RESEARCH ARTICLE

inventi Spreading Knowledge

Design and Evaluation of Transdermal Drug Delivery System of Nebivolol Hydrochloride

Garala K [1*], Kapadiya M[1], Patel J[1], Patel A[1], Raval M[2], Dharamsi A[3]

Abstracts: Nebivolol hydrochloride is a selective β_1-receptor antagonist with antihypertensive properties having plasma half life of 10 h and 12 % oral bioavailability. In the present work transdermal matrix patches of Nebivolol hydrochloride were prepared to improve its therapeutic efficacy and to avoid its extensive hepatic first pass metabolism of the drug. Nine formulations using 3^2 full factorial design (composed of Hydroxypropyl methyl cellulose K15M and Eudragit RL100 at a ratios of 1: 1) containing 15% w/w triethyl citrate as plasticizer were prepared. HPMC K15M and Eudragit RL100 were taken as independent variables. Folding endurance, % moisture content, tensile strength, *in vitro* drug release and flux were taken as dependent variables. Compatibility between drug and polymer was accessed by Fourier transform infrared spectroscopy (FTIR). The prepared TDDSs were evaluated for physicochemical parameters and *in vitro* skin permeation. F7 formulation exhibit maximum drug release of 99.53% for 24 h due to its higher amount of hydrophobic polymer concentration. Formulation F5 was optimized on the basis of results of dependent variables. The short term accelerated stability study was carried out for the optimized formulation and results revealed that all dependent variables and other parameters were within acceptable limits. Skin irritation study on albino rats have not shown any sign of erythema or edema. Result of high f_2 value showed similarity between *in vitro* drug release profile of reference and test formulation of before and after stability period. Thus, the prepared matrix transdermal film may prove to be a potential candidate to provide sustained drug release for 24 h.

INTRODUCTION

The transdermal route of administration has been recognized as one of the potential routes for local and systemic delivery of drugs. This route offers many advantages over the oral dosage form, such as improving patient compliance in long-term therapy, bypassing first-pass metabolism, sustaining drug delivery, maintaining a constant and prolonged drug level in plasma, minimizing inter and intra patient variability, and making it possible to interrupt or terminate treatment when necessary. [1] However, the highly organized structure of the stratum corneum forms an effective barrier to drug permeation, which must be modified if poorly penetrating drugs are to be administered. The use of chemical penetration enhancers would significantly increase the number of drug molecules suitable for transdermal delivery. [2]

Nebivolol hydrochloride is a selective β_1-adreno receptor blocker which exerts distinct hemodynamic profile, including reduced peripheral vascular resistance and neutral impact on cardiac output. Nebivolol hydrochloride is considered as safe and tolerated drug which reduce blood pressure in clinical trials in hypertensive patients by vasodilatory action. Nebivolol hydrochloride possesses low oral bioavailability (12-96%) which might be attributed to the first pass effect in extensive metabolizer to poor metabolizer. [3] Moreover, properties like low molecular weight (405.435 gm/mole), low half life (10 h), log P value (2.44) and low dose (2.5-20 mg) makes nebivolol hydrochloride an ideal candidate for transdermal delivery system. [4]

Nebivolol hydrochloride is a selective β_1-receptor antagonist. Activation of β_1-receptors by epinephrine increases the heart rate and the blood pressure, and the heart consumes more oxygen. Nebivolol hydrochloride blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure. In addition, β blockers prevent the release of renin, which is a hormone produced by the kidneys which leads to constriction of blood vessels. At high enough concentrations, this drug may also bind β_2-receptors. [5]

The aim of the present study is to prepare matrix type transdermal drug delivery system of nebivolol hydrochloride using HPMC K15M and Eudragit RL100 different type of hydrophilic and hydrophobic polymers.

MATERIALS AND METHODS

Materials

Nebivolol hydrochloride was obtained from Sun Pharmaceutical Industries Ltd, Baroda as a gift sample. HPMC K15M was obtained as a gift sample from Colorcon Asia Pvt Ltd., Goa, India. Eudragit RL100 was received as a gift sample from Evonik industries, Mumbai, India. Triethyl citrate was purchased from Sd fine Chemicals Limited, Mumbai. Glycerine was procured from RFCL Limited (Rankem), New Delhi. All other solvents (ethanol, acetone) are of analytical grade.

Calculation of Dose Design of Nebivolol hydrochloride [6-9]

The dose to be incorporated in a film was calculated using the following mathematical equation:

$$\text{Drug input (theoretical)} = C_{ss} \times k_e \times V_d \quad (1)$$
$$= 1.42\ \mu g\ L^{-1} \times 0.0693\ h^{-1} \times 695\ L$$
$$= 68.392\ \mu g\ hr^{-1}$$

Where, C_{ss} is concentration at steady state,
k_e is elimination rate constant and
V_d is volume of distribution.

$$\text{Dose required for a film} = \text{drug input} \times \text{delivery time} \quad (2)$$
$$= 68.392\ \mu g\ h^{-1} \times 24\ h$$
$$= 1.64\ mg \text{ per } 2 \times 2\ cm \text{ film}$$

[1]Atmiya Institute of Pharmacy, Kalawad Road, Rajkot, Gujarat, India.
E-mail: kevincgarala@gmail.com
*Corresponding author

[2]Saurashtra University, Rajkot, Gujarat, India.

[3]Swift School of Pharmacy, Rajpura, Punjab, India.

Table 1: 3^2 Full Factorial Experimental Design

Formulation	X_1	X_2
F1	-1	-1
F2	-1	0
F3	-1	+1
F4	0	-1
F5	0	0
F6	0	+1
F7	+1	-1
F8	+1	0
F9	+1	+1

*All the formulation containing 15% w/w of Triethyl citrate as plasticizer

Table 2: Values of Variables in 3^2 Factorial Design

Coded Variables	-1	0	+1
X_1 (HPMC K15M)	400	500	600
X_2 (Eudragit RL100)	400	500	600

Table 3: Evaluation Parameters of Dependent Variables

Batch	X_1	X_2	Y_1*	Y_2*	Y_3 *	Y_4*	Y_5 *
F1	400	400	182.00±1.00	5.27±0.88	0.43±0.002	95.41±0.18	11.76±0.06
F2	400	500	208.67±2.08	4.62±0.68	0.63±0.002	94.57±0.15	10.85±0.08
F3	400	600	276.33±2.08	4.05±0.99	0.71±0.001	94.34±0.17	10.6±0.05
F4	500	400	105.67±0.58	6.10±2.19	0.42±0.003	97.34±0.19	12.08±0.04
F5	500	500	205.33±2.08	5.50±1.21	0.53±0.003	96.54±0.12	11.99±0.07
F6	500	600	219.33±1.53	5.15±1.39	0.67±0.005	95.75±0.14	11.72±0.06
F7	600	400	98.33±0.58	6.94±4.05	0.31±0.002	99.53±0.16	13.57±0.05
F8	600	500	186.00±2.65	6.42±3.64	0.44±0.002	98.10±0.18	12.99±0.07
F9	600	600	213.33±2.52	6.31±0.17	0.65±0.002	97.71±0.11	12.16±0.08

*All the values are in mean ± SD (n = 3)

Formulation of Transdermal Films

In the present study, matrix type transdermal patches were prepared by solvent casting method. HPMC K15M was gradually added in hot boiled water with continuous stirring and allows cooling at room temperature. Eudragit RL100 was dissolved in acetone while nebivolol hydrochloride (29 mg) drug was dissolved in ethanol and both the solutions are mixed together and added into HPMC solution. Finally, triethyl citrate (15% w/w) was added into polymeric solution. Final mixture was shaken manually to form homogenous viscous solution and sonicated to remove entrapped air. Mixture was casted on plastic petri plate having area of 70.84 cm^2 (internal diameter 9.5 cm) and dried in hot air oven at 40°C for 24 h. [8] After 24 h, film was removed from plastic petri plate and cut into small films (4 cm^2) containing 1.64 mg of nebivolol hydrochloride and wrapped in aluminum foil and kept in a desiccator.

Experimental Design

A 3^2 randomized full factorial design was used in this study. In this design two factors (HPMC K15M and Eudragit RL100) were evaluated, each at three levels; experimental batches were performed at all nine possible combinations as shown in Table 1. The amount of HPMC K15M (X_1) and Eudragit RL100 (X_2) were selected as independent variables as shown in Table 2. The Folding endurance (Y_1), Tensile strength (Y_2), Percentage moisture content (Y_3), Percentage drug release (Y_4) and Flux (Y_5) were selected as dependent variables.

The data were subjected to contour and 3-D response surface plot in Design-Expert® 8.0.7.1 (a software developed by Stat-Ease®) to determine the effect of polymers on the release of drug and the dependent variable. The values of variables in 3^2 factorial design are described in Table 2.

A statistical model incorporating interactive and polynomial terms was used to calculate the responses as follows:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1 X_1 + b_{22} X_2 X_2 \quad (1)$$

Where, Y is dependent, b_0 is the arithmetic mean response of the all trials, and b_i (b_1, b_2, b_{12}, b_{11} and b_{22}) is the estimated coefficient for the corresponding factor X_i (X_1, X_2, X_1 X_2, X_{11} and X_{22}) which represents the average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X_1 X_1 and X_2 X_2) are included to investigate the nonlinearity.

Evaluation of Transdermal Films

Physicochemical Parameters

Film Thickness

Table 4 : Calculation for Testing the Model in Portions*

DF		SS	MS	R^2	
Folding Endurance					
Regression					
FM	5	23486.74707	4697.349414	0.939060867	F_{cal} = 3.00152
RM	1	17387.09002	17387.09002	0.695180809	F_{tab} = 9.12
Error					$F_{cal} < F_{table}$
FM	3	1524.141886	508.0472954		DF = (4,3)
RM	7	7623.798939	1089.114134		
% Moisture Content					
Regression					
FM	5	6.896936111	1.379387222	0.998655399	F_{cal} = 5.02273
RM	3	6.865841667	2.288613889	0.994153018	F_{tab} = 9.55
Error					$F_{cal} < F_{table}$
FM	3	0.009286111	0.00309537		DF =(2,3)
RM	5	0.040380556	0.008076111		
Tensile Strength					
Regression					
FM	5	0.150144444	0.030028889	0.968951671	F_{cal} = 0.24480
RM	2	0.148966667	0.074483333	0.961350925	F_{tab} = 9.28
Error					$F_{cal} < F_{table}$
FM	3	0.004811111	0.001603704		DF = (3,3)
RM	6	0.005988889	0.000998148		
Drug Diffusion					
Regression					
FM	5	23.90330004	4.780660009	0.995904565	F_{cal} = 3.13588
RM	2	23.59505255	11.79752627	0.983061774	F_{tab} = 9.28
Error					$F_{cal} < F_{table}$
FM	3	0.098296977	0.032765659		DF = (3,3)
RM	6	0.406544472	0.067757412		
Flux					
Regression					
FM	5	6.516202778	1.303240556	0.946175099	F_{cal} = 0.06843
RM	2	6.490833333	3.245416667	0.942491368	F_{tab} = 9.28
Error					$F_{cal} < F_{table}$
FM	3	0.370686111	0.123562037		DF = (3,3)
RM	6	0.396055556	0.066009259		

*DF, degree freedom; SS, sum of squares; MS, mean of squares; R, regression coefficient; FM, full model; RM, reduced model

Table 5: Summary of Results of Regression Analysis*

Coefficients	b_0	b_1	b_2[a]	b_{11}[a]	b_{22}[a]	b_{12}[a]
Folding Endurance						
FM	188.44	-28.223	53.831	17.3333	-17.5016	5.167
RM	188.33	53.831	-	-	-	-
% Moisture Content						
FM	5.5011	0.955	-0.4666	0.01833	0.12333	0.147
RM	5.5955	0.955	-0.4666	-	-	0.147
Tensile Strength						
FM	0.5411	-0.0616	0.145	-0.01166	-0.00166	0.015
RM	0.5322	-0.0616	0.145	-	-	-
Drug Diffusion						
FM	96.360	1.8371	-0.7466	0.06745	0.282	-0.187
RM	96.593	1.8371	-0.7466	-	-	-
Flux						
FM	11.904	0.9183	-0.4883	0.05833	0.03833	-0.062
RM	11.968	0.9183	-0.4883	-	-	-

*FM, full model; RM, reduced model; [a]Response is insignificant at $p \geq 0.05$

Thickness of the film was measured using screw micrometer (Usico, India). The procedure was repeated at mainly dependent on the hydroxylpropyl methyl cellulose and eudragit polymer concentration. Increase in polymer concentration increase the thickness path length which decreases the drug release rate.

Uniformity of Weight

Table 6: Evaluation Parameters of Experimental Design Batches*

Batch Code	Thickness (mm)	Weight Variation (gm)	% Moisture Uptake	% Elongation	% Flatness
F1	0.18 ± 0.02	0.063 ± 0.02	6.12 ± 1.03	14.00 ± 2.00	94.74 ± 0.05
F2	0.17 ± 0.01	0.072 ± 0.01	5.83 ± 0.13	23.33 ± 1.15	93.62 ± 0.08
F3	0.22 ± 0.02	0.058 ± 0.01	5.37 ± 1.56	28.67 ± 1.15	93.62 ± 0.03
F4	0.27 ± 0.02	0.061 ± 0.02	7.04 ± 0.50	14.67 ± 2.31	87.64 ± 0.08
F5	0.18 ± 0.02	0.061 ± 0.01	6.41 ± 1.31	18.00 ± 2.00	93.05 ± 0.10
F6	0.22 ± 0.02	0.071 ± 0.02	5.97 ± 0.18	24.00 ± 2.00	90.11 ± 0.08
F7	0.19 ± 0.02	0.062 ± 0.01	8.03 ± 0.66	8.00 ± 2.00	87.01 ± 0.10
F8	0.17 ± 0.01	0.067 ± 0.02	7.47 ± 2.55	17.33 ± 1.15	90.71 ± 0.13
F9	0.23 ± 0.02	0.079 ± 0.01	7.17 ± 0.64	26.00 ± 2.00	88.89 ± 0.13

*All the values are in mean ± SD (n = 3)

Table 7: % Drug Content in Transdermal Films of Nebivolol Hydrochloride

Formulation Code	Drug Content (%)*
F1	98.49 ± 1.44
F2	98.85 ± 0.62
F3	98.31 ± 0.83
F4	94.86 ± 0.86
F5	94.86 ± 1.13
F6	97.76±0.83
F7	97.03±1.13
F8	95.76±0.54
F9	96.49±0.83

*All the values are in mean ± SD (n = 3)

Table 8: Drug Diffusion and Flux Values of Transdermal Films of Nebivolol Hydrochloride

Formulation Code	% Drug Diffusion*	Flux (µg/cm²/h)*
F1	95.41 ± 0.18	11.76 ± 0.06
F2	94.57 ± 0.15	10.85 ± 0.08
F3	94.34 ± 0.17	10.6 ± 0.05
F4	97.34 ± 0.19	12.08 ± 0.04
F5	96.54 ± 0.12	11.99 ± 0.07
F6	95.75 ± 0.14	11.72 ± 0.06
F7	99.53 ± 0.16	13.57 ± 0.05
F8	98.10 ± 0.18	12.99 ± 0.07
F9	97.71 ± 0.11	12.16 ± 0.08

*All the values are in mean ± SD (n = 3)

Table 9: Results of Dependent Variables of Check-Point Batch

Test parameters	Result*
Folding endurance	188.12 ± 1.05
% Moisture content	5.58 ± 0.05
Tensile strength (kg/cm²)	0.528 ± 0.003
Drug diffusion (%)	96.55 ± 0.18
Flux (µg/cm²/h)	11.94 ± 0.07

*All the values are in mean ± SD (n = 3)

Weight variation was studied by individually weighing 10 randomly selected films and average weight was calculated. The individual weight should not deviate significantly from the average weight. [7]

Drug Content Determination

The film was dissolved in 25 mL of methanol and kept for 24 h on rotary flask shaker. The solution was filtered, suitably diluted and drug content per film was estimated using UV-Visible spectrophotometer at 281 nm. [7]

Moisture Content Study

The films were weighed (W_1) and kept in desiccator containing anhydrous calcium chloride until it showed a constant weight (W_2). The final weight was noted when there was no further change in the weight of the film. The moisture content was calculated according to the following equation. [10]

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_2} \times 100 \qquad (3)$$

Where, W_1 is the initial weight of each strip and W_2 is the final weight of each strip.

Moisture Uptake Study

The films were then transferred to another desiccator

Table 10: The Experimental Values and Predicted Values of Each Response

Response Parameters	Predicted Value	Observed Values	Relative Error (%)
Folding endurance	188.33	180.12	4.3593
% Moisture content	5.59	5.28	5.5456
Tensile strength (kg/cm^2)	0.53	0.521	1.6981
Drug release (%)	96.59	94.14	2.5364
Flux (µg/cm^2/h)	11.96	11.24	6.0200

Table 11: Standards for Skin Irritation Study

Erythema Formation	Rating
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness)	4
Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising	2
Moderate oedema (raised approx. 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Table 12: Dermal Observation of Skin Irritation Test

Rat No.	Reaction	Standard		Test	
		24 Hours	72 Hours	24 Hours	72 Hours
1	Erythema	2	3	0	0
	Edema	1	2	0	0
2	Erythema	2	2	0	1
	Edema	1	2	0	0
3	Erythema	2	2	0	0
	Edema	2	3	0	0
4	Erythema	1	3	0	0
	Edema	2	2	0	1
5	Erythema	1	3	0	0
	Edema	1	2	0	0
6	Erythema	1	3	0	0
	Edema	1	2	0	0

Table 13: Evaluation of Primary Irritation Index

Index	Evaluation
0.00	No irritation
0.04-0.99	Irritation barely perceptible
1.00-1.99	Slight irritation
2.00-2.99	Mild irritation
3.00-5.99	Moderate irritation
6.00-8.00	Severe irritation

containing saturated aluminum chloride ($AlCl_3$) solution (relative humidity 75%) at 25°C until a constant weight obtained. After equilibrium, the films were taken out and weighed (W_m). Moisture uptake capacity was calculated according to the following equation. [11]

$$\text{Moisture uptake (\%)} = \frac{W_2\text{-}W_1}{W_1} \times 100 \qquad (4)$$

Where, W_1 is the initial weight of each strip and W_2 is the final weight of each strip.

Flatness

Longitudinal strips were cut out from the prepared medicated patches and the lengths of each strip were measured and then the variation in lengths due to the non-uniformity in flatness was measured. Flatness was calculated by measuring constriction of strips, and a zero percent constriction was considered to be equal to a hundred percent flatness. [12-13]

$$\text{\% Constriction} = \frac{L_1\text{-}L_2}{L_1} \times 100 \qquad (5)$$

Table 14: Results of Stability Testing

Test Parameters	Result*
Thickness	0.17 ± 0.03
% Flatness	93.03 ± 0.10
% Moisture content	5.48 ± 1.19
% Moisture uptake	6.39 ± 1.30
Folding endurance	205.28 ± 2.05
Tensile strength	0.52 ± 0.004
Drug content (%)	94.83 ± 1.12
Drug release (%)	95.62 ± 1.58

*All the values are in mean ± SD (n = 3)

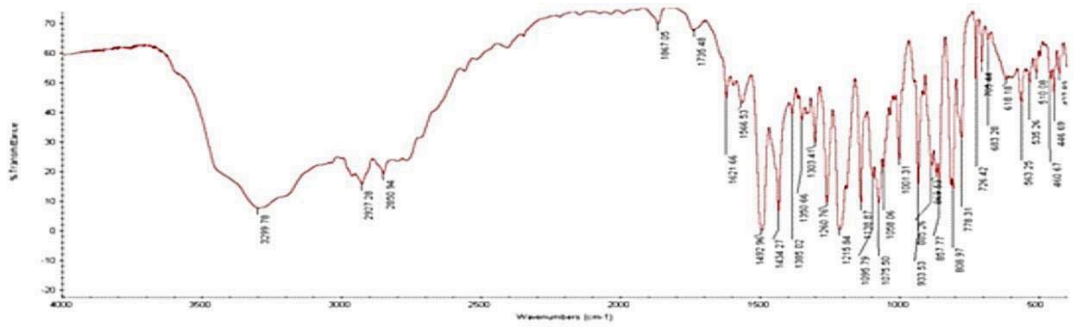

Figure 1(a): FTIR spectrum of Nebivolol hydrochloride

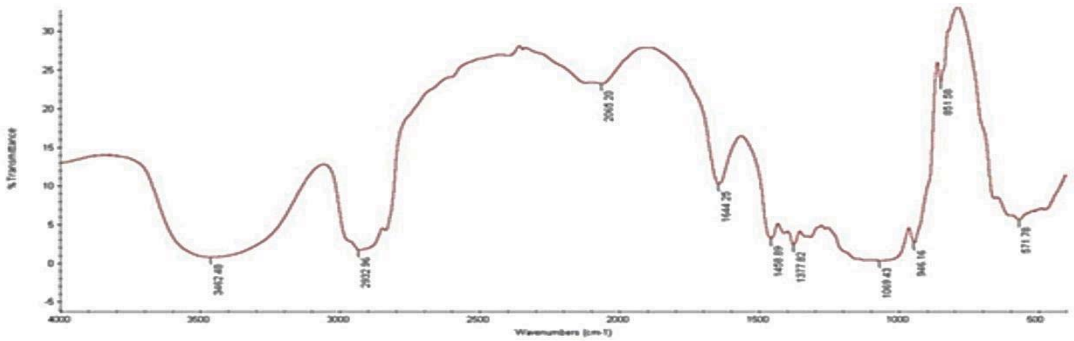

Figure 1(b): FTIR spectrum of HPMC K15M

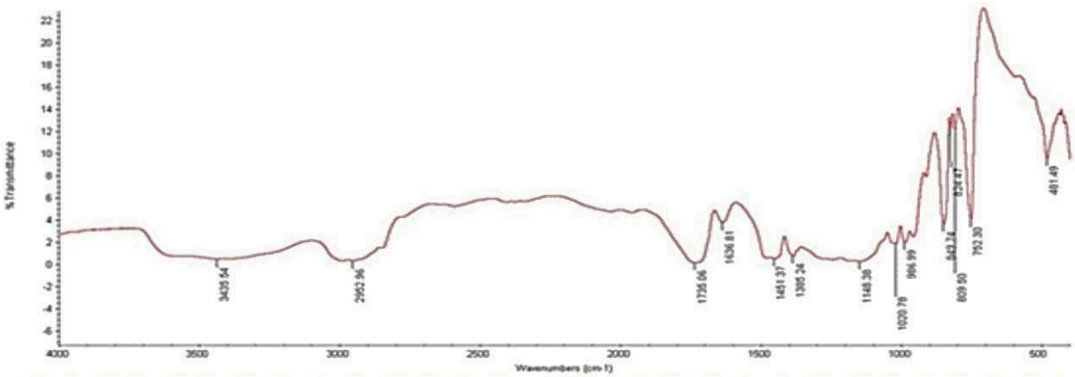

Figure 1(c): FTIR spectrum of Eudragit RL100

Where, L_1 is the initial length of each strip and L_2 is the final length of each strip.

Tensile Strength

To determine tensile strength, polymeric films were sandwiched separately by corked linear iron plates. One end of the film was kept fixed with the help of an iron screen and other end was connected to a freely movable thread over a pulley. The weights were added gradually to the pan attached with the hanging end of the thread. A pointer on the scale was used to measure the elongation of the film. The weight just sufficient to break the film was noted. The tensile strength was calculated using the following equation. [7]

$$\text{Tensile strength} = \frac{F}{a \times b} \times \frac{1+L}{l} \qquad (6)$$

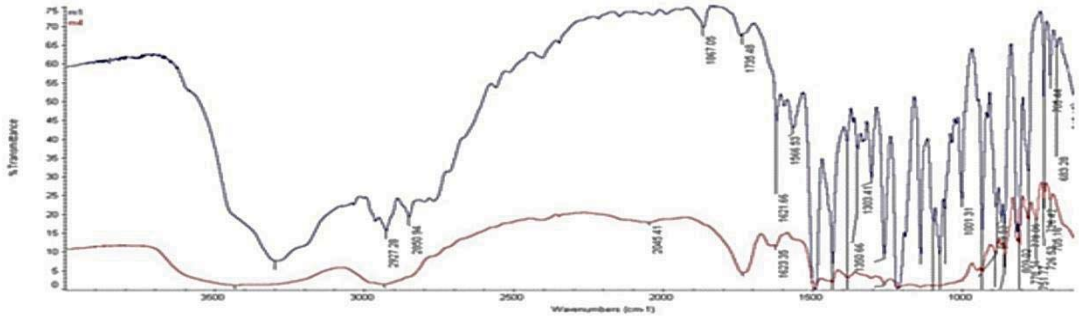

Figure 1(e): FTIR Spectrum of pure drug and transdermal film formulation

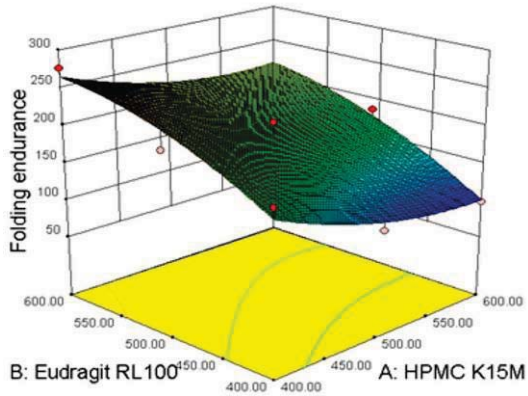

Figure 2 (a): Response surface plot showing effect of folding endurance of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

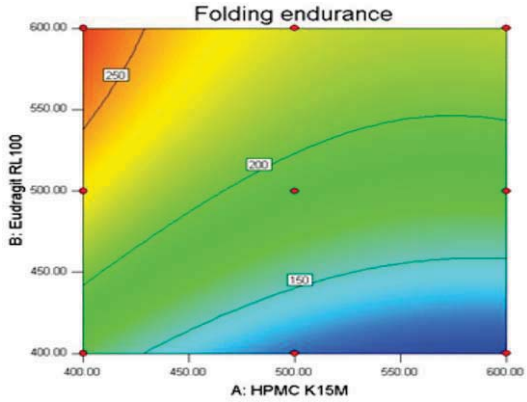

Figure 2 (b): Contour plot showing effect of folding endurance of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

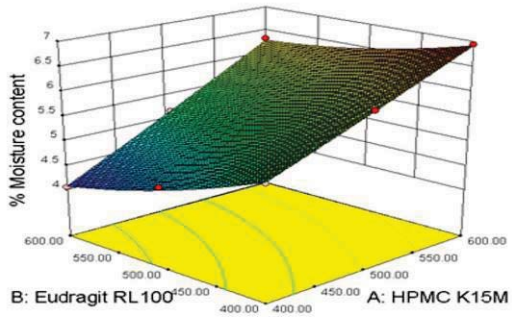

Figure 3 (a): Response surface plot showing effect of percentage moisture content of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

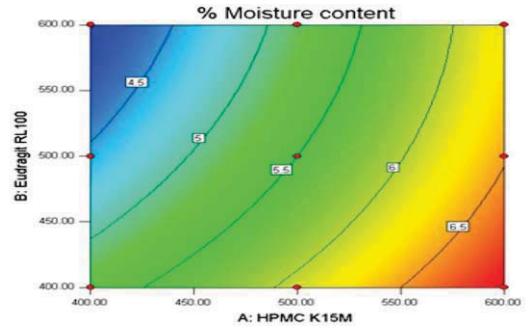

Figure 3 (b): Contour plot showing effect of percentage moisture content of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

Where, *F* is the force required to break, *a* is the width of film, *b* is the thickness of film, *L* is the length of film and *l* is elongation of film at break point.

Folding Endurance

Folding endurance was determined by repeatedly folding the film at the same place until it break. Three films were selected. The number of times the film was folded at the same place without breaking termed as folding endurance value. [7] The average of three determinations was recorded as results.

Elongation Break Test

The percentage elongation break was determined by noting the length just before the break point, the percentage elongation was determined from the below mentioned formula. [14]

$$\% \text{ Elongation} = \frac{L_1 - L_2}{L_1} \times 100 \qquad (7)$$

Where, L_1 is the final length of each strip and L_2 is the initial length of each strip.

***In vitro* Skin Permeation Study**

Preparation of Skin for *In vitro* Skin Permeation Study

Transdermal film formulations were studied for skin permeation using goat skin, obtained from the slaughter house after sacrificing the animal within 1 h. Then the hair was removed from the upper portion of skin surface using an electrical hair remover and these skins were thoroughly

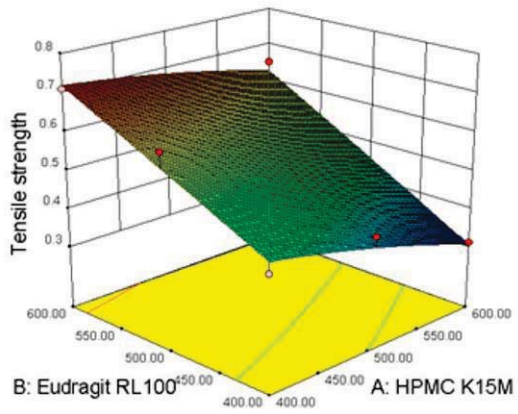

Figure 4 (a): Response surface plot showing effect of tensile strength of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

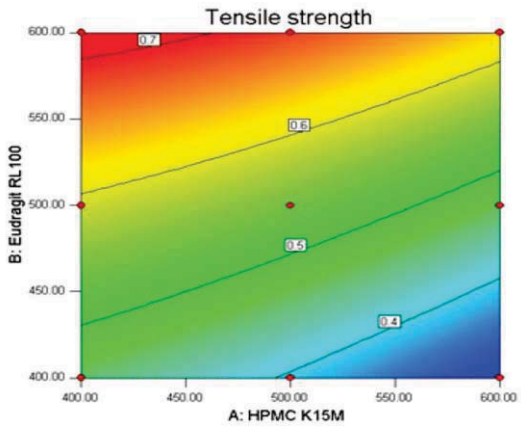

Figure 4 (b): Contour plot showing effect of tensile strength of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

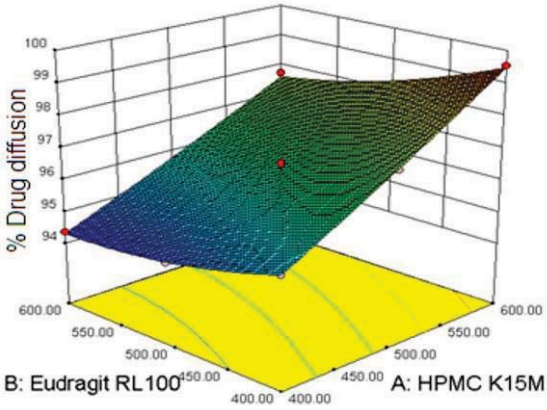

Figure 5 (a): Response surface plot showing effect of drug diffusion of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

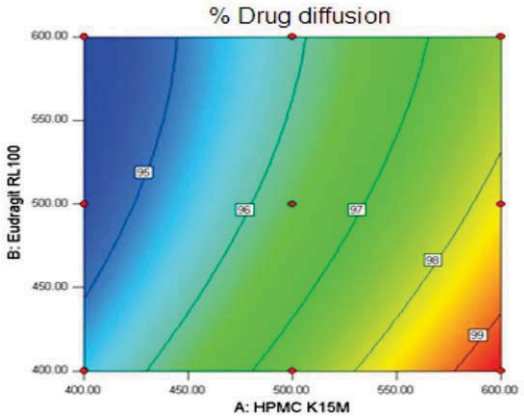

Figure 5 (b): Contour plot showing effect of drug diffusion of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

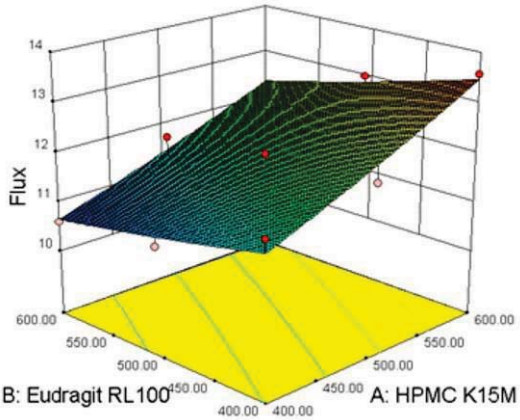

Figure 6 (a): Response surface plot showing effect of flux of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

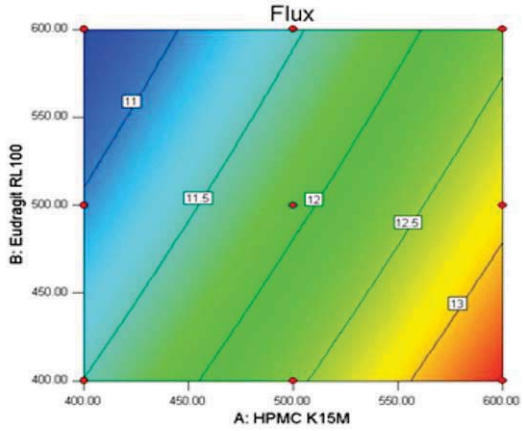

Figure 6 (b): Contour plot showing effect of flux of variables [HPMC K15M (X_1) and Eudragit RL100 (X_2)]

rinsed with distilled water and packed in aluminum foils. The skin samples were stored for a period of no more than one month at -20°C until use. [15]

In vitro Permeation Study

Goat skin was mounted with the stratum corneum uppermost in Franz diffusion cells. These cells provided a diffusional area of 4.9 cm^2 and the receptor compartment was filled with 16 mL phosphate buffer pH 7.4 containing 30 %v/v PEG-400. The temperature of diffusion medium was maintained at 37 ± 0.5°C and the receptor compartment was magnetically stirred at 300 rpm. A 4 cm^2 transdermal film was applied on goat skin. Stir the diffusion medium with magnetic bead. A 0.5 mL of the receptor medium was withdrawn at predetermined intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h) and replaced immediately with an equal volume of receptor solution to maintain a constant volume. [16] The amount of permeated drug was measured using UV visible spectrophotometer by measuring absorbance at λ_{max} 282.25 nm.

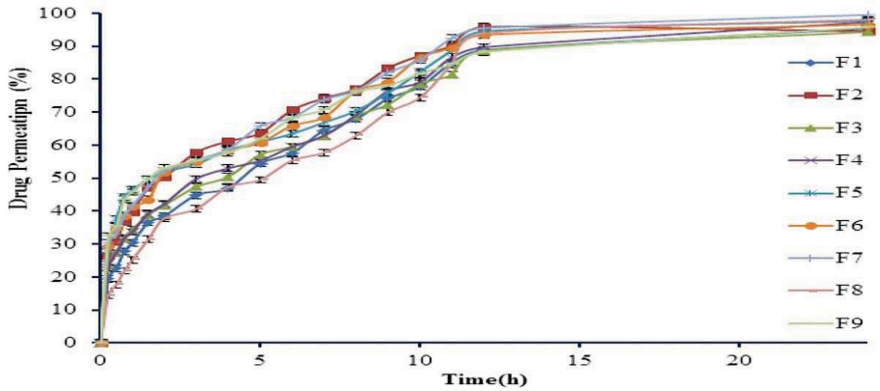

Figure 7: *In vitro* skin permeation profile

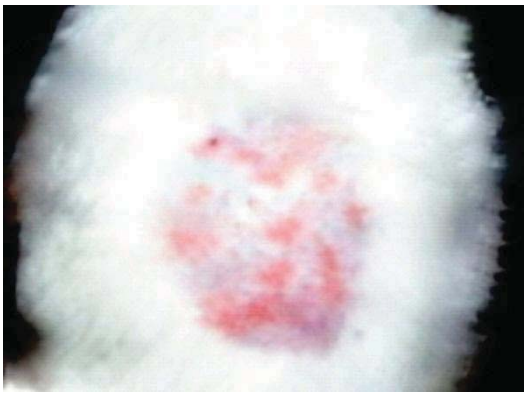

Figure 8(a): Group I: Formalin applied rat skin

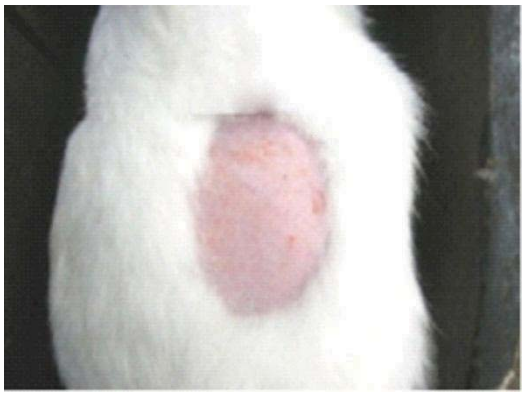

Figure 8(b): Group II: Transdermal formulation applied rat skin

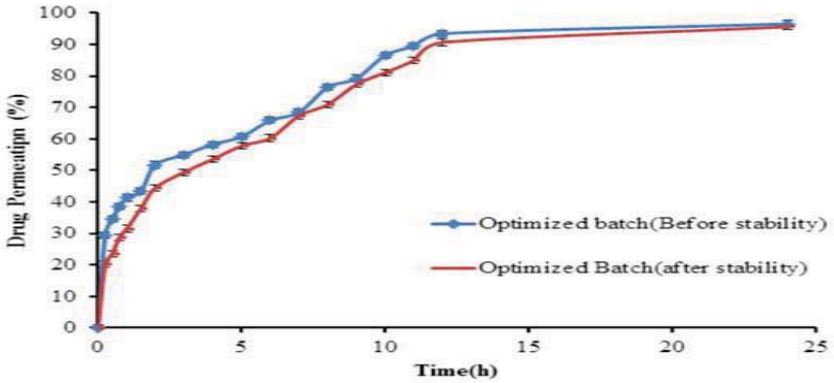

Figure 9: Comparison of drug diffusion profile of optimized formulation after and before stability

Skin Irritation Study

Skin irritation studies were performed on healthy rats. The dorsal surface of the rats was cleaned, and the hair was removed by shaving with an electric razor. The skin was cleansed with rectified spirit. Representative formulations were placed over the skin with the use of adhesive tape (3M™ 9772L PVC foam tape). Treated skin areas were evaluated according to a modified Draize scoring method and the irritation index was evaluated. The first or "Primary Irritation Index" (P.I.I.) was an average value reflecting irritation both immediately after dressing removal and 72 h later. The "Secondary Irritation Index" (S.I.I.) was determined after seven days of removal of formulation. The application sites were graded according to a visual scoring scale, always by the same investigator.In this study, the Primary Irritation Index was recorded. The rats were divided into two groups (n = 6). Group I received prepared formulation and Group II received 0.8% v/v aqueous solution of formalin as a standard irritant. At 24 and 72 h after test article application, the test sites were examined for dermal reactions in accordance with the Draize scoring criteria. [17]

Fourier Transform Infrared (FTIR) Spectroscopy

In the preparation of film formulation, drug and polymer may interact as they are in close contact with each other, which could lead to the instability of drug. Preformulation studies regarding the drug-polymer interaction are therefore very critical in selecting appropriate polymers. Infrared spectra of pure drug, pure polymer, physical mixture and prepared transdermal film were recorded using (FTIR 8400 Spectrophotometer, Shimadzu, Japan). All

the samples were dispersed in KBr and compressed into disc/pellet by application of pressure by KBr press. The pellets were placed in the holder and FTIR spectra were recorded by scanning between 4000-400 cm^{-1}.

Stability Study

Stability is defined as the ability of particular drug or dosage form in a specific container to remain within its physical, chemical, therapeutic, and toxicological specification. [18] Drug decomposition or degradation occurs during stability, because of chemical alteration of the active ingredients or due to product instability, lowering the concentration of the drug in the dosage form. The stability of pharmaceutical preparation should be evaluated by accelerated stability studies. Stability studies of the drug loaded transdermal film were carried out to determine the effect of contents on the stability of the drug. The accelerated stability studies were carried out according to ICH guidelines by storing the samples at 40 ± 2°C and 75 ± 5% RH for 3 month using stability chamber (Remi, India). [19] The samples were evaluated for physicochemical parameters namely thickness, flatness, folding endurance, tensile strength, moisture content and moisture uptake, drug content as well as drug release.

Similarity Factor (f_2)

Similarity factor f_2 is used to check the similarity between release profile of optimized formulation before and after the stability testing. It is adopted by FDA centre for drug evaluation and research (CDER) as an assessment criterion of similarity between different *in vitro* profile with value between 50 to 100 depicting similarity between the profiles. [20],[21] The similarity factor (f_2) can be explained by the following equation as defined by FDA:

$$f_2 = 50 \times \log\left[\left\{1+\frac{1}{n}\sum_{r=1}^{n} wt\,(Rt - Tt)\right\}^{-0.5} \times 100\right] \qquad (8)$$

where, wt = some dissolution time point

R_t = % released at time t of reference product (prechange)

T_t = % released at time t of test product (postchange)

The data obtained after one month accelerated stability study was compared were compared with the initial *in vitro* permeation data of the optimized batch, treated as a reference [22]

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy Analysis

FTIR spectra of Nebivolol hydrochloride [Figure 1(a)] exhibited principal peaks at 3299.78 cm^{-1} (2° amine group $-CH_2NH$, N-H stretching), 1138.87 cm^{-1} (Presence of Florine on aryl ring, C-F stretching), 1260.76 cm^{-1} ($-CH_2OH$ (2° alcohol group) C-O stretching), 1215.84 cm^{-1} Presence of (Aryl-O-CH_2) group C-O stretching and 1621.66 cm^{-1} (Aromatic C=C stretching peak). FTIR spectra of HPMC K15M [Figure 1(b)] exhibited principal peaks at 3462.48 cm^{-1} (O-H stretching vibration), peak at 2932.96 cm^{-1} was due to the C-H stretching vibration. FTIR spectra of Eudragit RL100 [Figure 1(c)] exhibited principal peaks at 2952.96 cm^{-1} due to CH aliphatic stretching and at 1735.06 cm^{-1} due to –C = O stretching. FTIR spectra of Transdermal film formulation Figure 1(d) exhibited principal peaks at 3431.56 cm^{-1} (2° amine group $-CH_2NH$, N-H stretching), 1260.71 cm^{-1} ($-CH_2OH$ (2° alcohol group) C-O stretching), 1215.54 cm^{-1} i.e. presence of (Aryl-O-CH_2) group C-O stretching and 1623.35 cm^{-1} (Aromatic C=C stretching peak). All these peaks clearly indicate that they are very much closely similar to the peaks of pure drug.

The interaction between the drug and the polymers often leads to identifiable changes in the FTIR profile of solid systems. The FTIR spectrum of HPMC presented a profile without distinctly high peaks. FTIR spectra for pure drug, polymers, and transdermal film formulation have been depicted in Figure 1(a) to 1(d). The spectrum of transdermal film formulation was equivalent to the addition spectrum of pure drug indicating no interaction occurring in the simple physical mixture of drug and polymer as shown in Figure 1(e).

Experimental Design

A statistical model incorporating interactive and polynomial terms was used to calculate the responses as follows:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1 X_1 + b_{22} X_2 X_2 \qquad (10)$$

Where, Y is dependent, b_0 is the arithmetic mean response of the all trials, and b_i (b_1, b_2, b_{12}, b_{11} and b_{22}) is the estimated coefficient for the corresponding factor X_i (X_1, X_2, X_1 X_2, X_{11} and X_{22}) which represents the average result of changing one factor at a time from its low to high value. The interaction term (X_1 X_2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X_1 X_1 and X_2 X_2) are included to investigate the nonlinearity.

The results of dependent variables (i.e., folding endurance (Y_1), % moisture content (Y_2), tensile strength (Y_3), drug diffusion (Y_4) and flux (Y_5) for 9 batches showed a variation in Table 3. The data indicate that the response of the drug is strongly dependent on the selected independent variables. The fitted equations (full and reduced) relating the responses folding endurance (Y_1), % moisture content (Y_2), tensile strength (Y_3), drug diffusion (Y_4) and flux (Y_5) to the transformed factor are shown in Table 5. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., negative or positive). Table 4 shows the results of analysis of variance (ANOVA), which was performed to identify insignificant factors. Data were analyzed using Microsoft Excel® 2007. [23]

Effect of Formulation Variable on Folding Endurance (Y_1)

Concerning Y_1, the results of multiple linear regression analysis showed that coefficient b_1 bear a negative sign and

coefficient b_2 bear a positive sign. The negative X_1 coefficient indicates that as the concentration of X_1 (HPMC K15M) increases; there is decrease in the folding endurance value of transdermal film. The positive X_2 coefficient indicates that as the concentration of X_2 (Eudragit RL 100) increase, the folding endurance value of transdermal film was increase. [24]

The fitted equation relating the response Y_1 to the transformed factor is shown in following equation,

$$Y_1 = 188.444 - 28.223X_1 + 53.831X_2 + 5.1675X_1X_2 + 17.333X_{11} - 17.501X_{22} \quad (11)$$

$$(R^2 = 0.9390)$$

The Y_1 for all batches F1 to F9 shows good correlation co-efficient of 0.9390. High folding endurance value is very important parameter for sustained release matrix transdermal film. Here, X_2 variable is responsible for high folding endurance value.

The relationship between formulation variables (X_1 and X_2) and Y_1 was further elucidated using contour and response surface plot. The effects of X_1 and X_2 on Y_1 are given in Figure 2 (a) and (b). From the response surface plot it was cleared that at highest levels of Eudragit RL100 (X_2), folding endurance (Y_1) was increased from 98.76 to 276.33 when HPMC K15M (X_1) was increased from -1 level to the +1 level.

Effect of Formulation Variable on Percentage Moisture Content (Y_2)

Concerning Y_2, the results of multiple linear regression analysis showed that coefficient b_1 bear a positive sign and coefficient b_2 bear a negative sign. The positive X_1 coefficient indicates that as the concentration of X_1 (HPMC K15M) increases; there is increase in the % moisture content of transdermal film. The negative X_2 coefficient indicates that as the concentration of X_2 (Eudragit RL 100) increase, the % moisture content of the transdermal film was decrease. [24] The fitted equation relating the response Y_2 to the transformed factor is shown in following equation,

$$Y_2 = 5.501 + 0.955X_1 - 0.466X_2 + 0.147X_1X_2 + 0.018X_{11} + 0.123X_{22} \quad (12)$$

$$(R^2 = 0.9986)$$

The Y_2 for all batches F1 to F9 shows good correlation co-efficient of 0.9986. Low moisture content is needed for longer time stability of matrix transdermal film. Here, X_1 variable is responsible for high moisture content value.

The relationship between formulation variables (X_1 and X_2) and Y_2 was further elucidated using contour and response surface plot. The effects of X_1 and X_2 on Y_2 are given in Figure 3 (a) and (b). From the response surface plot it was cleared that at highest levels of Eudragit RL100 (X_2), % moisture content (Y_2) was decreased from 6.94 to 4.05% when HPMC K15M (X_1) was increased from -1 level to the +1 level.

Effect of Formulation Variable on Tensile Strength (Y_3)

Concerning Y_3, the results of multiple linear regression analysis showed that coefficient b_1 bear a negative sign and coefficient b_2 bear a positive sign. The negative X_1 coefficient indicates that as the concentration of X_1 (HPMC K15M) increases; there is decrease in the tensile strength of transdermal film. The positive X_2 coefficient indicates that as the concentration of X_2 (Eudragit RL 100) increase, the tensile strength of transdermal film was increase. [24] The fitted equation relating the response Y_3 to the transformed factor is shown in following equation,

$$Y_3 = 0.541 - 0.061X_1 + 0.145X_2 + 0.015X_1X_2 - 0.011X_{11} - 0.001X_{22} \quad (13)$$

$$(R^2 = 0.9689)$$

The Y_3 for all batches F1 to F9 shows good correlation co-efficient of 0.9689. High tensile strength is needed to withstand mechanical pressure for matrix transdermal film. Here, X_2 variable is responsible for high tensile strength value. The relationship between formulation variables (X_1 and X_2) and Y_3 was further elucidated using contour and response surface plot. The effects of X_1 and X_2 on Y_3 are given in Figure 4 (a) and (b). From the response surface plot it was cleared that at highest levels of Eudragit RL100 (X_2), tensile strength (Y_3) was increased from 0.31 to 0.71 kg/cm^2 when HPMC K15M (X_1) was increased from -1 level to the +1 level.

Effect of Formulation Variable on Drug Diffusion (Y_4)

Concerning Y_4, the results of multiple linear regression analysis showed that coefficient b_1 bear a positive sign and coefficient b_2 bear a negative sign. The positive X_1 coefficient indicates that as the concentration of X_1 (HPMC K15M) increases; there is increase in the release of drug. The negative X_2 coefficient indicates that as the concentration of X_2 (Eudragit RL 100) increase, the drug release from the matrix was decrease. [24] The fitted equation relating the response Y_4 to the transformed factor is shown in following equation,

$$Y_4 = 96.360 + 1.837X_1 - 0.746X_2 + 0.187X_1X_2 + 0.067X_{11} + 0.282X_{22} \quad (14)$$

$$(R^2 = 0.9959)$$

The Y_4 for all batches F1 to F9 shows good correlation co-efficient of 0.9959. Higher permeation of drug and sustained release of drug for 24 hours is needed for matrix type transdermal film formulation. Here, X_1 variable is resposible for higher permeation of drug while X_2 variable is responsible for sustained the drug permeation. The relationship between formulation variables (X_1 and X_2) and Y_4 was further elucidated using contour and response surface plot. The effects of X_1 and X_2 on Y_4 are given in Figure 5 (a) and (b). From the response surface plot it was cleared that at highest levels of HPMC K15M (X_1), drug diffusion (Y_4) was increased from 94.34 to 99.53% when Eudragit RL100 (X_2) was increased from -1 level to the +1 level.

Effect of Formulation Variable on Flux (Y_5)

Concerning Y_5, the results of multiple linear regression analysis showed that coefficient b_1 bear a positive sign and

coefficient b_2 bear a negative sign. The positive X_1 coefficient indicates that as the concentration of X_1 (HPMC K15M) increases; there is increase in the flux of drug. The negative X_2 coefficient indicates that as the concentration of X_2 (Eudragit RL 100) increase, the flux of the drug from the matrix was decrease. [24] The relationship between formulation variables (X_1 and X_2) and Y_5 was further elucidated using contour and response surface plot. The effects of X_1 and X_2 on Y_5 are given in Figure 6 (a) and (b). From the response surface plot it was cleared that at highest levels of HPMC K15M (X_1), flux (Y_5) was increased from 10.60 to 13.57 μg/cm²/h when Eudragit RL100 (X_2) was increased from -1 level to the +1 level. The fitted equation relating the response Y_5 to the transformed factor is shown in following equation,

$$Y_5 = 11.904 + 0.918X_1 - 0.488X_2 + 0.062X_1X_2 + 0.058X_{11} + 0.038X_{22} \quad (15)$$

$$(R^2 = 0.9461)$$

The Y_5 for all batches F1 to F9 shows good correlation co-efficient of 0.9461. Higher permeation rate i.e. flux is needed for matrix type transdermal film formulation. Here, X_1 variable is responsible for higher permeation rate of drug while X_2 variable is responsible for sustained the drug permeation.

ANOVA (Analysis of Variance)

R^2 value for folding endurance (Y_1), % moisture content (Y_2), tensile strength (Y_3), drug diffusion (Y_4) and flux (Y_5) are 0.9390, 0.9986, 0.9689, 0.9959 and 0.9461 respectively, indicating good correlation between dependent and independent variables. The reduced models were developed for response variables by omitting the insignificant terms with $P > 0.05$. The terms with $P < 0.05$ were considered statistically significance and retained in the reduced model. The coefficients for full and reduced models for response variables are shown in Table 5.

Full and Reduced Model for Folding endurance

The significance levels of the coefficients b_1, b_{11}, b_{22} and b_{12} were found to be P = 0.0546, 0.3563, 0.3523 and 0.6777 respectively; hence, they were omitted from the full model to generate a reduced model. The results of statistical analysis are shown in Table 5. The coefficient b_2 was found to be significant at $P < 0.05$; hence, it was retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_1, b_{11}, b_{22} and b_{12} contribute significant information to the prediction of folding endurance. The results of model testing are shown in Table 4. The critical value of F for α = 0.05 is equal to 9.12 (df = 4, 3). Since the calculated value (F = 3.00) is less than critical value (F = 9.12), it may be concluded that the terms b_1, b_{11}, b_{22} and b_{12} do not contribute significantly to the prediction of folding endurance and can be omitted from the full model to generate the reduced model. [23]

Full and Reduced Model for % Moisture content

The significance levels of the coefficients b_{11} and b_{22} were found to be P = 0.6729 and 0.0518 respectively; hence, they were omitted from the full model to generate a reduced model. The results of statistical analysis are shown in Table 5. The coefficients b_1, b_2 and b_{12} were found to be significant at $P < 0.05$; hence, they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_{11} and b_{22} contribute significant information to the prediction of percentage moisture content. The results of model testing are shown in Table 4. The critical value of F for α = 0.05 is equal to 9.55 (df = 2, 3). Since the calculated value (F = 5.02) is less than critical value (F = 9.55), it may be concluded that the terms b_{11} and b_{22} do not contribute significantly to the prediction of percentage moisture content and can be omitted from the full model to generate the reduced model. [23]

Full and Reduced Model for Tensile strength

The significance levels of the coefficients b_{11}, b_{22} and b_{12} were found to be P = 0.7080, 0.9567 and 0.5081 respectively; hence, they were omitted from the full model to generate a reduced model. The results of statistical analysis are shown in Table 5. The coefficients b_1 and b_2 were found to be significant at $P < 0.05$; hence, they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_{11}, b_{22} and b_{12} contribute significant information to the prediction of tensile strength. The results of model testing are shown in Table 4.

The critical value of F for α = 0.05 is equal to 9.28 (df = 3, 3). Since the calculated value (F = 0.244) is less than critical value (F = 9.28), it may be concluded that the terms b_{11}, b_{22} and b_{12} do not contribute significantly to the prediction of tensile strength and can be omitted from the full model to generate the reduced model. [23]

Full and Reduced Model for Drug Diffusion

The significance levels of the coefficients b_{11}, b_{22} and b_{12} were found to be P = 0.6346, 0.1148 and 0.1305 respectively; hence, they were omitted from the full model to generate a reduced model. The results of statistical analysis are shown in Table 5. The coefficients b_1 and b_2 were found to be significant at $P < 0.05$; hence, they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_{11}, b_{22} and b_{12} contribute significant information to the prediction of percentage release of drug. The results of model testing are shown in Table 4.

The critical value of F for α = 0.05 is equal to 9.28 (df = 3, 3). Since the calculated value (F = 3.13) is less than critical value (F = 9.28), it may be concluded that the terms b_{11}, b_{22} and b_{12} do not contribute significantly to the prediction of percentage release of drug and can be omitted from the full model to generate the reduced model. [23]

Full and Reduced Model for Flux

The significance levels of the coefficients b_{11}, b_{22} and b_{12} were found to be P = 0.8295, 0.8872 and 0.7456 respectively; hence, they were omitted from the full model to generate a reduced model. The results of statistical

analysis are shown in Table 5. The coefficients b_1 and b_2 were found to be significant at $P < 0.05$; hence, they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_{11}, b_{22} and b_{12} contribute significant information to the prediction of flux. The results of model testing are shown in Table 4. The critical value of F for $\alpha = 0.05$ is equal to 9.28 (df = 3, 3). Since the calculated value (F = 0.06) is less than critical value (F = 9.28), it may be concluded that the terms b_{11}, b_{22} and b_{12} do not contribute significantly to the prediction of flux and can be omitted from the full model to generate the reduced model. [23]

Physicochemical Evaluation of Transdermal Films

Film Thickness

The thickness of the films varied due to increase in concentration of polymers which ranges from 0.17 to 0.27 mm. The values obtained for all the formulations are given in the Table 6. The low value of standard deviation indicated physical uniformity of the films. The uniformity of film thickness was evidenced by the low values of the SD and coefficient of variation. The films were generally thin, therefore aesthetically satisfactory and more acceptable. [25] It is important here to denote that the results of determination of film thickness indicate that the process employed to prepare the films was suitable, reproducible, and capable of producing films with minimal variability.

Uniformity of Weight

The weight of the films varied from 0.058 to 0.079 gm. The values obtained for all the formulations are given in the Table 6. The low value of standard deviation indicated uniform distribution of polymers in the films.

Flatness

An idyllic film should be formulated in such a way that it possesses a smooth surface and should not constrict with time. Flatness studies were performed to judge the same. The result of and thickness shown in Table 6 and low value of standard deviation indicates good uniformity of weight. The result of the flatness study showed that none of the formulations had many differences in the strip lengths before and after their cuts indicating good uniformity of the polymers throughout the transdermal films. It indicates much closed to 100% flatness observed in the formulated films. Thus, very minute amount of constriction was observed in the film of any formulation and it indicates smooth flat surface of the films and these formulations can maintain uniform surface when they are administered onto skin.

Folding Endurance

The folding endurance of the films varied from 98 to 276. The folding endurance measures the ability of film to withstand rupture. The folding endurance was measured manually and results indicated that the films would not break and would maintain their integrity with general skin folding when used. The results of folding endurance were shown in Table 3. It was found to be high in films containing a higher amount of the Eudragit RL100. [26]

Tensile Strength

The tensile strength of the films varied from 0.31 to 0.71 kg/cm^2. The tensile strength results indicate the strength of film and the risk of film cracking. But, no sign of cracking in prepared transdermal films was observed, which might be attributed to the addition of plasticizer, triethyl citrate. The results of tensile strength are shown in Table 3. Tensile strength test results showed that the film contains HPMC K15M in higher amount were less strengthens. There is increase in tensile strength with increase in Eudragit RL100 in the polymer blend. [27]

Elongation break

The percentage elongation of the films varied from 8.00 to 28.67%. Percentage elongation results indicate the elasticity of the film at risk of brittleness. The results of percentage elongation are shown in Table 6 which showed that due to addition of plasticizer i.e. triethyl citrate, the films were exhibit excellent elasticity.

Moisture Content Study

The physicochemical studies like moisture content and moisture uptake provide the information regarding the stability of the formulation. The results of the moisture content studies for different formulations are shown in Table 3. The moisture content varied to a small extent in all the trials. However there was an increase in the moisture content with an increase in the hydrophilic polymer, HPMC K15M in matrix transdermal films. The moisture content of the prepared transdermal film was low, which could help the formulations remain stable and from being a completely dried and reduce brittleness during storage. [28]

Moisture Uptake Study

Percentage moisture uptake was calculated from the weight difference relative to the initial weight after exposing the prepared films to 84% relative humidity (saturated aluminum chloride solution). The results of moisture uptake studies for different formulations are shown in Table 6. The percentage moisture uptake was also found to increase with increasing concentration of hydrophilic polymer, HPMC K15M. [28] The moisture uptake of transdermal formulations was also low, which could protect the formulations from microbial contamination and also reduce bulkiness of films.

Drug Content

The drug content of all the formulations were determined spectrophotometrically at 281 nm which varied from 94.86 to 98.85%. The standard deviation was found to be less which indicated uniform distribution of drug throughout the formulated films. The results of content uniformity are shown in Table 7.

***In vitro* Permeation Study**

The *in vitro* permeation study was carried out on goat skin. The effect of concentration of the polymers on the cumulative amount of drug permeation profile was shown in Figure 7. Changing the composition and dimension of polymer matrix can alter the rate of drug release. The films containing plasticizer triethyl citrate and Eudragit RL100 were provided a drug release between 94.34 to 99.53% at the end of 24 h. The matrix films provided drug release for a period of 24 hours. The maximum drug release was found in formulation F7 (99.53%). The slope of the straight line obtained after plotting the mean cumulative amount released per film versus time was taken as the experimental flux for Nebivolol hydrochloride. The flux obtained for all the formulations was in the range of 10.6-13.57 μg/cm^2/h, but formulation F7 showed not only the maximum flux, i.e. 13.57 μg/cm^2/h, but it also exhibited sustained release up to 24 h. The drug diffusion and flux value of F1 to F9 formulations were shown in Table 8.

The effect of rate controlling membranes on permeation of Nebivolol hydrochloride from transdermal films was studied and it was found that film with Eudragit RL100 provided a maximum release of 99.53% at the end of 24 hours. These transdermal films were able to deliver 1.64 mg of Nebivolol hydrochloride at the end of 24 h.

In the present study, it was observed that as the concentration of hydrophilic polymer (HPMC) increased in the formulations, the drug release rate increased substantially. The addition of hydrophilic component to an insoluble film former tends to enhance the release rates [29]

Analysis of Check Point Batch

From the Design-Expert® 8.0.7.1 (software developed by Stat-Ease®) F5 batch was optimized where X_1: X_2 factors were in 500: 500 mg are coded.

Comparison Between Observed and Predicted Results of Checkpoint Batch

In order to assess the reliability of the equations that describes the influence of the factors on the folding endurance, tensile strength, percentage moisture content, drug diffusion and flux of transdermal film. Predicted results of check point batch are shown in Table 9. The experimental values and predicted values of each response are shown in Table 10.

The % relative error between predicted values and experimental values of each response was calculated using the following equation: [30]

$$\% \text{ Relative error} = \frac{|\text{Predicted value} - \text{Experimental value}|}{\text{Predicted value}} \times 100 \qquad (16)$$

The % relative error obtained from checkpoint batch was in the range of 1.6981 – 6.0200. It can be seen that in all cases there was a reasonable agreement of predicted values and experimental values, since low values of the relative error were found. This confirmed the role of a derived reduced polynomial equation, proved the validity of the model, and as certained the effects of HPMC K15M and Eudragit RL 100 on dependent variables. [31]

Skin Irritation

The dermal observation of skin irritation study is shown in Table 11. From Figure 8 (a) there was no sign of either erythema or edema after 24 hours of application, but in Figure 8 (b) there was slight erythema or edema observed in some rats after the application of 72 hours.

The animals were applied with new formalin solution each day upto 7 days and finally the application sites were graded according to a visual scoring scale, always by the same investigator. The erythema and edema scale observed was given in Table 12. Skin irritation studies on rats gave 0 scales for erythema as well as 0 level scales for edema as compared to the standard formalin solution were showed in Table 12. Table 13 showed the different range value of primary irritation index for indication of skin irritation. According to the range value, irritation could be identified whether an animal was irritated or not. [32]

Primary Irritation Index (PII) Calculation

The primary irritation index for test was calculated from equation 9 and it was found to be 0.2 which produce 'barely irritation' (index 0.04-0.99). [33] According to Draize test, formulation producing scores of or less are considered negative or no irritation. Hence, the developed transdermal formulation was free from skin irritation.

$$\text{PII} = \frac{(\Sigma \text{ erythema at 24 hours and 72 hours})}{(\text{number of test sites x 2 scoring intervals})} + \frac{(\Sigma \text{ oedema at 24 hours and 72 hours})}{(\text{number of test sites x 2 scoring intervals})} \qquad (9)$$

Stability Studies

F5 formulation of Nebivolol hydrochloride film is optimized from statistical design application and was selected for the stability studies. The influence of temperature and humidity on the physicochemical properties of the formulations that was stored for 90 days were also determined.

The results of all physicochemical parameters like thickness, flatness, folding endurance, tensile strength, moisture content and moisture uptake, drug content as well as drug release after the stability period are given in Table 14. The data of optimized formulation, after stability period, was found to be nearly same as those of film, before the stability period and fairly stable as revealed by the stability studies conducted as per ICH guidelines. Hence stability study indicates that the formulation is quiet stable at accelerated conditions.

Similarity Factor (f_2)

By probing the *in vitro* permeation profiles of both the batches the similarity factor value found was 59.0 which indicated that both the profiles are similar.

From the Figure 9 it was also cleared that there is no any significant difference between diffusion pattern after and before stability.

CONCLUSION

HPMC-Eudragit blend with triethyl citrate at 15% w/w concentration for all the formulations exhibited good

flexibility, tensile strength, smooth and non sticky appearance. Based on the results of dependent variables and *in vitro* skin permeation study formulation F5 was considered as the best formulation which exhibited the drug release of 96.54% at the end of 24 h. The optimized formulation was found to be stable at in short term accelerated stability testing at 40 ± 0.5°C and 75 ± 5% RH for 3 month.

REFERENCES AND NOTES

1. Chien Y W. Transdermal Therapeutic System. In Controlled Drug Delivery Fundamentals and Applications,2nd ed.; Robinson, J. R., Lee, V. H. L., Eds.;New York: Marcel Dekker, 1987; p. 524–552.
2. Kanikannan N, Andega S, Burton S, Babu R J, Singh M. Formulation and in vitro evaluation of transdermal patches of melatonin. Drug Dev Ind Pharm 2004;30(2):205–212.
3. http://www.medscape.com/viewarticle/575672_6
4. http://www.medscape.com/viewarticle/742527_3
5. http://drugbank.ca/drugs/DB04861
6. Mittal A, Sara U V S, Ali A. Formulation and evaluation of monolithic matrix polymer films for transdermal delivery of nitrendipine. Acta Pharm 2009;59:383–93.
7. Sahana G N, Sarala N, Kumar T N. Nebivolol - pharmacological aspects. Int J Biol Med Res 2011;2(2):577-580.
8. Banu V T S, Som S, Khaleel M, Havannavar N T. An approach to the formulation of transdermal film of oxybutynin. Res J Pharm Biol Chem Sci 2010;1(3):412-421.
9. Sharma R, Mehra G R. Preparation, characterization, in vitro and in vivo evaluation of transdermal matrix films of celecoxib. Acta Pharm 2011;53:67-76.
10. Gupta R, Mukharjee B. Development and in vitro evaluation of diltiazem hydrochloride transdermal patches based on povidone-ethyl cellulose matrices. Drug Dev Ind Pharm 2003;29:1-7.
11. Devi K, Saisivum S, Maria G R, Deepti P U. Design and evaluation of matrix diffusion controlled transdermal patches of verapamil hydrochloride. Drug Dev Ind Pharm 2003;5:495-503.
12. Arora P, Mukherjee B. Design, development, physiochemical, and in vitro evaluation of transdemal patches containing diclofenac diethylammonium salt. J Pharm Sci 2002;91(9):2076 –2089.
13. Mutalik S, Udupa N. Glibenclamide transdermal patches patches: Physicochemical, pharmacodynamic and pharmacokinetic evaluations. J Pharm Sci 2004;93(6):1577–1594.
14. Lec S T, Yac SH, Kim S W, Berner B. One way membrane for transdermal drug delivery system. II. System optimization. Int J Pharm 1991;77(2-3):231–237.
15. Malakar J, Sen S O, Nayak A, Sen K. Development and evaluation of microemulsions for transdermal delivery of Insulin. ISRN Pharm 2011:1-7.
16. Okur N U, Apaydın S, Yavaşoglu N K, Yavaşoglu A, Karasulu HY. Evaluation of skin permeation and anti-inflammatory and analgesic effects of new Naproxen microemulsion formulations. Int J Pharm 2011;416(1):136 44.
17. Shinde A J, Garala K C and More H. Formulation and evaluation of transdermal patches of metoprolol tartarate. The Indian Pharmacist 2009;8(84):69-74.
18. Aulton M E. Aulton's Pharmaceutics. The Design and Manufacture of Medicines, ISBN 978-0-443-10108-3, Edinburgh, Churchill Livingstone, 2007
19. Shams M S, Alam M I, Ali A, Sultana Y, Aqil M. Pharmacodynamics of a losartan transdermal system for the treatment of hypertension. Drug Dev Ind Pharm 2010;36(4):385–392.
20. US Department of Health and Human Services, Food And Drug Administration, Centre for Drug Evaluation and Research (1997). Guidance for Industry: Dissolution Testing of Immediate Release Solid Dosage Forms. Available: www.fda.gov/./Drugs/Guidancecompliance/Regulatory/Information/Guidance/ucm070 246.pdf
21. EMEA: Committee for Proprietary Products, London, UK (1999). Note for Guidance on Quality of Modified Release Product: A Oral Dosage form., Available: www.ema.europa.eu/pdfs/human/qwp/060496en.pdf
22. Dua K, Ramana M V, Sara U V S, Agrawal D K, Pabreja K, Chakravarthi S. Preparation and Evaluation of Transdermal Plasters Containing Norfloxacin: A Novel Treatment for Burn Wound Healing. Eplasty 2010;10:364-382.
23. Prajapati S T, Patel C G, Patel C N. Formulation and Evaluation of Transdermal Patch of Repaglinide. International Scholarly Research Network. 2011:1-9.
24. Garala K C, Shah P H. Influence of crosslinking agent on the release of drug from the matrix transdermal patches of HPMC/Eudragit RL 100 polymer blends, Journal of Macromolecular Science, Part A, 2010;47(3):273-281.
25. Woolfson A D, McCafferty D F, Moss G P. Development and characterization of a moisture-activated bioadhesive drug delivery system for a percutaneous local anesthesia. Int J Pharm 1998;169:83–94.
26. Vijaya R, Sureshkumar S, Umamaheswari S, Prakash M, Senbagapriya T, Umamaheswari A. Preparation of amitriptyline hydrochloride films using eudragit rl 100 and hydroxy propyl methyl cellulose polymers and their in-vitro evaluation for effective transdermal delivery. Int J life Sci Pharm Res 2012;2(2):7-15.
27. Shinde A J, Shinde A L, Paithane M B, More H N. Design and evaluation of gliclazide transdermal patch containing film former. Int J Pharm Tech 2008;2(3):792-809.
28. Tirunagari M, Jangala V R, Khagga M. Matrix-Type Transdermal Drug Delivery System of Trandolapril: In Vitro and Ex Vivo Characterization. PDA J. Pharm. Sci and Technol 2010;64:44-53.
29. Bodmeier R, Paeratakul O. Drug release from laminated polymeric films prepared from aqueous latexes. J Pharm Sci 1990;79(1):32–36.
30. Mashru R C, Sutariya V B, Sankalia M G, Parikh P. Development and Evaluation of Fast-Dissolving Film of Salbutamol Sulphate. Drug Dev Ind Pharm 2005;31:25–34.
31. Paterakis P G, Korakianiti E S, Dallas P P, Rekkas D M. Evaluation and simultaneous optimization of some pellets characteristics using 3^3 factorial design and the desirability function. Int J Pharm 2002;248(1 –2):51–60.
32. Draize J H, Woodard G, Calvery H O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1994;82:377-390.
33. Aroonrerk N, Kamkaen N. Anti-inflammatory activity of quercus infectoria, glycyrrhiza uralensis, kaempferia galanga and coptis chinensis, the main components of thai herbalremedies for aphthous ulcer. J Health Res 2009;23(1):17-22.

Cite this article as: Garala K, Kapadiya M, Patel J, Patel A, Raval M, Dharamsi A. Design and Evaluation of Transdermal Drug Delivery System of Nebivolol Hydrochloride. Inventi Impact: NDDS, 2013(1):77-91, 2013.