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Optimization of matrix diffusion mediated transdermal therapeutic system for buspirone: Effect of variables on *in vitro* release, *ex vivo* permeation and pharmacotechnical properties

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Abstract

The purpose of present study was to develop and optimize matrix type transdermal patches for buspirone, a low bioavailable drug. Transdermal patches were optimized using a three-factor, three- level Box–Behnken design, the independent variables selected were d-limonene, transcutol and propylene glycol; dependent variables (responses) were cumulative amount released in 24 h (Q24R; Y1), flux (Y2), and tensile strength (Y3). Mathematical equations and response surface plots were used to relate the dependent and independent variables. All formulations followed zero order release kinetics and the release pattern was found to follow non-fickian model. The regression equations were generated for responses Y_1, Y_2 and Y_3. 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 7 confirmatory runs indicated high degree of prognostic ability of response surface methodology. The optimized formulation (B-OPT) showed a flux of 92.5 $\mu g\ cm^{-2}\ h^{-1}$, which could meet target flux. Matrix diffusion mediated transdermal patches having suitable mechanical properties for BUSP were developed and optimized using Box–Behnken statistical design.

Keywords: Matrix type TDDS, buspirone, Box-Behnken, optimization, mechanical properties, tensile strength

Introduction

Buspirone (BUSP) is an anxiolytic drug that has dopaminergic, noradrenergic and serotonin-modulating properties. BUSP is rapidly absorbed from the gastrointestinal tract but systemic bioavailability is low (4%) because of extensive first pass metabolism (Iftekhar and Chandra 1999). Most of the metabolites are inactive, although oxidative dealkylation produces an active metabolite, 1-(2–pyrimidinyl)-piperazine which is about 20 to 25% as potent as parent drug. The major metabolite is 5–hydroxybuspirone. The metabolites are excreted mainly in urine (65%) and faeces (35%) (Clarke's 2007). The mean elimination half life of unchanged BUSP after a single 10–40 mg oral dose is merely 2–3 h (Dollery 1999). The low oral bioavailability restricts its use. Therefore, current BUSP treatment generally involves taking three daily oral doses of between 5 and 20 mg each. Due to the chronic nature of therapy required, a decrease in the number of daily doses would be desirable, as it would greatly enhance patient compliance. To overcome the problem of first pass metabolism, improve bioavailability and for effective treatment of anxiety, an alternative long-acting formulations could be beneficial. Transdermal route of administration may be a good alternative to circumvent these problems. For certain drugs, transdermal delivery offers a number of advantages with respect to oral or parental administration: improved patient compliance, reduced side-effects, elimination of first-pass effect, interruption or termination of treatment when unnecessary (Vaddi et al. 2001), sustaining drug delivery, maintaining a constant and prolonged drug

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level in plasma, minimizing inter and intra patient variability (Keith 1983, Chien 1987). BUSP possesses ideal characteristics, such as a low molecular weight (422 Da), low dose range (15-30 mg), short plasma half-life (2.4 h) and poor oral bioavailability (4%) (Clarke's 2007) for formulation as a transdermal patch.

There are no reports on transdermal delivery systems for BUSP. However two reports were based on iontophoresis (Mohammad et al. 2003, Victor et al. 2003). Designing drug delivery systems with minimum number of experiments is very crucial for pharmaceutical scientists (Hamed and Sakr 2001). In this study, we demonstrate the use of response surface methodology (Box Behnken design) to optimize the matrix type transdermal patches for BUSP.

The objective of the present study was to develop matrix type transdermal patches for BUSP using HPMC as polymeric matrix. The independent variables selected are d-limonene (DLM), transcutol (TRA) and propylene glycol (PG); dependent variables are cumulative amount released in 24 h (Y_1, $Q_{24}R$), flux (Y_2) and tensile strength (T.S, Y_3).

Materials and Methods

Materials

Buspirone hydrochloride, hydroxypropylmethylcellulose E 15 (HPMC) and Transcutol (Gattefosse, France) were gift samples from Dr Reddys Laboratories, Hyderabad, India. D-limonene and Dulbeccos buffer (pH 7.4) were purchased from Himedia, Mumbai, India. Propylene glycol was purchased from Merck, Mumbai, India. All other chemicals and solvents were of analytical reagent grade.

Development of transdermal systems

Matrix type transdermal patches containing BUSP were prepared by solvent evaporation technique, using HPMC E15 as polymeric matrix. Fixed amount (2.5 g) of HPMC was weighed and allowed for swelling for about 6 h in 20 mL of solvent mixture (1:1 ratio of dichloromethane, methanol). Required quantities of d-limonene (DLM), transcutol (TRA) and propylene glycol (PG) were added as penetration enhancers and plasticizer respectively. Then the drug solution (200 mg of BUSP in 5 mL of solvent mixture) was added to the polymeric solution, casted on to anumbra petri dish of surface area about 70 cm^2, allowed for air drying over night followed by vacuum drying for 8-10 h. Control (B-C) was prepared by incorporating 10% v/w of PG. The entire sheet was cut into small patches with an area of 3.56 cm^2 and used for characterization.

In vitro release studies

The drug release studies from BUSP transdermal patches were performed using Franz diffusion cells with a surface area of 3.8 cm^2. Commercially available water impermeable adhesive backup membrane was placed over the patches (3.56 cm^2). Then the transdermal patch was placed in a dialysis membrane (Himedia, Mumbai, India) so as to face the release surface of the patch towards receptor compartment and was further placed between donor and receptor compartments. Phosphate buffer pH 5.6 (25 mL) was placed in receptor compartment. The whole assemble was placed on multi magnetic stirrer (Cintex, Mumbai, India). The contents of receptor compartment were stirred at 400 rpm and study was conducted at 32 ± 0.5°C. Samples (1 mL) were collected up to 24 h and the drug released was estimated using high performance liquid chromatography (HPLC) (Ramesh et al. 2009). Mathematical expressions, zero order (Donbrow and Samuelov 1980), first order (Wagner 1969), Higuchi (Higuchi 1963) and to analyze the release pattern Peppas equation (Korsmeyer et al. 1983) were applied to analyze the release mechanism from the transdermal patches.

Preparation of rat abdominal skin

The animal study was conducted in accordance with the approval of the animal ethical committee, Kakatiya University, India. Albino rats weighing 150-200 g were sacrificed using anesthetic ether. The hair of test animals was carefully trimmed with electrical clippers and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique (Levang et al. 1999), 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 cells were used for *ex vivo* permeation studies. The rat skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The stratum corneum side of the skin was kept in intimate contact with the release surface of the transdermal patch under test. A dialysis membrane was placed over the skin, so as to secure the patch tightly not to get dislodged from skin. 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. Samples of 1 mL were collected at preset time point up to 24 h and replenished with an equal volume of PBS pH 7.4. The amount of drug permeated was estimated using HPLC (Ramesh et al. 2009) and concentration was corrected for sampling effects according to Eq.1 (Hayton and Chen 1982).

$$C^1_n = C_n (V_T/V_T - V_S) (C^1_{n-1}/ C_{n-1}) \qquad \text{Eq. 1}$$

Where C^1_n is the corrected concentration of n^{th} sample, C_n is the measured concentration of BUSP in n^{th} sample, C^1_{n-1} is measured concentration of BUSP in $(n-1)^{th}$ sample, V_T is total volume of receiver fluid and V_S is the volume of sample drawn.

The steady state flux (Jss) was calculated from slope of the steady state portion of line in the plot of drug amount permeated Vs time. Permeability coefficient (Kp) was calculated by dividing the flux with the amount of drug in the patches. The lag time was calculated from intercept on time axis in the plot of cumulative amount permeated Vs time. The target flux was calculated using Eq 2.

$$\text{Target flux} = \frac{Css\ CLt\ B.W}{A} \qquad \text{Eq. 2}$$

Css, the BUSP concentration at therapeutic level (2.5 μg L^{-1}) and CLt the total body clearance, 1700 mL h^{-1} (Iftekhar and Chandra 1999), BW the standard human body weight of 60 kg, A represents surface area of the diffusion cell (i.e 3.56 cm^2). The calculated target flux value for BUSP was 71.6 μg cm^{-2} h^{-1}.

Measurement of mechanical properties

Mechanical properties of the films were evaluated using a microprocessor based advanced force gauze (Ultra Test Mecmesin, West Sussex, UK) equipped with a 25 kg load cell. Film strip with dimensions 60x10 mm was held between two clamps positioned at a distance of 3 cm. During measurement, the top clamp at a rate of 2 mm s^{-1} pulled the strips to a distance till the film broke. The force and elongation was measured when the film broke. The mechanical properties were calculated according to the following formula (Peh and Wong 1999).

$$\text{Tensile strength (T.S)} = \frac{\text{Force at break}}{\text{Initial cross sectional area of the sample}} \qquad \text{Eq. 3}$$

$$\text{Elongation at break} = \frac{\text{Increase in length}}{\text{Original lenght}} X \frac{100}{\text{Cross sectional area}} \qquad \text{Eq. 4}$$

Experimental design

Box-Behnken statistical design was used to optimize the formulation factors and evaluate main effects, interaction effects and quadratic effects on the amount of BUSP released in 24 h ($Q_{24}R$), flux and pharmacotechnical property, tensile strength (TS). Response surface methodologies (RSM), such as Box-Behnken model and Central Composite Design (CCD) possible curvature in the response function (Govender et al. 2005, Chopra et al. 2007) were used. A 3-factor, 3-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). This cubic design is characterized by set of points lying at the midpoint of each edge and center point of the multidimensional cube (Box and Behnken, 1960). A design matrix comprising of 13 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_1X_2 and X_i^2 (i = 1, 2 or 3) represent the interaction and quadratic terms, respectively. The dependent and independent variables selected were shown in Table 1 along with their low, medium and high levels. The proportion of DLM (X1), TRA (X2) and PG (X3) used to prepare the 13 formulations and the respective observed responses are given in Table 1.

Table 1. Variables and observed responses in Box-Behnken design for BUSP transdermal patches

Code	Independent variables			Dependent variables			$Q_{24}P$	E/B	Kp ($x 10^{-3}$) /cm
	X_1	X_2	X_3	Y_1	Y_2	Y_3			
B1	-1	0	1	8945.1	86.9	0.92	7275.3	67.1	6.38
B2	1	0	1	6765.4	79.8	1.62	6645.2	45.3	4.54
B3	-1	-1	0	7481.3	88.1	1.15	7117.8	57.1	4.79
B4	0	0	0	9481.8	79.4	1.27	6417.6	55.3	8.52
B5	-1	1	0	8743.7	88.4	1.02	7056.1	63.2	5.92
B6	1	0	-1	9021.8	55.5	1.23	4717.5	53.7	7.70
B7	0	1	1	9527.1	67.8	0.89	5487.2	69.5	7.93
B8	1	1	0	9246.1	61.6	0.95	5248.3	65.4	8.00
B9	-1	0	-1	7519.0	44.1	1.71	4015.5	41.0	5.38
B10	0	0	0	9517.6	77.7	1.29	6714.4	54.7	8.49
B11	0	1	-1	9318.4	46.3	1.53	4121.1	49.1	8.02
B12	1	-1	0	6829.1	41.8	1.12	3911.8	56.4	4.39
B13	0	-1	-1	9814.2	85.1	0.87	6833.6	72.4	8.65

Independent variables	Levels used, Actual (Coded)		
	Low (-1)	**Medium (0)**	**High (+1)**
X_1 = D-Limonene (mL)	4	8	12
X_2 = Transcutol (mL)	2.5	5	7.5
X_3 = Propylene glycol (mL)	0	10	20

Note: $Q_{24}R$-Cumulative amount (µg) of BUSP released *in vitro*; $Q_{24}P$-Cumulative amount (µg) of BUSP permeated *ex vivo*; Jss- steady state flux (µg h^{-1} cm^{-1}); T.S-Tensile Strength (Kg^{-2}); E/B-Elongation at Break (mm^{-2}); Kp- Permeation coefficient (cm^{-1})

Check point analysis and optimization model validation

Statistical validation of the polynomial equations generated by Design Expert was established on the basis of ANOVA provision in the software. The models were evaluated in terms of statistically significant coefficients and R^2 values. Various feasibility and grid searches were performed to find the optimum parameters and seven optimum check point formulations were selected to validate the experimental model and polynomial equations. The optimized check point 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.

Skin irritation studies

The skin irritation study was performed on six rabbits. The hair of rabbits on dorsal side was shaved with electrical shaver and B-OPT was applied with help of adhesive tape USP. The development of erythema was monitored for seven days.

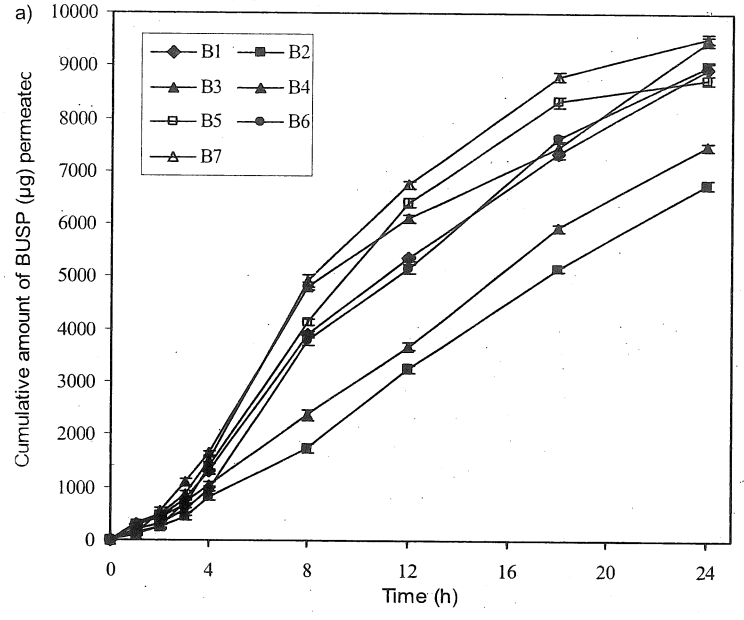
Drug-polymer interaction study

To study the possible interaction between BUSP and polymeric material of the patches, infrared (IR) spectroscopy was carried out on pure substances and their physical mixtures. The IR spectra were recorded using IR Spectrophotometer (Perkin Elmer FT-IR, Perkin Elmer Inst. USA) by KBr pellet method.

Results and Discussion

In vitro release studies

In our preliminary study, HPMC at an amount of 2.5 g produced films with good content uniformity without separation of BUSP, below 2.5 g drug separation was observed. Therefore 2.5 g as polymer amount was taken. DLM and TRA were incorporated as penetration enhancers and PG as plasticizer. The *in vitro* release profiles of BUSP transdermal patches are shown in Figures 1a and 1b. Cumulative amount of BUSP released ($Q_{24}R$) (Table 1) for all experimental formulations were calculated and the release was found to follow zero-order kinetics (R^2= 0.972-0.999). The correlation coefficients of the experimental formulations ranged from 0.684-0.799 and 0.970-0.995 for first order and Higuchi square-root model, respectively. The release pattern was found to follow non-fickian model as it was evidenced from release exponent ($0.557 \leq n \geq 0.773$), explaining that the release pattern is mediated by the process of diffusion. The $Q_{24}R$ ranged from 6765.4 to 9814.2 µg indicating that the release of BUSP from transdermal patches was markedly influenced by the composition. Formulation B13 showed maximum amount of BUSP released (9814.2 µg) among the experimental formulations. B-C (control) (DLM and TRA free) showed a release of 4137.2 µg.



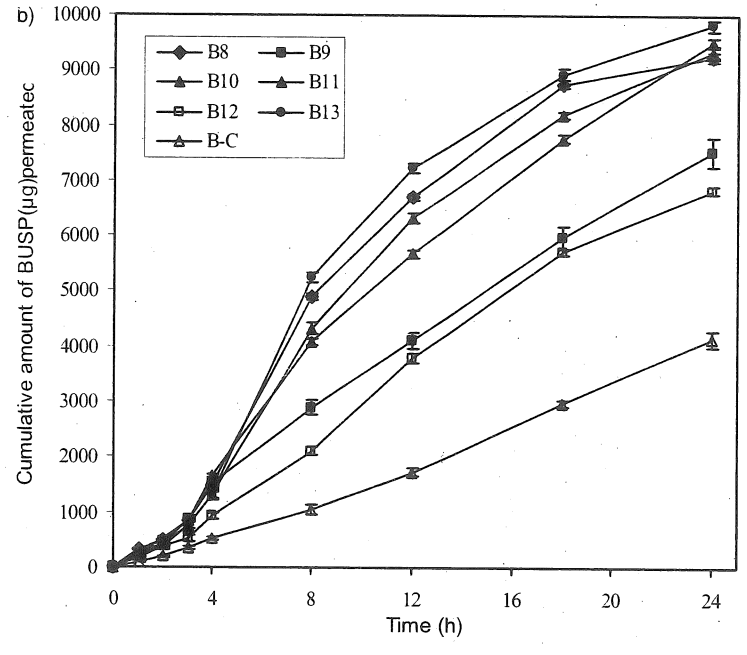


Figure 1. *In vitro* release profiles of BUSP from transdermal patches, values represented are mean ± SD (n=3)

Ex vivo skin permeation experiments

The permeation profiles of BUSP-transdermal patches across rat abdominal skin are shown in Figures 2a and 2b. The permeation parameters, Q_{24} and flux (Table 1) for all experimental formulations were calculated and the permeation was found to follow zero-order kinetics ($R^2 > 0.983$). The cumulative amount ranged from 3911.8 to 7275.3 µg, flux ranged from 44.1 to 88.4 µg cm^{-2} h^{-1} indicating that the permeation parameters of BUSP from transdermal patches were influenced by the composition. Formulation B5 showed the highest flux (88.4 µg cm^{-2} h^{-1}) among the experimental formulations. B-C (control) showed a cumulative amount of 1735.5 µg permeated with a flux of 20.5 µg cm^{-2} h^{-1}.

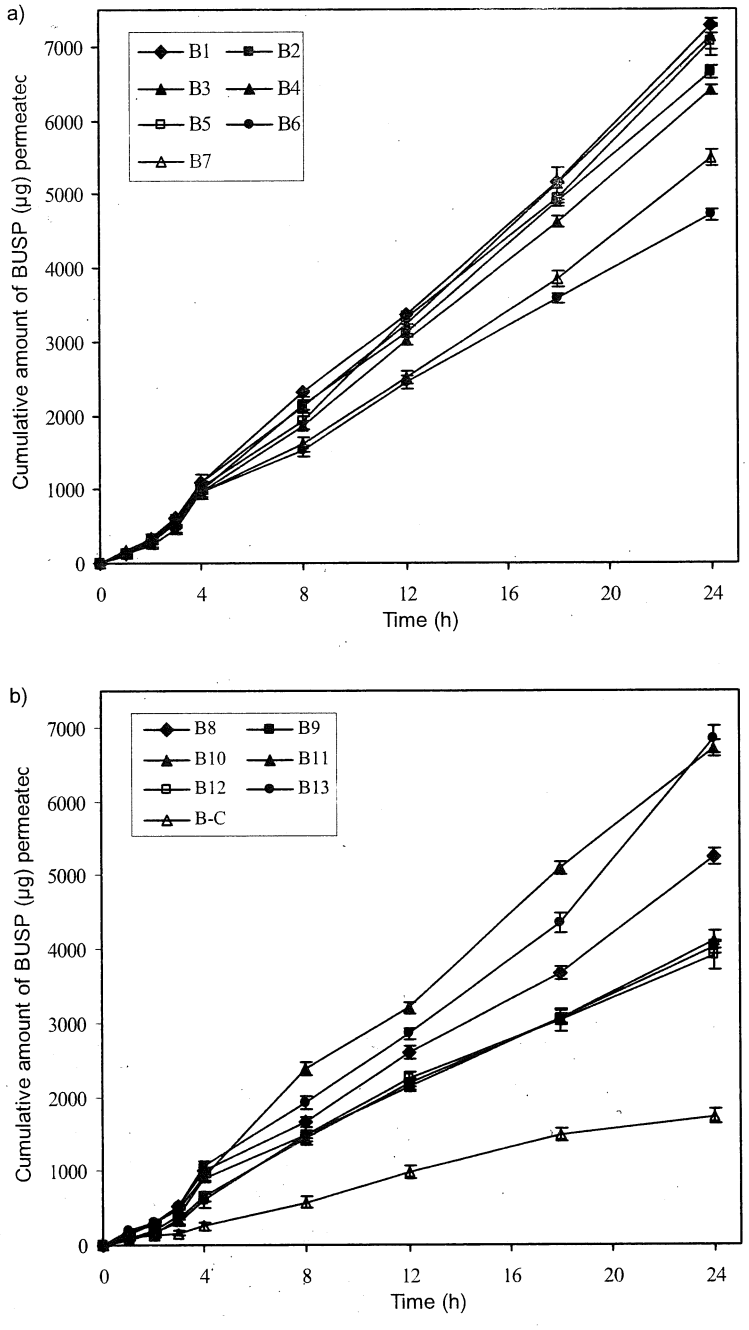


Figure 2. *Ex vivo* permeation profiles of BUSP from BUSP transdermal patches, values represented are mean ± SD (n=3)

The independent variables and responses for all 13 experimental runs are given in Table 1. The contour plots and 3D response surface plots drawn using Design Expert software are shown in Figure 3. Design Expert software was used to optimize the formulation and to develop the mathematical (quadratic) equations (5-7).

The responses, $Q_{24}R$ (Y_1) and flux (Y_2) were found to be significantly higher (Y_1, 8743.7–9814.2 µg; Y_2, 77.7 to 88.4 µg h^{-1} cm^{-2}) only when DLM and TRA were used at 4 or 8% v/w and 2.5 or 5% v/w concentration level respectively. The tensile strength (T.S) (Y_3) was found to be ranging from 0.89-1.71 Kg mm^{-2} at high to low levels of PG. The ranges of other responses, Y_1 and Y_2 were 6765.4-9814.2 µg and 41.8-88.4 µg cm^{-2} h^{-1}, respectively.

The responses of formulations ranged from low drug release of 6765.4 µg (BUSP2, high level of DLM and PG and medium level of TRA) to a higher release of 9814.2 µg (BUSP13, low level of TRA and PG and medium level of DLM). For estimation of quantitative effects of the different combination of factors and factor levels on $Q_{24}R$, flux and T.S, the response surface models were calculated with Design-Expert software by applying coded values of factor levels. The model described could be represented as:

$$Y_1(Q_{24}R)=9499.7+277.8X_1+896.4X_2+531.2X_3+288.7X_1X_2-158.4X_1X_3-403.5X_2X_3-574.7X_1^2-849.9X_2^2-100X_3^2 \quad \text{Eq. 5}$$

$$Y_2(\text{Flux}) = 85.0-7.8X_1-11.9X_2-4.9X_3+6.2X_1X_2-0.1X_1X_3-5.4X_2X_3-12.4X_1^2-4.9X_2^2-2.4X_3^2 \quad \text{Eq. 6}$$

$$Y_3(\text{T.S})=1.28-0.08X_1-0.07X_2-0.29X_3-0.01X_1X_2+0.11X_1X_3-0.03X_2X_3-0.16X_1^2-0.06X_2^2+0.06X_3^2 \quad \text{Eq. 7}$$

Fitting of data to the model

Formulation B5 showed a significantly higher flux (88.4 µg cm^{-2} h^{-1}) with cumulative amount of 7056.1 µg and 8743.7 µg released *in vitro* and permeated *ex vivo* respectively among the formulations. The responses observed for 13 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, standard deviation and % coefficient of variation are given in Table 2 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 factor and response. It is evident that the independent variable X_1, X_2 and X_3 are having positive effect on the response Y_1.

Table 2. 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	Adeq Precision	SD	% CV
Y_1	0.943	0.772	6.29	517.8	5.99
Y_2	0.967	0.868	7.82	5.95	8.73
Y_3	0.975	0.901	10.23	0.09	7.36

The three-dimensional response surface plots (Figures 3d-3f) were drawn to estimate the effects of independent variables on response and to select the optimal formulation. The required flux to reach therapeutic concentration calculated was found to be about 71.6 µg cm^{-2} h^{-1}. Hence, the penetration rate of optimal formulations in the optimization process was set at above 71.6 µg cm^{-2} h^{-1}. Formulation, B5

showed maximum flux of 88.4 μg cm^{-2} h^{-1} and could meet the target flux indicating that the concentrations may be enough to elicit the pharmacological effect.

Data analysis

Formulations B1, B4, B5, B10 and B13 had the highest $Q_{24}R$ and flux. Table 3 shows the observed and predicted values with residuals and percent error of responses for all the formulations. The $Q_{24}R$ and flux obtained at various levels of the 3 independent variables (X_1, X_2 and X_3) was subjected to multiple regression analysis to yield a second-order polynomial equation. The correlation coefficient (R^2) of equation 5 was found to be 0.943, indicating good fit (Table 2). "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable, the ratio of 6.29 (Table 2) indicates an adequate signal. The $Q_{24}R$ values measured for the different formulations showed wide variation (i.e., values ranged from a minimum of 6765.4 μg in B2 to a maximum of 9814.2 μg in B13). The results clearly indicate that the $Q_{24}R$ value is strongly affected by the variables selected for the study. The main effects of X1, X2, and X3 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 $Q_{24}R$ changes when two variables are simultaneously changed. The negative coefficients for all 3 independent variables indicate an unfavorable effect on $Q_{24}R$ while the positive coefficients for interactions between 2 variables indicate a favorable effect on $Q_{24}R$. It was observed that the 3 independent variables showed positive coefficient indicating the variables have favorable effect on $Q_{24}R$. Among the 3 independent variables, the lowest coefficient value is for X1 (277.8), indicating that this variable is insignificant in prediction of Q_{24}.

The value of correlation coefficient (R^2) of equation 6 was found to be 0.967, indicating good fit (Table 2). The "Adeq Precision" was found to be 7.82, indicating an adequate signal. The flux values of B1, B4, B5, B10 and B13 were found to be more among the formulations and could meet the target flux. The flux values were found to be increased from low to medium levels of X_1; low to high level of X_2 and low to medium levels of X_3. However the 2 variables at constant level of third variable showed an effect on flux. The flux values measured for the different formulations showed wide variation (i.e., values ranged from a minimum of 41.8 μg cm^{-2} h^{-1} in B12 to a maximum of 88.4 μg cm^{-2} h^{-1} in B5). The interaction terms (X1X2, X1X3, X2X3, $X1^2$, $X2^2$, and $X3^2$) show how the flux changes when 2 variables are simultaneously changed. The positive coefficient (X_1X_2) for the interactions between 2 variables indicates a favorable effect on flux. Among the 3 independent variables, the lowest coefficient value is for X1 (–11.9), indicating that this variable is insignificant in prediction of flux.

The R^2 value of equation 7 was found to be 0.975, indicating good fit (Table 2). The "Adeq Precision" was found to be 10.23, indicating an adequate signal. The tensile strength (T.S) of B2, B4, B5, B6, B9, B10, B11 and B12 were found to be high among the experimental formulations. The T.S values were found to be increased from low to high levels of X_1; low to medium levels of X_2 and high to low levels of X_3. The results attributed to that the plasticizing property of PG influence the T.S. The elongation at break (E.B) values was found to be decreased with increasing T.S. The E.B values ranging from 41 (B9) to 72.4 mm^2 (B13). The interaction terms (X_1X_3 and X_3^2) show how the T.S changes when 2 variables are simultaneously changed. The positive coefficients (X_1X_3 and X_3^2) for the interactions between 2 variables indicate a favorable effect on T.S. Among the 3 independent variables, the lowest coefficient value is for X_3 (–0.29), indicating that this variable is insignificant in prediction of T.S. The tensile testing gives an indication of the strength and elasticity of the film, reflected by the parameters, T.S and E.B.A soft and weak polymer is characterized by a low TS and E.B; a hard and brittle polymer is defined by a moderate T.S and high low E.B; a soft and tough polymer is characterized by a moderate T.S and high E.B; where as a hard and tough polymer is characterized by a high T.S and E.B (Aulton et al. 1981). Hence, it is suggested that a suitable transdermal film should have a relatively high T.S and E.B. In the present study all the experimental formulations showed moderate T.S and high E.B values.

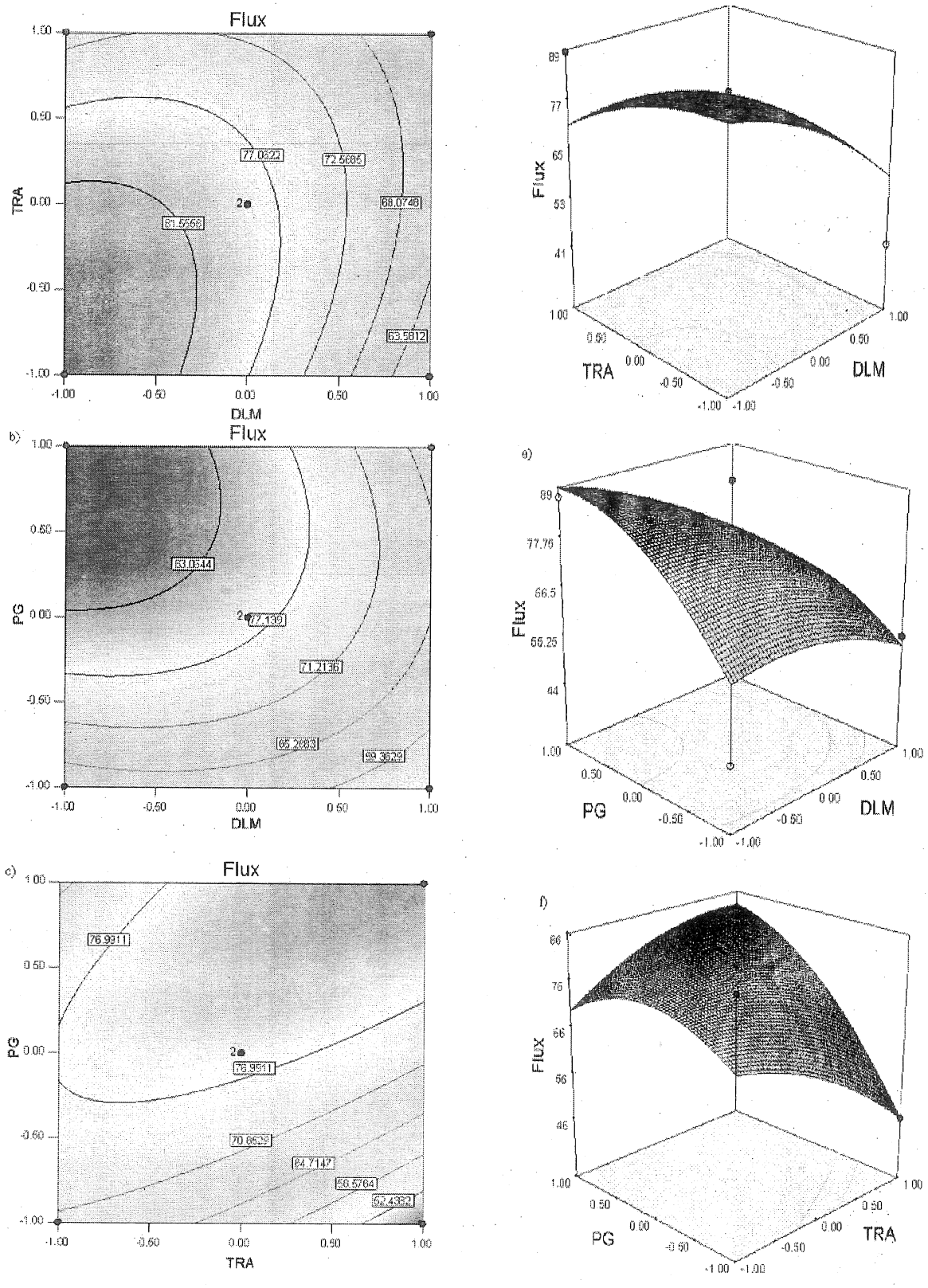


Figure 3. Contour plot showing effect of (2-a) DLM (X_1) and TRA (X_2); (2-b) DLM (X_1) and PG (X_3); (2-c) TRA (X_2) and PG (X_3) on response Y_2 (Flux); Corresponding response surface plots (2d-f.)

Contour plots and response surface analysis

Two-dimensional contour plots and three-dimensional response surface plots are shown in Figure 3, which are useful to study the interaction effects of the factors on responses 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 (Figure 3). Factors X_2 and X_3 have curvilinear relationship at all levels of the two variables on response Y_2. Response surface plots show the relationship between these factors even more clearly.

Optimization

The optimum formulation was selected based on criteria of attaining maximum value of $Q_{24}R$, flux and tensile strength by applying constraints on Y_1 (8000≤ Y≤10,000 μg), Y_2 (72≤Y≤100 μg cm^{-2} h^{-1}) and Y_3 (0.90≤Y≤1.30 h). Upon trading of various response variables and comprehensive evaluation of feasibility and exhaustive grid search, the formulation composition with DLM concentration of 8.8%, TRA, 5.42% and PG 13.5% was found to fulfill the maximum requisite of an optimum formulation. The optimized formulation (B-OPT) showed a 9769.9 μg, 92.5 μg cm^{-2} h^{-1} and 1.20 kg mm^{-2} as Q24R, flux and tensile strength respectively. The flux of optimized formulation was found to meet the target flux (71.6 μg cm^{-2} h^{-1})

Validation of response surface methodology

Seven checkpoint formulations were obtained from RSM, the composition and predicted responses were shown in Table 3. To confirm the validity of 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 3, the predicted error was below 20%, 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 experimental and predicted responses were shown in Fig. 4. The linear correlation plots drawn between experimental and predicted values demonstrated high values of r^2 ($Q_{24}R$, 0.972; flux, 0.871; tensile strength, 0.734) indicating goodness of fit.

Skin irritation study

The skin irritation studies could not find any irritation, erythyma indicating that B-OPT is non-irritant.

Drug-polymer interaction study

The IR spectral analysis of BUSP alone showed that the principal peaks were observed at wave numbers of 1679.2, 1444.6 and 1360.3. In the IR spectra of the physical mixture of BUSP and HPMC were 1676.3, 1445.5 and 1362.4. These results suggest that there is no interaction between the drug and polymer used in the present study. The peaks of BUSP are in comparable to that of reported literature (Clarke's 2007).

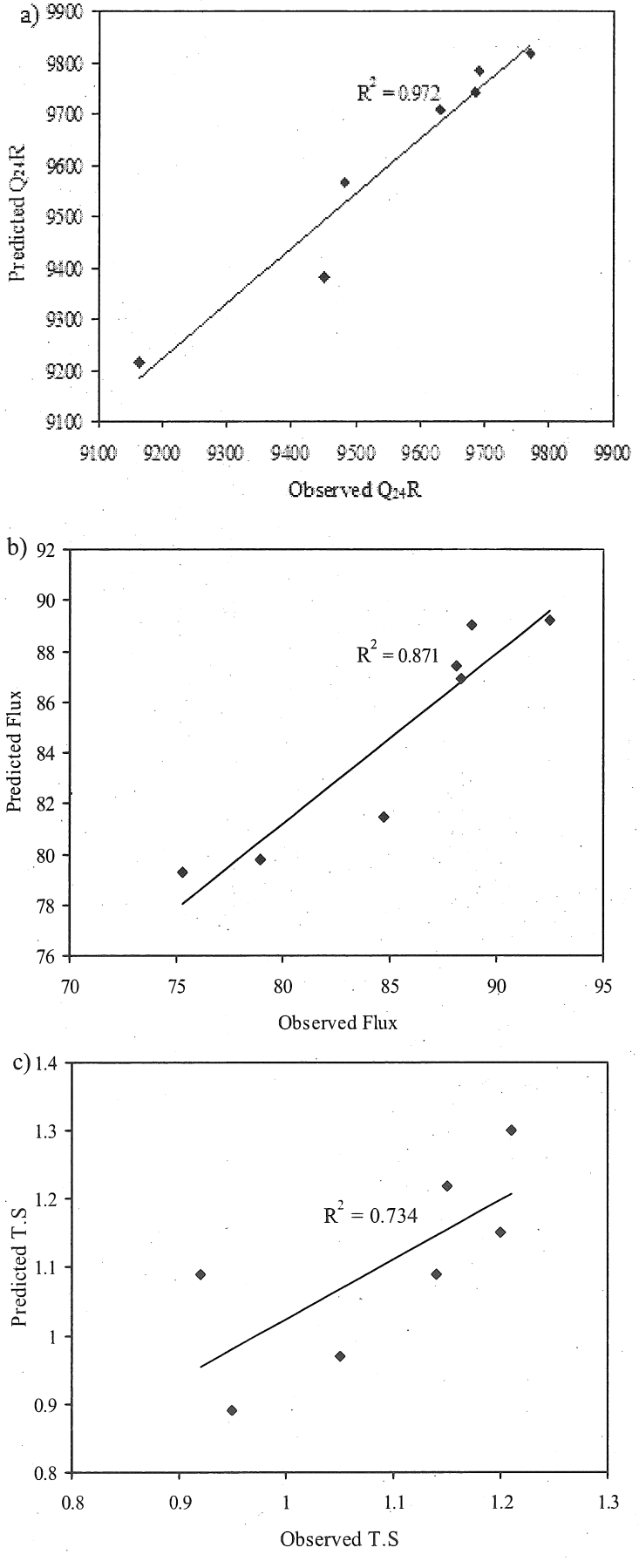


Figure 4. Linear correlation plots (a, b, c) between actual and predicted values

Table 3. 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
6.55:4.7:9.85	Y1	9164.2	9214.6	-0.55
	Y2	75.3	79.3	-5.31
	Y3	1.21	1.30	-7.44
10.6:6.95:19.1	Y1	9690.1	9783.1	-0.96
	Y2	88.3	86.9	1.59
	Y3	0.95	0.89	6.32
9.75:3.91:18.4	Y1	9450.8	9379.1	0.76
	Y2	84.7	81.47	3.81
	Y3	1.14	1.09	4.39
9.75:6.2:7.7	Y1	9683.1	9739.1	-0.57
	Y2	88.8	89.0	-0.22
	Y3	1.15	1.22	-6.08
11.3:5.6:16.4	Y1	9628.7	9706.5	-0.81
	Y2	88.1	87.4	0.79
	Y3	1.05	0.97	7.61
5.8:5.7:15.2	Y1	9483.1	9563.4	-0.84
	Y2	79.0	79.8	-1.01
	Y3	0.92	1.09	-18.48
8.8:5.42:13.5	Y1	9769.9	9815.4	-0.46
	Y2	92.5	89.2	3.56
	Y3	1.20	1.15	4.17

Conclusions

The present study conclusively demonstrates the use of a Box–Behnken statistical design is valid for predicting amount released in 24 h, flux, and tensile strength in optimization of matrix type transdermal patches. The derived polynomial equations and contour plots aid in predicting the values of selected independent variables for the preparation of optimum formulation with desired properties. The results demonstrated that the formulation was nonirritating and did not cause any erythema upon transdermal administration. Further studies are recommended for the optimized formulations by pharmacokinetic or pharmacodynamic studies in humans or animals to prove their therapeutic utility.

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