity permeated and flux values increased with increasing concentrations of either oil or Smix (up to medium levels) at constant concentration of water phase.

Optimized formulations Compositions			Response variables	Predicted value	Experimental value	Difference prediction error
X_1	X_2	X_3				
6.45	59.44	35.48	Y_1	8511	7680	831
			Y_2	224	206	18
			Y_3	0.470	0.430	0.040
6.45	59.41	35.48	Y_1	8511	7700	811
			Y_2	224	202	22
			Y_3	0.470	0.440	0.030
6.49	59.49	35.48	Y_1	8507	7690	817
			Y_2	223	203	20
			Y_3	0.470	0.425	0.045
6.5	59.39	35.48	Y_1	8508	7660	848
			Y_2	224	208	16
			Y_3	0.470	0.430	0.040
6.61	59.11	35.48	Y_1	8502	7702	800
			Y_2	224	206	18
			Y_3	0.470	0.435	0.035
6.45	58.88	35.47	Y_1	8515	7710	805
			Y_2	224	205	19
			Y_3	0.490	0.450	0.040
6.64	58.79	35.48	Y_1	8501	7675	826
			Y_2	224	209	15
			Y_3	0.490	0.443	0.047
6.45	58.62	35.48	Y_1	8516	7667	849
			Y_2	224	207	17
			Y_3	0.500	0.451	0.049
6.45	59.74	33.8	Y_1	8501	7657	844
			Y_2	223	210	13
			Y_3	0.480	0.436	0.044
6.45	59.68	33.11	Y_1	8499	7684	815
			Y_2	223	204	19
			Y_3	0.490	0.442	0.048

Table 5. Composition of checkpoint formulations, the experimental and predicted values of response variables and difference prediction error.

Optimization and Validation

The optimized formulation was selected on the basis of results finding maximum value of cumulative quantity permeated, flux and low lag time. The formulation with composition of oil 17%, Smix 52% and water 31% was found to be optimized with high value of cumulative quantity permeated, flux and lag time using various response variables and comprehensive evaluation of feasibility and exhaustive grid search. RSM was used to obtain ten checkpoint formulations and shown on Table 5. Theses formulations were formulated and checked for permeation studied. The optimized parameters and predicted responses were validated with the prepared formulation and checked for permeation studies.

Stability studies

Centrifugation result showed that the selected formulations were physically stable. The formulations were stable at 40°C and no phase separation and degradation of Lornoxicam during 6 months study.

Skin Irritation study

Microemulsion did not show any significant irritation or erythema but little rubefaction appeared on third or fourth day which was then disappeared on seventh day.

CONCLUSION

In the present study new oil in water microemulsion system of Lornoxicam was established and optimized using Box-Behnken statistical design which was used to predict the cumulative quantity permeated through rabbit skin, flux and lag time. The optimized microemulsion (20% oil, 54% Smix, and 27% water) showed highest permeation and flux of 8503.3 and 229, respectively than the control. The formulation has droplet size of 54.7nm, pH of 4.7. The DMSO act as permeation enhancer. So the given system of microemulsion is most suitable carrier for the transdermal delivery of Lornoxicam.

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