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Original Article

Use of Box-Behnken Experimental design for Optimization of process Variables in Iontophoretic delivery of Repaglinide

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ABSTRACT

Objective: In this study, a Box-Behnken design (BBD) of response surface method was used to investigate and optimize the process parameters of Repaglinide. Repaglinide is a suitable candidate for iontophoretic delivery due to its first pass metabolism and frequent dosing, hence transdermal delivery was required to improve bioavailability and patient compliance. The goal was to identify the optimum levels of independent variables intended for the dependent variable. **Materials and Method:** Independent variables selected were current intensity (X_1), medium/pH (X_2), polymer (HPMC) concentration (X_3). The dependent variables studied were amount of drug permeated in 4 h (Y_1: Q_4), 24 h (Y_2: Q_{24}) and lag time (Y_3). The BBD provided an excellent relationship between the independent and dependent variables. Mathematical equations, response surface and contour plots were used to show the interactions and discuss the results in graphic model. **Results:** The regression equation generated for the iontophoretic permeation was $Y_1 = 898.68 + 114.51X_1 + 325.29X_2 - 0.83X_3 + 142.62X_1X_2 - 27.03X_1X_3 - 47.84X_2X_3 - 8.85X_1^2 - 90.26X_2^2 - 302.09X_3^2$; $Y(2) = +3446.82 + 275.42X_1 + 1092.93X_2 + 37.23X_3 + 136.49X_1X_2 - 114.52X_1X_3 - 241.01X_2X_3 - 164.78X_1^2 - 487.25X_2^2 - 990.88X_3^2$. The results of statistics between factorial and theoretical profiles were used to select optimized critical process parameters. **Conclusion:** Thus, it was concluded that optimal use of amount of polymer and current intensity in the permeation of drug could increase the bioavailability and the interactions can be defined as a regression model which is statistically significant.

Key words: Box-Behnken, Factorial design, Iontophoresis, Optimization, Repaglinide, Transdermal.

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INTRODUCTION

Repaglinide is an oral blood glucose-lowering drug used to treat NIDDM (noninsulin-dependent diabetes mellitus). It drops the blood level glucose by stimulating the release of insulin. It has an oral bioavailability of 56% due to extensive hepatic first-pass metabolism and extreme short half-life of 1 h.[1,2] To overcome the problem of hepatic metabolism and to improve bioavailability for the effective treatment of diabetes, alternate long acting formulations may be beneficial.[3] Repaglinide topical route of administration may be helpful to the patient to circumvent these problems. It is further justified that requirement of drug for maintaining unfluctuating plasma concentrations for the management of blood sugar in diabetic patients for a long period. Repaglinide is a suitable candidate for the development of transdermal dosage form because of its melting point of 130-131°C and mol. wt. 452.58 daltons and a low dose 2.0-16 mg/day.[2,4] Because of low permeability and barrier properties of skin a classical administration is not adequate, however, only a small amount of drug molecules can penetrate passively through the skin. Polar, neutral and ionic molecules show limited penetration. Ions permeate the skin at much lower rate compared to neutral compounds. Techniques such as chemical enhancers and iontophoresis were successfully used to enhance ionic drug permeation.[5] Iontophoresis defined as the facilitation of (ionizable) drug permeation across the skin by an applied electric potential. It is a noninvasive and painless means of delivering various drugs into the body.[6] Increased drug permeation is due to the following mechanisms: Electro-repulsion (for charged solutes), electro-osmosis (for uncharged solutes), thus enhancing the flux of charged drugs. Currently, 0.5 mA/cm^2 is tolerable for patients, the onset of action is rapid with iontophoresis in contrast to hours in passive diffusion. Since the amount of drug permeated through iontophoresis is proportional to intensity of current applied, the benefit includes the possibility of pre-programming the drug delivery and time in constant or pulsatile fashion. Generally to develop a formulation, traditional experiments require more time and materials, so an issue was to design the pharmaceutical formulation within shorter time and minimum trials showing maximum penetration rate.[7] A widely accepted approach in the development of drug delivery is Response surface method (RSM) which aims at approximate regression model that is closest to the actual regression model. In this work, transdermal formulation was developed an approach to illustrate the interactions caused by three different factors such as current intensity, medium and polymer concentration by the aid of Box-Behnken experimental design. The responsibility of each factor to the drug permeation has been defined and the results have been discussed statistically.

MATERIALS AND METHODS

Materials

Repaglinide was received as a gift sample from Symid Laboratories, Hyderabad, India. HPMC E15 was purchased from Qualikem fine chemicals Ltd, Hyderabad, India. PVP K30 was obtained from SD fine-Chem. Ltd, Mumbai, India. All other materials and chemicals used were of analytical grade.

Methods

Drug-Excipients Compatibility study

A Fourier Transform Infra-Red spectrophotometer (FTIR Spectrum BX series 2.19 version) equipped with spectrum v 2.19 software was used to study the non-thermal analysis of drug-excipient (binary mixture of drug: excipient 1:1 ratio) compatibility. The spectrum for each sample was recorded over the 450-4000 cm^{-1} spectral region with a resolution of 4 cm^{-1}.

Preparation of Repaglinide transdermal patch

Matrix type transdermal patches containing Repaglinide were prepared by solvent evaporation technique, using different ratios of HPMC E15. The polymers were weighed accurately in requisite ratios and dissolved in solvent mixture (1:1 ratio of methanol and dichloromethane), allowed for swelling for about 6 h. 15% v/w dibutyl phthalate was incorporated as plasticizer, then the drug solution was added to the polymeric solution followed by addition of d-Limonene as penetration enhancer and casted onto the anumbra petri plate with total area of 43.56 cm^2 allowed for air drying overnight followed by vacuum drying for 8-10 h. The entire sheet was cut into small patches of required area and stored in desiccator for further studies. Composition of preliminary batches is shown in Table 1.

Preparation of rat abdominal skin

The male albino rats weighing 150-200 g were sacrificed using and the hair was carefully trimmed short (<2 mm) with a trimmer taking extreme precaution not to damage the skin and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique.[8] The epidermis was washed and hydrolyzed with water and used for *ex vivo* permeability studies.

Preparation of electrodes

The silver-silver chloride electrodes used for this study were prepared by dipping silver wire of 0.5 mm diameter into molten silver chloride to form a uniform thin coat. Before start using, the electrodes were immersed in 0.1 M HCl.

Iontophoresis of Repaglinide

In the iontophoretic experiment, a portable iontophoresis system is applied to generate a weak current of 0.5 mA/cm^2 using silver/silver chloride electrodes.[9] Pure silver wire as anodal electrode, and silver chloride (AgCl) electrode as cathodal electrode was connected to a power source. Initially the receptor compartment filled with phosphate buffer was used for skin integrity test for 3 h by placing the skin between two compartments with methyl red solution in donor compartment. Thereafter, the skin was washed thoroughly and mounted between the compartments with patch positioned above the skin. Now a power supply was used to deliver a constant direct current for about 2 h via electrodes. The active electrode effectively repels the drug and forces into the skin.[10] The samples were withdrawn at predetermined time intervals and analyzed. The effect of various iontophoresis limits including applied current density, pulsatile condition was studied.[11]

Optimization of variables using Experimental design

The experiments were performed according to the Box-Behnken design which is a kind of response surface methodology (RSM), a collection of statistical and mathematical techniques useful for modeling and analyzing the problems in which a response of interest is influenced by several variables and the objective is to optimize this response.[10-12] It is an empirical technique developed for analyzing and studying the relationship between set of controlled experimental factors and observed results.[13] To analyze a process mutually with a response Y which mainly depends on the input factors X_1, X_2, …, X_n, the correlation between the response and the input process parameters are described as $Y = f(X_1, X_2, \ldots, X_n) + \varepsilon$, where f is the response function and ε is the error. Since, the correlation between the response and input variables are described as a surface of the X_1, X_2, coordinates in the graphical sense, hence named the response surface study.[10-12] In this design, 3 independent factors were evaluated, each at 3 levels, and experimental trials were performed for all 13 possible combinations. Current density (X_1) medium (X_2) and HPMC concentration (X_3) were chosen as independent variables strength, permeation of drug at 4 h ($Q4$) Y_1, amount of drug permeated at 24 h ($Q24$) Y_2 and lag time h Y_3 were dependent variables. The formulation layout for the factorial design batches (F1 to F9) are shown in Table 2 and 3.

Table 1: Composition of Repaglinide Transdermal Patches

Formulation Code	Drug (mg)	HPMC E15 (mg)	d-Limonene (% v/w)	Plasticizer (% v/w)
F1	34	238	5	15
F2	34	272	5	15
F3	34	340	5	15

Each patch contains 4 mg of Repaglinide.

Table 2: Variables in BB statistical design

Factor	Levels		
	(-1) Low	(0) Medium	(+1) High
Independent Variables			
X1 Current density mA	0.05	0.25	0.50
X2 Medium used pH	5.8 pH	Water	7.4 pH
X3 Polymer concentration mg	238	272	340

Dependent Variables

Y1 = Q4 Cumulative amount of drug permeated in 4 h μg/cm^2

Y2 = Q24 Cumulative amount of drug permeated in 24 h μg/cm^2

Y3 = Lag time h

Characterization of Transdermal patches (Table 4)

Film thickness, weight variation

The thickness of patches were measured using screw gauge and the weight was determined using electronic balance. The experiment was performed in triplicate and the mean was calculated.

Drug content[14]

A specified area of patch was dissolved in 100 mL buffer and shaken continuously for 24 h, filtered and the resultant solution is analyzed by UV spectrophotometer at 283 nm.

Folding endurance[15]

Folding endurance was determined manually by folding and unfolding the medicated patch at the same place repeatedly till it broke. The number of times the strip could be folded at the same place without breaking gave the folding endurance.

Percentage moisture content[17]

The films were weighed individually and placed in a desiccator containing calcium chloride for 24 h. After 24 h, the films were reweighed and percentage moisture content was determined from formula mentioned below

Percentage moisture content=Initial weight−Final weight/
Final weight×100.

Percentage Moisture absorbed[17]

The weighed films were kept in a desiccator containing saturated solution of potassium chloride for 24 h in order to maintain 84% RH. After 24 h, the films were reweighed and the percentage moisture uptake was determined from the formula mentioned below

Percentage moisture uptake = Final weight − Initial weight/
Initial weight × 100.

In vitro drug release

In vitro drug release studies were performed by using a Franz diffusion cell. The cellulose acetate membrane was mounted between the donor and receptor compartments. The patch was placed on the membrane and

Table 3: Observed responses in the iontophoresis of Repaglinide by BB statistical design

Batch	Independent Variables			Dependent Variables (mean ± SD)		
	current mA (X_1)	Medium (X_2)	Polymer mg (X_3)	Q4 (Y_1) µg/cm²	Q24 (Y_2) µg/cm²	Lag time (Y_3) h
B01	0	0	0	898.68	3446.82	0.42
B02	0	1	1	550.16	2126.06	0.62
B03	1	0	1	658.9	2564.93	0.52
B04	0	1	-1	781.24	2988.6	0.45
B05	1	0	-1	580.87	2264.54	0.82
B06	-1	1	0	951.3	3828.25	0.41
B07	0	-1	1	327.11	1430.8	0.23
B08	1	-1	0	362.61	1488.35	0.36
B09	-1	0	1	648.68	2546.82	0.22
B10	0	-1	-1	366.83	1329.3	0.37
B11	1	1	0	1630.28	4955.78	0.63
B12	-1	-1	0	254.11	906.8	0.31
B13	-1	0	-1	462.52	1788.35	0.32

Table 4: Evaluation Parametersof Preliminary patches

Trial code	Weight variation(mg)	Thickness variation(mm)	Folding endurance	Drug content (mg)	% Moisture absorbed	% Moisture content
B1	25.25 ± 0.67	0.19 ± 1.52	232.48 ± 0.64	3.35 ± 0.96	5.92 ± 1.25	4.38 ± 0.77
B2	29.26 ± 0.59	0.22 ± 1.27	290.7 ± 0.74	3.36 ± 1.29	6.14 ± 1.53	4.82 ± 0.85
B3	37.55 ± 0.55	0.25 ± 0.65	362.45 ± 0.53	3.39 ± 0.84	8.27 ± 0.95	6.97 ± 1.17

Values expressed as mean ± S.D, n=3.

Table 5: Summary of results of regression analysis for responses

Quadratic model	R^2	Adjusted R^2	Predicted R^2	SD	%CV
Y_1	0.9267	0.6040	0.5721	.21	29.66
Y_2	0.9599	0.6797	0.5424	.61	22.94
Y_3	0.8332	0.6187	0.5689	.09	21.29

clamped together. The receptor compartment was filled with phosphate buffer pH 7.4 and the whole assembly was placed on magnetic stirrer and maintained at 37°C. Aliquots of 1 mL sample were withdrawn and replenished with an equal volume of phosphate buffer at predetermined time intervals and analyzed at 283 nm by UV spectrophotometer.

Ex vivo permeation studies

Franz diffusion cell was used for *ex vivo* permeation studies. The isolated rat abdominal skin was mounted between the donor and receptor compartments. The patch was placed on the rat skin and clamped together. The receptor compartment was filled with phosphate buffer pH 7.4 maintained at 37°C by stirring magnetically at 200 rpm. Aliquots of 1 mL sample were withdrawn at predetermined time intervals and analyzed by HPLC. The amount of drug permeated was calculated and Flux was determined from the slope of the curve between the amount of drug permeated versus time.

Release kinetic data

The release profile were fitted in to various mathematical models such as Zero order, First order, Hixon and Crowell,[18] Higuchi and Korsmeyer.[19]

RESULTS AND DISCUSSION

The study was to identify the controlling factors responsible for the permeation of Repaglinide. A set of initial trials were carried to establish the range for each process variable to optimize the iontophoretic delivery of Repaglinide across abdominal skin. The initial trials conducted revealed the current density in the range of 0.05-0.5 mA/cm² as low and high current density respectively, based on these observation the centre level 0.25 mA/cm² was also used in the design (Table 4). Thirteen experiments were carried out based on BBD and the experimental runs and observed responses were given in the Table 3. The range of the responses were found to be 254.11 µg/cm² in B12 and 951.3 µg/cm² in B06; 906.8 µg/cm² in B12 and 3828.25 µg/cm² in B06 as Y_1 and Y_2 respectively. The predicted and actual values were reasonably good and were tested using analysis of variance (ANOVA). The ANOVA indicated a significant effect ($p<0.05$) of factors on response.

The further elucidation was carried out using response surface plots and contour plots. The effects of current density and medium and their interaction on Q_1 at fixed level of polymer concentration are given in 1a and 1b. At high levels of medium the Y_1 increased from 550.16 to 1630.28 µg/cm² when current density was increased from 0.25 to 0.5 mA/cm². Similarly at low levels of medium Y_1 increased from 254.11 to 366.83 µg/cm² when current density was increased from 0.05 to 0.25 mA/cm². As shown in Figure 2a and 2b, with high pH medium the response Y_2 was found to be increased from 2126.06 to 4955.78 µg/cm² when current density was increased from 0.25 to 0.5 mA/cm² and with low pH medium Y_1 increased from 906.8 to 1329.3 µg/cm² when current density was increased from 0.05 to 0.25 mA/cm². The cumulative amount of drug permeated in 4 h and 24 h were found to be increased from 254.11 and

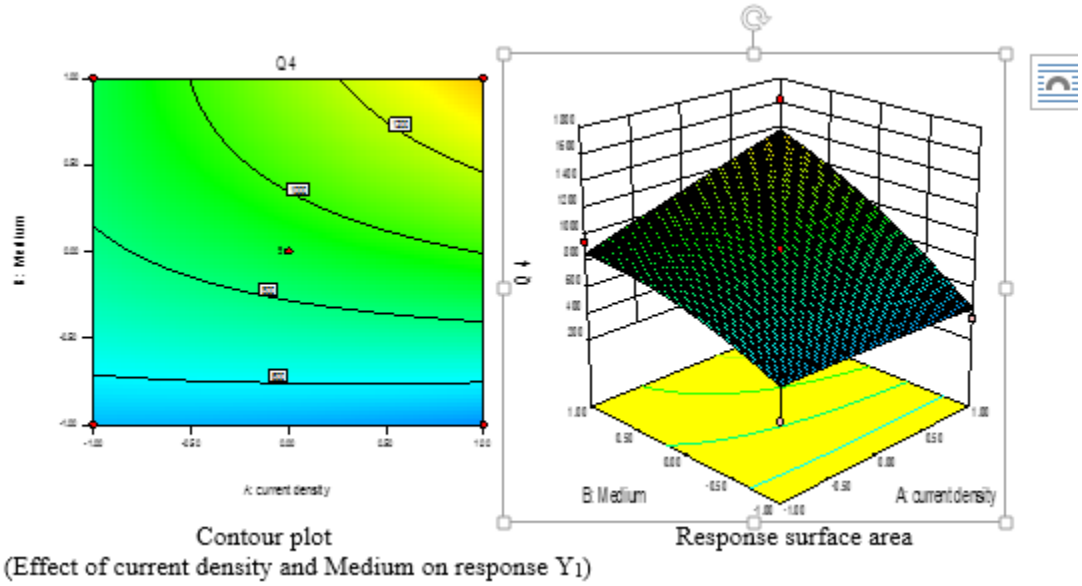

Figure 1: Effect of current density and polymer on Q4.

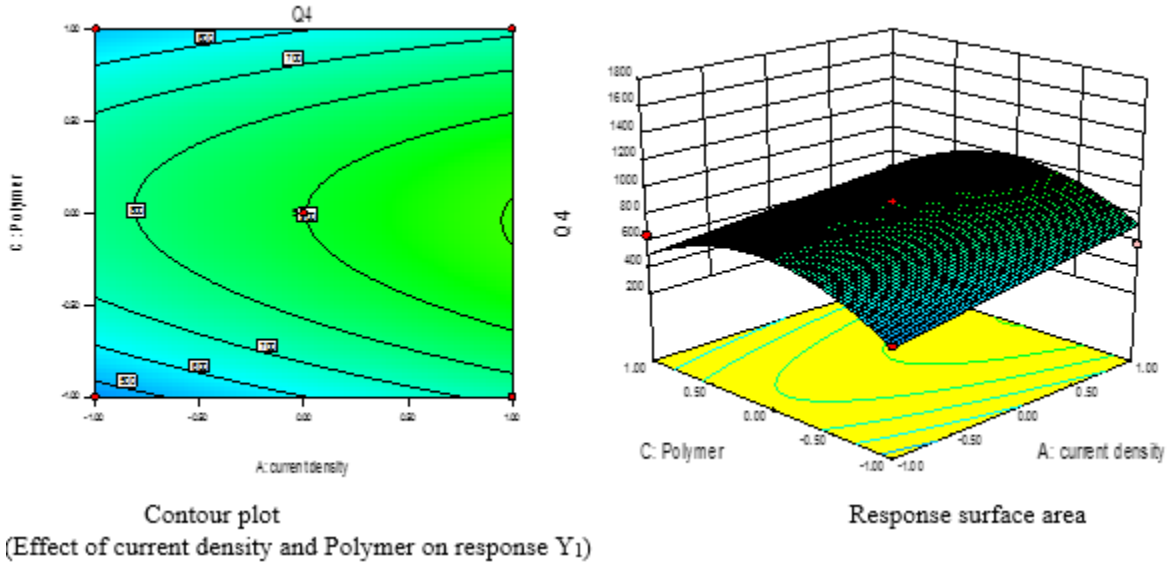

Figure 2: Effect of current and medium on response Y1.

1630.28 µg/cm^2 to 906.8 and 4955.78 µg/cm^2 in B12 and B11 respectively, using 272 mg of HPMC polymer. The results suggest that decrease in the pH of the medium decreased the iontophoretic permeation of Repaglinide, therefore the permeation of drug increases with increase in current density and increasing the pH of the medium.

Data analysis

By applying regression analysis methods, the predicted response have been obtained and given as

$Y_1=898.68+114.51X_1+325.29X_2-0.83X_3+142.62X_1X_2-27.03X_1X_3-47.84X_2X_3-8.85X_1^2-90.26X_2^2-302.09X_3^2$

$Y(2) = +3446.82 + 275.42\ X_1 + 1092.93\ X_2 + 37.23\ X_3 + 136.49\ X_1X_2 - 114.52\ X_1X_3 - 241.01\ X_2X_3 - 164.78\ X_1^2 - 487.25\ X_2^2 - 990.88\ X_3^2$

$Y(3) = +0.42 + 0.13X_1 + 0.11X_2 - 0.046X_3 + 0.042X_1X_2 - 0.050\ X_1X_3 + 0.077X_2X_3 + 0.030X_1^2 - 0.022X_2^2 + 0.020\ X_3^2$.

Where Y_1, Y_2 and Y_3 are the predicted response and X_1, X_2 and X_3 are the coded values of the test variables, current density, medium and polymer concentration.

The value of R^2 was found to be 0.9267, (Table 5) indication good fit. The results clearly indicate that permeation of drug is strongly affected by the selected variables. The positive coefficients shows the interactions between the variables (X_1X_2) indicate favourable effect on Q_4 and the negative coefficients indicate an unfavourable effect on Q_4. The lowest coefficient value is for X_2^2 indicating that this variable is insignificant in prediction of Q_4. Among the selected independent variables and their

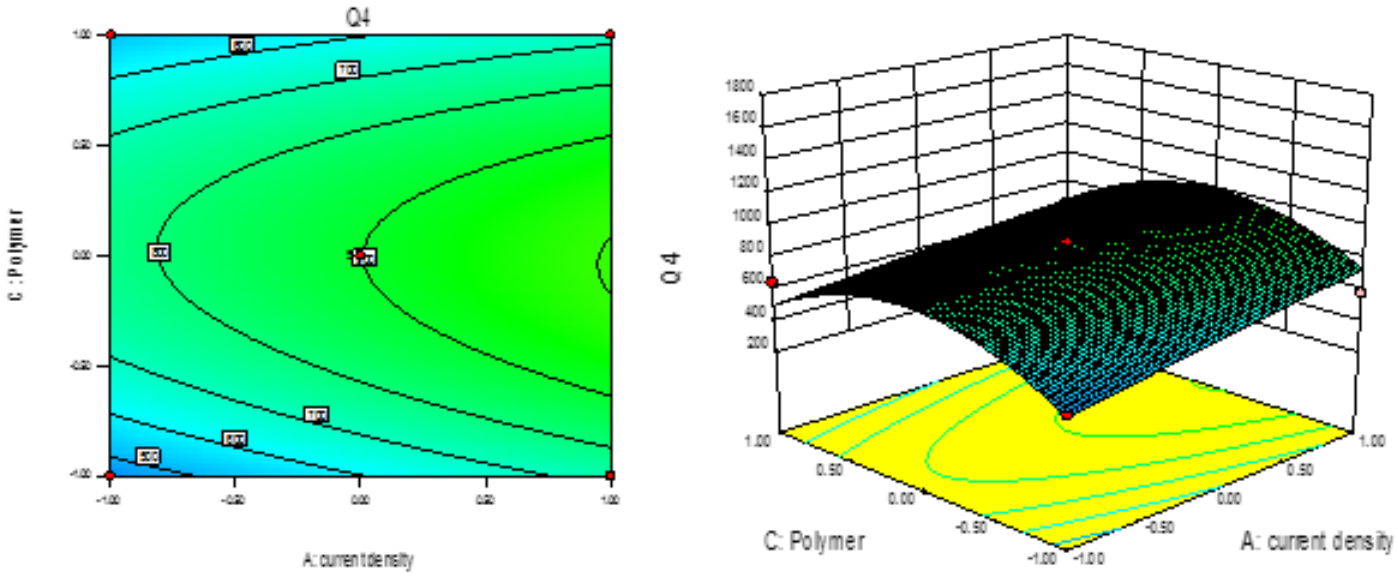

Figure 3: Effect of current and polymer on Y2.

interactions, only X_1 and X_2 were found to be significant ($p<0.05$), indicating favourable effect on lag time. The experiments conducted with high current density and 7.4 pH medium yielded low lag time.

The contour plots and response surface plots are presented in Figure 1a-1b and 2a-2b, which were useful to study the interactions between factors on responses. They are also useful to study the effect of two factors at one time on the response keeping third factor constant level. All the relationships among three variables are curvilinear relationship but Figure 1a exhibits a slight linear relationship in the form of straight line from low to medium level of current density. The response surface plots showed the relationship even more clearly. The results revealed that Q_4 and Q_{24} were found to be increased with increasing current density at high level of pH medium and medium level of polymer. Check point analysis were conducted and evaluated. The validity of the results predicted by regression model were compared and revealed that they were as expected. The validity of the results which were predicted by regression model was confirmed by conducting repeated experiments. The results obtained from replication of three confirmed that the average cumulative amount of drug permeated Q_{24} (3818 μg/cm^2) obtained was close to the predicted value (3810 μg/cm^2) and the predicted error was found to be 0.209%, this indicates excellent correlation between experimental values and predicted values.

CONCLUSION

The permeation of drug was influenced by medium of the donor compartment and current density. The results of the study confirm that the factors current density and medium pH significantly influence the response variables. The application of BBD statistical technique for optimization of process parameters helps in reaching optimum level with minimum experiments in shortest time.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

ABBREVIATIONS USED

BBD: Box behnken design; **NIDDM:** Non insulin dependent diabetes mellitus; **HPMC:** Hydroxy propyl methyl cellulose.

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