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Research Article

Optimization of Hydrogels for Transdermal Delivery of Lisinopril by Box–Behnken Statistical Design

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Abstract. The aim of this study was to investigate the combined influence of three independent variables on the permeation kinetics of lisinopril from hydrogels for transdermal delivery. A three-factor, three-level Box–Behnken design was used to optimize the independent variables, Carbopol 971 P (X_1), menthol (X_2), and propylene glycol (X_3). Fifteen batches were prepared and evaluated for responses as dependent variables. The dependent variables selected were cumulative amount permeated across rat abdominal skin in 24 h (Q_{24}; Y_1), flux (Y_2), and lag time (Y_3). *Aloe* juice has been first time investigated as vehicle for hydrogel preparation. The *ex vivo* permeation study was conducted using Franz diffusion cells. Mathematical equations and response surface plots were used to relate the dependent and independent variables. The regression equation generated for the cumulative permeation of LSP in 24 h (Q_{24}) was $Y_1 = 1{,}443.3 - 602.59X_1 + 93.24X_2 + 91.75X_3 - 18.95X_1X_2 - 140.93X_1X_3 - 4.43X_2X_3 - 152.63X_1^2 - 150.03X_2^2 - 213.9X_3^2$. The statistical validity of the polynomials was established, and optimized formulation factors were selected by feasibility and grid search. Validation of the optimization study with 15 confirmatory runs indicated high degree of prognostic ability of response surface methodology. The use of Box–Behnken design approach helped in identifying the critical formulation parameters in the transdermal delivery of lisinopril from hydrogels.

KEY WORDS: Box–Behnken; factorial design; hydrogel; lisinopril; optimization.

INTRODUCTION

Lisinopril (LSP) is an angiotensin-converting enzyme inhibitor used for the treatment of hypertension and congestive heart failure and to alleviate strain on hearts damaged as a result of a heart attack. LSP is slowly and incompletely absorbed after oral administration with a bioavailability of 25–30% (1,2). LSP is available only in the form of oral tablets in the market. However, this formulation has a major disadvantage since it is incompletely absorbed from the gastrointestinal tract. To overcome the problem of incomplete absorption, low oral bioavailability, and for the effective treatment of chronic hypertension, alternative long-acting formulations could be beneficial. Transdermal route of administration may be a good alternative to circumvent these problems and is recognized as one of the potential route for the local and systemic delivery of drugs. LSP was selected as a model drug for the investigation because of its clinical need, low oral dose (2.5–20 mg), low molecular mass (405.5 g/mol), and low oral bioavailability (25%). Avoidance of hepatic first-pass elimination, decrease in side effects, improved patient compliance, interruption or termination of treatment when unnecessary, as well as maintaining suitable plasma concentration for longer duration through a non-invasive zero-order delivery are the well-documented advantages of this route of administration (3). However, the highly organized structure of stratum corneum forms an effective barrier to the permeation of drugs, which must be modified if poorly penetrating drugs are to be administered. The use of penetration enhancers would significantly increase permeation of drugs through skin. Terpenes present in naturally occurring volatile oils appear to be clinically acceptable enhancers (4); a wide variety of terpenes have been shown to increase the percutaneous absorption of number of drugs (5). In the present study, menthol was used as penetration enhancer.

In the development of transdermal dosage form, an important issue was to design an optimized pharmaceutical formulation with appropriate penetration rate within a short time period and minimum trials. Traditional experiments require more effort, time, and materials when a complex formulation needs to be developed. Various experimental designs (6–8) are useful in optimizing formulations, requiring less experimentation and providing estimates of the relative significance of different variables. Response surface methodology (RSM) is a widely practiced approach in the development and optimization of drug delivery devices (9,10). In this investigation, we explored the utility of RSM for the optimization of hydrogels. Based on the principle of design of experiments, the methodology encompasses the use of

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various types of experimental designs, generation of polynomial equations, and mapping of the response over the experimental domain to optimize the hydrogels. The technique requires minimum experimentation and time, thus proving to be far more effective and cost effective than the conventional methods of formulating dosage forms.

The work reported in this paper, a Box–Behnken design was used to optimize hydrogels containing lisinopril as drug and Carbopol 971P as gelling agent. Independent variables selected were Carbopol 971P (X_1), menthol (X_2), and propylene glycol (X_3) to evaluate their separate and combined effects on permeation of LSP in 24 h (Q_{24}) across rat abdominal skin, flux, and lag time as dependent variables.

MATERIALS AND METHODS

Materials

Lisinopril was a gift sample from M/s Dr Reddy's Laboratories, Hyderabad, India. Menthol was purchased from Merck, Mumbai, India. Carbopol 971P was gift sample from Lubrizol Advanced Materials India Pvt. Ltd, Mumbai, India. All other chemicals used were of analytical grade.

Preparation of *Aloe* Juice and LSP Hydrogels

Aloe vera juice was prepared from the full size mature leaves of *Aloe vera*. They were cut from the plant, and the green rind was removed. From the cut leaf bases, the yellow juice was allowed to drain into a container to remove the exudates. The colorless parenchyma was ground in a blender (Remi, Mumbai, India) and centrifuged at 3,000 rpm for 30 min to separate the fibers. The supernatant (*Aloe vera* juice) was used for the preparation of hydrogels.

The composition of the gels was detailed in Tables I and II. Carbopol hydrogels were prepared by dispersing in small aliquots of Carbopol 971P (CP-971P) into aloe juice containing 10% *v/v* ethanol and propylene glycol. After continuous stirring at 1,000 rpm for 1–2 min, LSP and menthol were added, and the contents were stirred for 6 h. The pH was adjusted to 7 with 1% *v/v* triethanolamine.

Formulation LSP7W using water as vehicle was prepared to study the effect of vehicle on permeation of LSP from hydrogel. It was prepared by dispersing CP-971P in water containing 10% *v/v* ethanol and propylene glycol. After continuous stirring at 1,000 rpm for 1–2 min, LSP and menthol were added, and the contents were stirred for 6 h. The pH was adjusted to 7 with 1% *v/v* triethanolamine.

pH Evaluation

The pH of the hydrogels was recorded with pH meter (Elico, India), by bringing the glass electrode in contact with the hydrogel and allowing it to equilibrate for 1 min. Experiments were performed in triplicate to check for the neutralization of gels. pH evaluation was carried out for all experimental formulations in triplicate.

Rheological Measurements

The rheological properties of hydrogels were measured using Brookfield Programmable DVIII+ Digital Rheometer (Brookfield Engineering Laboratories Inc., Massachusetts, USA). The rheological measurements were performed using a

Table I. Variables and Observed Responses in Box–Behnken Design for Hydrogels

	Independent variables			Dependent variables							Levels used, Actual (Coded)		
Batch	X_1% (*w/w*)	X_2% (*w/w*)	X_3% (*v/w*)	Y_1 (μg)	Y_2 (μg h^{-1} cm^{-2})	Y_3 (h)	pH	Gel index	Assay (%)	K (cm^{-1})	Low (−1)	Medium (0)	High (+1)
LSP1	−1	−1	0	1,597.3	13.89	1.86	6.4	0.89	99.8	1.38			
LSP2	1	−1	0	589.1	5.65	7.01	6.9	2.11	101.0	0.56			
LSP3	−1	1	0	1,730.2	15.98	1.84	6.8	1.05	98.6	1.58			
LSP4	1	1	0	646.1	6.50	5.58	7.3	2.40	102.3	0.64			
LSP5	−1	0	−1	1,943.7	17.67	1.79	6.8	1.03	99.4	1.75			
LSP6	1	0	−1	861.3	9.39	4.19	7.4	2.21	99.6	0.93			
LSP7	−1	0	1	2,429.7	22.67	1.17	6.8	1.11	100.8	2.25			
LSP8	1	0	1	783.6	6.91	6.01	7.0	2.35	98.6	0.68			
LSP9	0	−1	−1	1,282.3	10.01	2.09	6.9	1.58	100.2	0.99			
LSP10	0	1	−1	1,569.2	11.10	2.12	6.7	1.52	98.6	1.10			
LSP11	0	−1	1	1,454.0	11.45	1.93	7.1	1.86	101.6	1.13			
LSP12	0	1	1	1,723.2	14.51	1.91	7.0	1.74	99.1	1.44			
LSP13	0	0	0	1,468.9	12.29	2.14	6.8	1.65	99.0	1.22			
LSP14	0	0	0	1,415.3	11.75	2.25	7.1	1.72	99.7	1.16			
LSP15	0	0	0	1,445.7	11.67	1.80	6.9	1.68	100.0	1.16			
Independent variables													
X_1=Carbopol 971P (% *w/w*)											0.25	0.63	1
X_2=Menthol (% *w/w*)											4	8	12
X_3=Propylene glycol (% *v/v*)											5	10	15

Table II. Composition of Checkpoint Formulations, the Predicted and Experimental Values of Response Variables, and Percentage Prediction Error

Optimized formulation composition (X_1/X_2/X_3)	Response variable	Experimental value	Predicted value	Percentage prediction error
0.285:10.12:8.34	Y1	1,873.47	1,873.40	0.004
	Y2	18.09	17.03	5.860
	Y3	1.66	1.78	−7.229
0.648:11.00:12.30	Y1	1,551.96	1,481.65	4.530
	Y2	13.99	12.17	13.009
	Y3	2.13	2.19	−2.817
0.448:8.64:8.12	Y1	1,594.98	1,679.32	−5.288
	Y2	13.67	14.16	−3.584
	Y3	1.58	1.49	5.696
0.515:5.22:11.12	Y1	1,479.38	1,515.63	−2.450
	Y2	12.20	12.31	−0.902
	Y3	1.83	1.78	2.732
0.438:10.75:9.71	Y1	1,626.25	1,700.96	−4.594
	Y2	15.04	14.38	4.388
	Y3	1.68	1.64	2.381
0.345:9.35:11.75	Y1	2,014.73	1,923.91	4.508
	Y2	16.53	17.35	−4.961
	Y3	1.43	1.37	4.196
0.558:6.98:7.82	Y1	1,390.42	1,504.84	−8.229
	Y2	12.21	12.37	−1.310
	Y3	1.84	1.72	6.522
0.340:4.10:12.48	Y1	1,659.80	1,720.56	−3.661
	Y2	14.24	14.64	−2.809
	Y3	1.40	1.47	−5.000
0.276:11.68:10.66	Y1	1,812.36	1,882.14	−3.850
	Y2	16.78	17.15	−2.205
	Y3	2.08	1.99	4.327
0.484:9.52:5.90	Y1	1,651.27	1,693.58	−2.562
	Y2	14.12	13.77	2.479
	Y3	1.37	1.40	−2.190
0.254:5.40:11.45	Y1	1,785.16	1,840.24	−3.085
	Y2	15.62	16.85	7.875
	Y3	1.71	1.54	9.942
0.384:4.05:5.90	Y1	1,453.12	1,510.65	−3.959
	Y2	12.27	12.42	−1.222
	Y3	1.60	1.70	−6.250
0.289:5.64:6.62	Y1	1,564.55	1,692.00	−8.146
	Y2	13.01	14.88	−14.373
	Y3	1.90	1.73	8.947
0.375:10.60:9.50	Y1	1,896.95	1,768.47	6.773
	Y2	14.82	15.31	−3.306
	Y3	1.46	1.67	−14.384
0.288:7.52:8.84	Y1	1,782.05	1,807.08	−1.405
	Y2	15.82	16.34	−3.287
	Y3	1.52	1.65	−8.553

controlled stress rheometer with the cone (24 mm) and plate geometry. The viscosity was determined by torque sweep from 10% to 110%. All the measurements were performed in triplicate at 25°C. The equilibrium time before every measurement was 5 min, and the sample volume used was approximately 1 mL. Calculation of rheological properties was performed using Rheocalc 32 software (Brookfield Engineering Laboratories Inc., USA).

Determination of Drug Content

Weighed quantity of about 1.0 g of hydrogel was dissolved in 100 mL of distilled water, sonicated for 10 min using bath sonicator, and filtered through 0.45-μm membrane filter. The filtrate was suitably diluted, and the drug content in the sample was determined using high-pressure liquid chromatography (HPLC).

Preparation of Rat Abdominal Skin

Albino rats weighing 150–200 g were killed using anesthetic ether. The hair of test animals was carefully removed with electrical clippers, and the full thickness of skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique (11), which involved soaking the entire abdominal skin in water at

60°C for 45 s, followed by careful removal of the epidermis. The epidermis was washed with water and used for *ex vivo* permeability studies.

Ex vivo Permeation Studies

Franz diffusion cell with a surface area of 3.56 cm^2 was used for *ex vivo* permeation studies. The rat abdominal skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. About 1.0 g of gel was placed in donor compartment. The receiver phase is 12 mL of phosphate buffer saline (PBS) pH 7.4, stirred at 400 rpm on a magnetic stirrer; the whole assembly was kept at 37±0.5°C. The amount of drug permeated was determined by removing 1 mL of sample at appropriate time intervals up to 24 h; the volume was replenished with an equal volume of PBS pH 7.4. The drug content in the samples was determined by HPLC, and the concentration was corrected for sampling effects according to the Eq. 1 (12).

Cumulative amounts of drug permeated in microgram per square centimeter were plotted against time, drug flux (μg h^{-1} cm^{-2}) at steady state was calculated by dividing the slope of the linear portion of the curve by the area of the exposed skin surface (3.56 cm^2), and the permeability coefficient was deduced by dividing the flux by initial drug load as shown in Table I.

$$C_n^1 = C_n(V_T/V_T - V_S)(C_{n-1}^1/C_{n-1}) \quad (1)$$

where C_n^1 is the corrected concentration of the nth sample, C_n is the measured concentration of lisinopril in the nth sample, C_{n-1} is the measured concentration of the lisinopril in the $(n-1)$th sample, V_T is the total volume of the receiver fluid, and V_S is the volume of the sample drawn. The theoretical (required) flux was calculated using Eq. 2 (13).

$$J_{Target} = \frac{C_{SS}\ CL_T\ BW}{A} \quad (2)$$

A represents the surface area of the hydrogel applied over the excised skin (i.e., 3.56 cm^2), BW the standard human body weight of 60 kg, C_{SS} the LSP concentration at the therapeutic level (70 ng/mL), and the CL_T the total clearance (6.36 L/h) (14); the calculated target flux value for LSP was 7.50 μg h^{-1} cm^{-2}.

HPLC Analysis of LSP

Analysis of samples was performed with a Shimadzu HPLC system equipped with LC-10AT pump, UV-Vis spectrophotometric detector (SPD-10A), and C18 column (Phenomenex; 25 cm×4.6 mm; 5 μm) at ambient temperature. The mobile phase used was a mixture of phosphate buffer (25 mM potassium dihydrogen ortho phosphate, pH 5.0) and acetonitrile at a ratio of 88:12. A flow rate of 1 mL/min was maintained, and the detection wavelength was 215 nm. A calibration curve was plotted for LSP in the range of 50–2,500 ng/mL. A good linear relationship was observed between the concentration of LSP, and the peak area of LSP with a correlation coefficient (r^2=0.999). The required studies were carried out to estimate the precision and accuracy of the HPLC method of analysis of LSP and were found to be within limits [percent coefficient of variation (% CV) was less than 15%]. Sample preparation briefly involved the filtration of sample through 0.4 μ membrane filter, diluted with mobile phase, and 20 μL was spiked into column.

Experimental Design

Box–Behnken statistical screening design was used to statistically optimize the formulation factors and evaluate main effects, interaction effects, and quadratic effects on the amount permeated in 24 h (Q_{24}), flux, and lag time. RSM, such as the Box–Behnken, model possible curvature in the response function (15,16). A three-factor, three-level Box–Behnken design was used to explore quadratic response surfaces and constructing second order polynomial models with Design Expert (Version 7.1, Stat-Ease Inc., Minneapolis, MN, USA). The Box–Behnken design was specifically selected, since it requires fewer runs than a central composite design in cases of three or four variables. This cubic design is characterized by set of points lying at the mid point of each edge of a multidimensional cube and center point replicates (n=3), whereas the "missing corners" help the experimenter to avoid the combined factor extremes (17). A design matrix comprising of 15 experimental runs was constructed. The non-linear computer generated quadratic model is given as $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$, where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y; and X_1, X_2, and X_3 are the coded levels of independent variables. The terms X_1, X_2 and X_i^2 (i=1, 2, or 3) represent the interaction and quadratic terms, respectively (15,16). The dependent and independent variables selected were shown in Table I along with their low, medium, and high levels, which were selected based on the results from preliminary experimentation. The concentration of CP-971(X_1), menthol (X_2), and PG (X_3) used to prepare the 15 experimental trials, and the respective observed responses are given in Table I.

Optimization Data Analysis and Optimization Model Validation

Statistical validation of the polynomial equations generated by Design Expert was established on the basis of analysis of variance provision in the software. A total of 15 runs were generated. The models were evaluated in terms of statistically significant coefficients and R^2 values. Various feasibility and grid searches were conducted to find the optimum parameters. Various 3-D response surface graphs were provided by the Design Expert software. By intensive grid search performed over the whole experimental region, 15 optimum checkpoint formulations were selected to validate the chosen experimental domain and polynomial equations. The optimized checkpoint formulation factors were evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with the predicted values to calculate the percentage prediction error.

RESULTS

Characterization of Gels

The prepared hydrogels were slight yellowish in color with slight aromatic odor. The pH of all the 15 hydrogel formulations prepared as per Box–Behnken design and optimized formulations were found in the range of 6.4–7.4 after neutralization with triethanolamine. The content of LSP in hydrogels varied from 98.6% to 102.3%.

Rheological Measurements

CP-52 spindle was used for the viscometric characterization of hydrogels, as the working range for this spindle as reported by manufacturers is 400–7,500 cps (at 15 rpm spindle speed) or 4,500–35,000 cps (at 0.25 rpm). The decrease in viscosity of the gels observed with an increasing shear rates can be described well by an exponential function, and hence, the obtained data were analyzed using the ''Power Law'' (18) as expressed by the following equation, respectively:

$$\tau = k\, D^n \tag{3}$$

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. The GI value for different formulations is presented in Table I. The gel index was found to be ranging from 0.89 to 2.40.

Experimental Design

The independent variables and the responses for all 15 experimental runs are given in Table I. The effect of variables on responses is shown in Fig. 1. The 15 experimental formulations of hydrogels were prepared using CP-971P polymer. The responses, Q_{24} (Y_1) and flux (Y_2), were found to be significantly higher (Y_1, 1,282.3–2,429.7 μg; Y_2, 9.39 to 22.67 μg h^{-1} cm^{-2}) only when the CP-971P and menthol were used at 0.25% or 0.63% and 8% or 12% *w/w* concentration level, respectively. The lag time (Y_3) was found to be significantly lower (Y_3, 1.17–2.25 h) at low to high levels of menthol and PG. The ranges of other responses, Y_1, Y_2, and Y_3 were 589.1–2,429.7 μg, 5.65–22.67 μg h^{-1} cm^{-2}, and 1.17–7.01 h, respectively.

The responses of these model formulations ranged from a low drug penetration of 589.1 μg (LSP2, CP-971 1%, menthol 4%, and PG 10%) to a higher penetration of 2,429.7 μg (LSP7, CP-971P 0.25%, menthol 8%, and PG 15%). For estimation of quantitative effects of the different combination of factors and factor levels on the Q_{24}, flux, and lag time, the response surface models were calculated with Design Expert software by applying coded values of factor levels. The model described could be represented as:

$$\begin{aligned} Y_1(Q_{24}) = {} & 1,443.3 - 602.59X_1 + 93.24X_2 + 91.75X_3 \\ & - 18.95X_1X_2 - 140.93X_1X_3 - 4.43X_2X_3 \\ & - 152.63X_1^2 - 150.03X_2^2 - 213.9X_3^2 \end{aligned} \tag{4}$$

$$\begin{aligned} Y_2(\text{Flux}) = {} & 11.90 - 5.22X_1 + 0.89X_2 + 0.92X_3 \\ & - 0.31X_1X_2 - 1.87X_1X_3 + 0.49X_2X_3 \\ & + 0.50X_1^2 - 1.89X_2^2 + 1.76X_3^2 \end{aligned} \tag{5}$$

$$\begin{aligned} Y_3(\text{lag time}) = {} & 2.06 + 2.01X_1 - 0.18X_2 + 0.10X_3 \\ & - 0.35X_1X_2 + 0.61X_1X_3 - 0.01X_2X_3 \\ & + 1.64X_1^2 + 0.36X_2^2 - 0.42X_3^2 \end{aligned} \tag{6}$$

Ex vivo Skin Permeation Experiments

The amount of LSP permeated from 15 experimental runs was found to be ranging from 589.1 to 2,429.7 μg in 24 h with a flux of 5.65 to 22.67 μg h^{-1} cm^{-2}. The lag times were found to be decreased significantly from 7.01 to 1.17 h. Experimental run 7 (LSP7) showed maximum amount of LSP (2,429.7 μg) permeated through rat abdominal skin among all the experimental runs. Formulation LSP7W composed of water as vehicle in hydrogel preparation was prepared to investigate the effect of vehicle on permeation. The formulation showed 1,087.1 μg of LSP permeated in 24 h with a flux of 11.5 μg h^{-1} cm^{-2} and permeation coefficient of 1.2 cm/h. The rat skin permeation profile of LSP exhibited zero order permeation at a constant penetration rate (r^2, 0.902–0.992). The independent variables and response (Q_{24}, flux and lag time) of these model formulations were shown in Table I. Figure 2 shows the permeation profiles of hydrogel formulations through excised rat abdominal skin.

DISCUSSION

Ex vivo Skin Permeation Experiments

In our preliminary study, menthol showed a potential enhancement effect on LSP permeation through rat abdominal skin. However, menthol had low solubility in aqueous systems; a co-solvent is required to improve its solubility. Additionally, some reports (19) have indicated that specific combinations of vehicles and enhancers such as menthol in ethanol, and PG had shown an increased in drug penetration. In our previous investigation *Aloe* juice had showed an improvement in percutaneous absorption of LSP (20). Therefore, in this study, the combination of *Aloe* juice containing 10% *v/v* ethanol and PG was used in the preparation of Carbopol-based hydrogels. PG, menthol, and *Aloe* juice were used as multi-enhancers to produce the synergistic enhancement effect on penetration rate of lisinopril and to decrease the used amount of enhancers. Ethanol was used to solubilize menthol, and it also possesses penetration enhancement properties. Formulation LSP7 showed maximum Q_{24} and flux among the hydrogels and was also showed statistically significant ($P<0.05$) difference compared to that of Q_{24} and flux of LSP7W. The results showed that the use of *Aloe* juice in hydrogel preparation showed significant improvement in skin permeation of LSP. In order to quickly obtain an optimal formulation with fewer experimental trials and quantify the effect of these enhancers,

Table III. Summary of Results of Regression Analysis for Responses Y_1, Y_2, and Y_3 for Fitting to Quadratic Model

Quadratic model	R^2	Adjusted R^2	Predicted R^2	SD	% CV	Regression equations of the fitted quadratic model
Response (Y_1)	0.9804	0.9451	0.6921	118.05	8.45	$Y_1=1{,}443.3-602.59X_1+93.24X_2+91.75X_3-18.95X_1X_2-140.93X_1X_3-4.43X_2X_3-152.63X_1^2-150.03X_2^2-213.9X_3^2$
Response (Y_2)	0.9782	0.9392	0.6638	1.10	9.11	$Y_2=11.90-5.22X_1+0.89X_2+0.92X_3-0.31X_1X_2-1.87X_1X_3+0.49X_2X_3+0.50X_1^2-1.89X_2^2+1.76X_3^2$
Response (Y_3)	0.9783	0.9329	0.6849	0.45	15.54	$Y_3=2.06+2.01X_1-0.18X_2+0.10X_3-0.35X_1X_2+0.61X_1X_3-0.01X_2X_3+1.64X_1^2+0.36X_2^2-0.42X_3^2$

a computer optimization technique, including uniform design, Design Expert 2007, was used.

Ex vivo Permeation Kinetics

In order to develop a transdermal drug delivery system for localized and systemic delivery, it is necessary to check the release/permeation profile. The description of permeation profiles by a model function has been attempted using different kinetics (zero order, first order and Higuchi square-root model) and using the following equation (Eq. 7) derived by Korsmeyer *et al.* (21).

$$Mt/M\alpha = Kt^n \quad (7)$$

where $Mt/M\alpha$ is the fractional permeation of drug, Mt is the amount released at time t, $M\alpha$ is the total amount of drug contained in the hydrogel, t is time, K is the kinetic constant, and n is the diffusional release exponent indicative of the operating release mechanism. The drug permeation from Hydrogels followed zero order as the most appropriate model describing permeation kinetics (correlation coefficient between 0.902 and 0.992). On the other hand, n values ($0.119<n>0.254$) indicated that amount of permeated drug was by Fickian diffusion (22).

Fitting of Data to the Model

A three-factor, three-level Box–Behnken statistical experimental design as the RSM requires 15 experiments. Formulation LSP7 showed a significantly higher amount of drug permeation (Y_1, Q_{24}) and higher flux (Y_2) among the experimental runs. All the responses observed for 15 formulations prepared were simultaneously fit to first order, second order, and quadratic models using Design Expert 7.1.5. It was observed that the best fit model was quadratic model and the comparative values of R^2, SD, and %CV are given in Table III along with the regression equation generated for each response. A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between the factor and the response (23). It is evident that all the three independent variables, viz., the concentration of CP-971P (X_1), menthol (X_2), and PG (X_3) have positive effects on the three responses, viz., Q_{24} (Y_1), flux (Y_2), and lag time (Y_3).

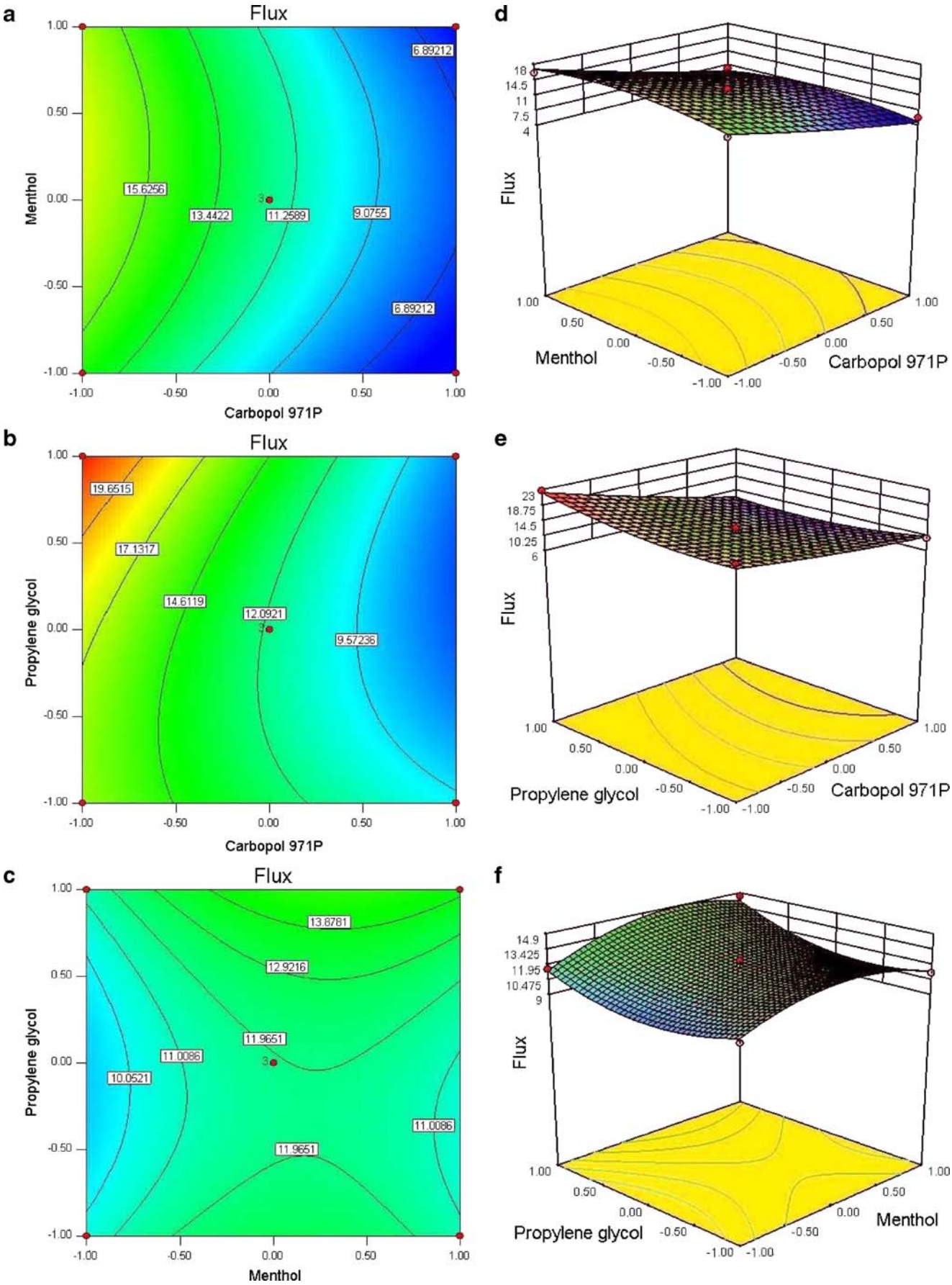
The quantitative effects of the different combination of factors and factor levels on the Q_{24}, flux, and lag time were calculated using response surface models. The significant P values ($P<0.05$), R^2, adjusted R^2, and coefficient of variation values of this model indicated that the assumed regression model was significant and valid for each considered response. The values of the coefficients in the model are related to the effect of these variables on the response. From this model, menthol, PG in *Aloe* juice containing 10% *v/v* ethanol as vehicle system was the best, indicating that the combination of above system had the greatest potential influence on the penetration of lisinopril from hydrogel system. The penetration enhancers used in the investigation works by different mechanisms. Menthol acts by modifying the solvent nature of the stratum corneum and improves drug partitioning into the tissue (24). PG alters the thermodynamic activity of the drug from the reservoir, which would in turn modify the driving force for diffusion, solvent may partition into the tissue facilitating uptake of the drug into skin, and there may be some minor disturbance to intercellular lipid packing within the stratum corneum bilayers (24). The incorporation of ethanol in *Aloe* juice also improved the LSP permeation across skin apart from solubilizing effect. The probable mechanism for the enhancement could be due to the permeation of ethanol into the stratum corneum and can alter the solubility properties of the tissue with a consequent improvement for drug partitioning into the membrane (25). In addition, ethanol as a volatile solvent may extract some of the lipid fraction from the stratum corneum and improve drug flux through skin. Therefore, the incorporation of multi-enhancers in the hydrogels could enhance the LSP permeation that could meet the target flux.

The 3-D response surfaces (Fig. 1d–f) were drawn to estimate the effects of the independent variables on response and to select the optimal formulation. The required flux to reach therapeutic concentration calculated was found to be about 7.5 μg h^{-1} cm^{-2}. Hence, the penetration rate of optimal formulations in the optimization process was set at above 7.50 μg h^{-1} cm^{-2}. Formulation LSP7 showed a flux of 22.67 μg h^{-1} cm^{-2} could meet the target flux (7.50 μg h^{-1} cm^{-2}), calculated from the pharmacokinetic parameters of LSP, indicating that the concentrations may be enough to elicit the pharmacological effect.

Data Analysis

Formulations LSP7, LSP5, LSP12, and LSP3 had the highest Q_{24} and flux. Table II shows the observed and predicted values with residuals and percent error of responses

Fig. 1. Contour plot showing effect of **a** Carbopol 971P (X_1) and menthol (X_2); **b** Carbopol 971P (X_1) and propylene glycol (X_3); **c** menthol (X_2) and propylene glycol (X_3) on response Y_2 (flux); corresponding response surface plots **d–f** ▶


a
Flux
6.89212
15.6256
13.4422
11.2589
9.0755
6.89212
Menthol
Carbopol 971P
b
Flux
19.6515
17.1317
14.6119
12.0921
9.57236
Propylene glycol
Carbopol 971P
c
Flux
13.8781
12.9216
11.9651
11.0086
10.0521
11.0086
11.9651
Propylene glycol
Menthol
d
Flux
Menthol
Carbopol 971P
e
Flux
Propylene glycol
Carbopol 971P
f
Flux
Propylene glycol
Menthol

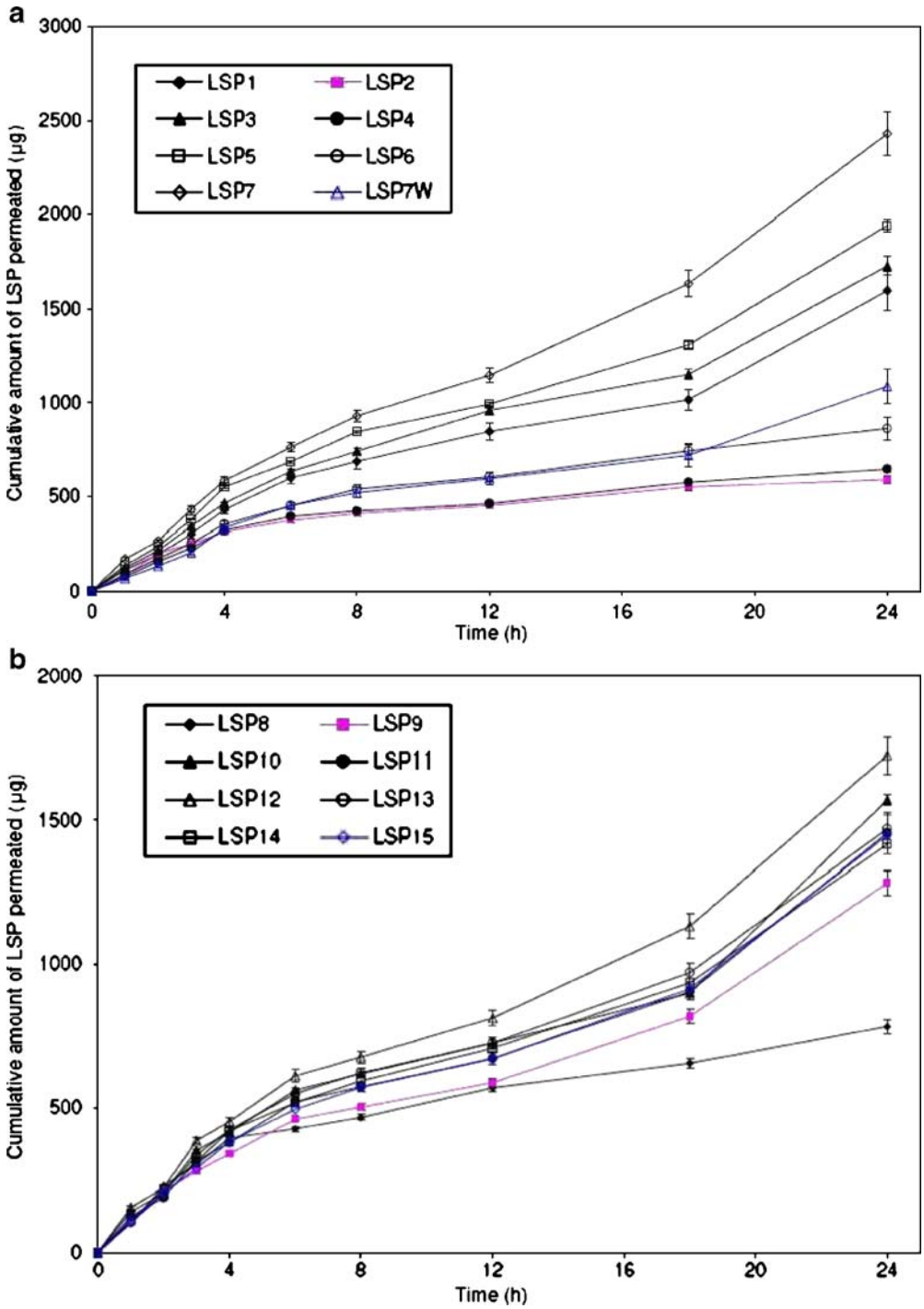


Fig. 2. **a**, **b** Permeation profiles of lisinopril from hydrogels

for all the formulations. The Q_{24} and flux (dependent variable) obtained at various levels of the three independent variables (X_1, X_2, and X_3) was subjected to multiple regression to yield a second-order polynomial equation (full model). The value of the correlation coefficient (r^2) of Eq. 4 was found to be 0.9804, indicating good fit (Table III). The Q_{24} values measured for the different formulations showed wide variation (i.e., values ranged from a minimum of 589.1 µg in LSP2 to a maximum of 2,429.7 µg LSP7). The results clearly indicate that the Q_{24} value is strongly affected by the variables selected for the study. The main effects of X_1, X_2, and X_3 represent the average result of changing one variable at a time from its low level to its high level. The interaction terms (X_1X_2, X_1X_3, X_2X_3, X_1^2 , X_2^2 , and X_3^2) show how the Q_{24} changes when two variables are simultaneously changed. The negative coefficients for all three independent variables indicate an unfavorable effect on the Q_{24}, while the positive coefficients for the interactions between two variables indicate a favorable effect on Q_{24}. Among the three independent variables, the lowest coefficient value is for X_1 (−602.59), indicating that this variable is insignificant in prediction of Q_{24}.

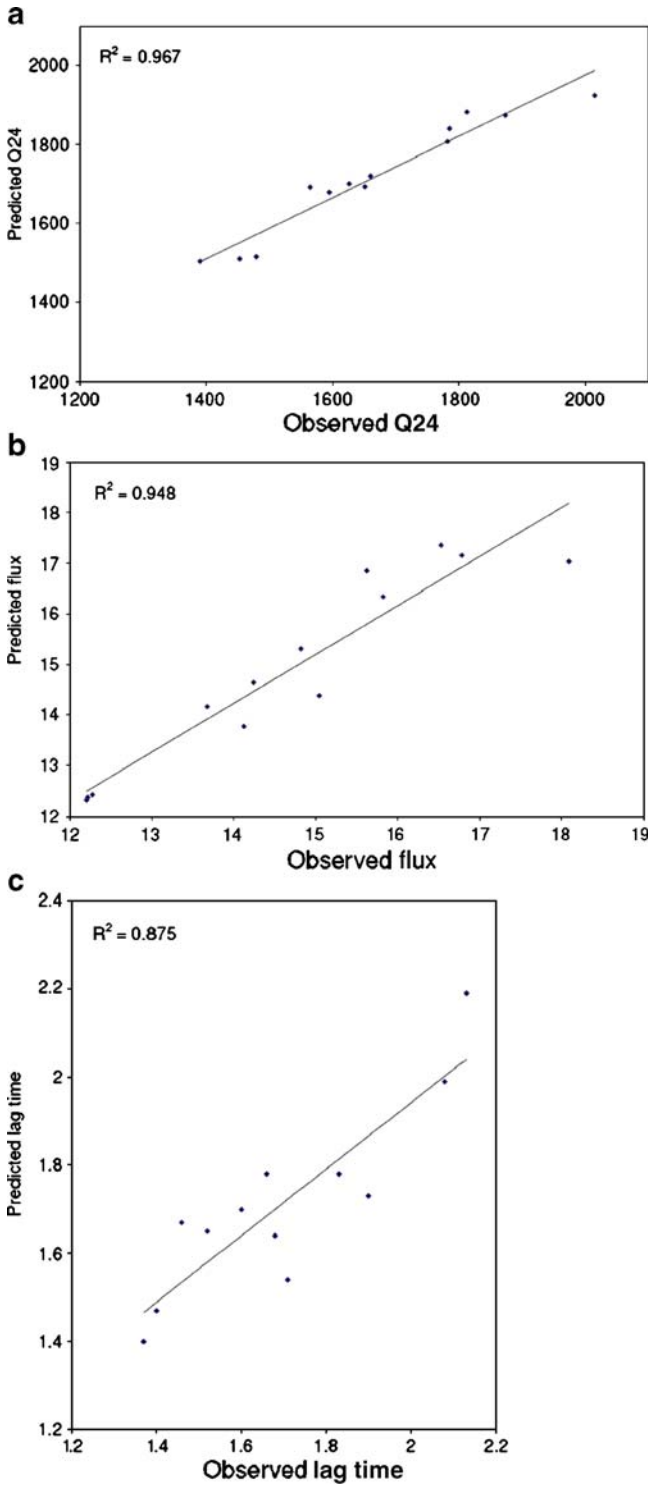


Fig. 3. Linear correlation plots **a–c** between actual and predicted values

The value of the correlation coefficient (r^2) of Eq. 5 was found to be 0.9782, indicating good fit (Table III). The flux values of LSP7, LSP5, LSP12, and LSP3 were found to be more among the experimental trials. The flux values were found to be increased from medium to high levels of variables X_2 and low to high levels of X_3. The flux values measured for the different formulations showed wide variation (i.e., values ranged from a minimum of 5.65 μg h^{-1} cm^{-2} in LSP2 to a maximum of 22.67 μg h^{-1} cm^{-2} in LSP7). The interaction terms (X_1X_2, X_1X_3, X_2X_3, X_1^2 , X_2^2 , and X_3^2) show how the flux changes when two variables are simultaneously changed. The positive coefficients (X_1X_3) for the interactions between two variables indicate a favorable effect on flux. Among the three independent variables, the lowest coefficient value is for X_1 (−5.22), indicating that this variable is insignificant in prediction of flux.

The value of the correlation coefficient (r^2) of Eq. 6 was found to be 0.9783, indicating good fit (Table III). Among the independent variables selected and their interactions, only X_1 and X_3 (Eq. 6) were found to be significant ($P<0.05$), indicating a major contributing effect of X_1 and X_3 on lag time. A positive value of the coefficient for X_1 (CP-971P) and X_3 (propylene glycol) indicates a favorable effect on lag time. The hydrogels composed of high propylene glycol and low polymer concentration yielded low lag time.

Contour Plots and Response Surface Analysis

Two-dimensional contour plots and 3-D response surface plots are shown in Fig. 1, which are very useful to study the interaction effects of the factors on the responses. These types of plots are useful in study of the effects of two factors on the response at one time. In all the presented figures, the third factor was kept at a constant level. All the relationships among the three variables are non-linear, although they exhibit a nearly linear relationship of factor X_2 with factors X_1 and X_3, in the form of almost straight lines up to the medium level of menthol concentration (Fig. 1). At higher concentrations of menthol, these become curvilinear or non-linear. Factors X_2 and X_3 have curvilinear relationship at all levels of the two variables on the response Y_2. Response surface plots show the relationship between these factors even more clearly. The Q_{24} and flux were found to be increased with increasing concentrations of either menthol (up to medium level) or PG at constant concentration of polymer.

Optimization

The optimum formulation was selected based on the criteria of attaining the maximum value of Q_{24}, target flux, and low value of lag time by applying constraints on Y_1 ($1{,}000 \le Y \le 2{,}500$), Y_2 ($7.5 \le Y \le 25$), and Y_3 ($0.50 \le Y \le 2.50$). Upon trading of' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with CP-971P concentration of 0.64%, menthol 7.90%, and propylene glycol 14.9% was found to fulfill the maximum requisite of an optimum formulation because of maximum Q_{24}, target flux, and low lag time values.

Validation of Response Surface Methodology

Fifteen checkpoint formulations were obtained from the RSM, the composition and predicted responses of which are listed in Table II. To confirm the validity of the calculated optimal parameters and predicted responses, the optimum formulations were prepared according to the above values of the factors and subjected to *ex vivo* permeation studies. From

the results presented in Table II, the predicted error is below 15%, indicating that the observed responses were very close to the predicted values. Percentage prediction error is helpful in establishing the validity of generated equations and to describe the domain of applicability of RSM model. Linear correlation plots between the actual and the predicted response variables were shown in Fig. 3. The linear correlation plots drawn between the predicted and experimental values demonstrated high values of R^2 (Q_{24}, 0.967; flux, 0.948; and lag time, 0.875) indicating goodness of fit. Thus, the low magnitudes of error as well as the R^2 values in the present investigation prove the high prognostic ability of the RSM.

CONCLUSIONS

Optimization of hydrogel formulation is a complex process that requires one to consider a large number of variables and their interactions with each other. The present study conclusively demonstrates the use of a Box–Behnken statistical design is valid for predicting the Q_{24}, flux, and lag time in optimization of hydrogel formulations. The derived polynomial equations and contour plots aid in predicting the values of selected independent variables for preparation of optimum hydrogel formulations with desired properties. Hydrogels could be prepared having suitable flux. Further studies are recommended to prove the therapeutic efficacy by pharmacokinetic or/and pharmacodynamic studies in humans.

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