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DEVELOPMENT AND EVALUATION OF TRANSDERMAL SYSTEMS CONTAINING BISOPROLOL FUMARATE

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ABSTRACT

Hypertension is a major disease caused by mental stress and work tension. To duplicate the benefits of intravenous drug infusion without its potential hazards, The novel drug delivery system has brought renaissance into the pharmaceutical industry for controlled drug delivery. In this investigation it was planned to formulate transdermal formulations containing Bisoprolol fumarate (hydrophilic) using two natural gums viz.., Xanthan gum and Almond gum as a reservoir gels planned to characterize the candidate drugs for physico-chemical properties. Membrane-moderated TTS was prepared with rate controlling Eudragit RL 100 polymer, with reservoir gels, and provided with a backing laminate. The films was characterized by WVT studies and SEM photomicrographs. Further, *in vitro* permeation of the candidate drugs was conducted in keshary-chien diffusion cells across depilated abdominal skin of male Swiss albino rat. The data was corrected with Hayton-chien equation, to remove any sample induced bias. Also the data was subjected to regression analysis and ANOVA. A value of $p<0.05$ shall be considered statistically significant. Various permeation parameters like, flux, diffusivity, and permeability coefficient was determined. Stability of the TTS of Bisoprolol fumarate also studied at 40 ºC / 75 %RH.

KEYWORDS: Bisoprolol fumarate, Xanthan gum, Almond gum, Eudragit RL 100.

INTRODUCTION

Hypertension is a major disease caused by mental stress and work tension. It is commonly seen in plus forty age group of either sex and a major cause of cardiac arrest and brain hemorrhage. Most of the antihypertensives are available in the form of conventional tablets and capsules. Further the conventional dosage forms used for the control of infection, pain and fertility may cause side effects like nausea, vomiting, gastric irritation and toxicity if they are consumed for long duration.[1] To duplicate the benefits of intravenous drug infusion without its potential hazards, several technical advancements have been made. They have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling rate of drug delivery, sustaining the duration of therapeutic activity and /or targeting the delivery of drug to a particular tissue.[2] This process has been brought into sharp focus in recent years by the efforts of pharmaceutical films to develop transdermal delivery devices to treat motion sickness, angina, hormone deficiency and hypertension.[3] The novel drug delivery system has brought renaissance into the pharmaceutical industry for controlled drug delivery. The novel drug delivery systems include transdermal drug delivery system, muco-adhesive drug delivery system, ocular drug delivery; nasal drug delivery system etc.Systemic drug delivery through the skin may have several advantages over conventional drug therapy.[4-8] In this investigation it was planned to formulate transdermal formulations. Bisoprolol fumarate (hydrophilic) using two natural gums viz.., xanthan gum and almond gum as a reservoir gels planned to characterize the candidate drugs for physico-chemical properties. Membrane-moderated TTS shall be prepared with rate controlling Eudragit RL 100 polymer, with reservoir gels, and provided with a backing laminate.

MATERIALS AND METHODS

Materials: Bisoprolol fumarate is a gift sample from Hetero Drugs Ltd, Hyderabad. Eudragit RL100 from Rohm Polymers, Xanthan gum from Signet Chemical Corporation, Mumbai. Almond gums from INR Chem Mumbai.Mercury from Central drug house Pvt. Ltd., Mumbai are procured.The others solvents and chemicals used were of analytical grade.

Animals Used: The male Swiss albino rats, weighing 170 to 190 Gms, were obtained from Sri Venkateshwara Enterprises, Bangalore. Permission to carryout permeation studies on animal skin was obtained from institutional animal ethical committee (IAEC).

Certificate is obtained. The animal had free access to food and water.

Analytical method used for the estimation of drug either in bulk or in diffusion samples or in gels

The UV Spectrophotometric analytical method was developed for bisoprolol fumarate pure drug using a double beam U.V. spectrophotometer.

Method used to estimate Bisoprolol fumarate

The drug Bisoprolol fumarate was dissolved in distilled water to get 1mg /ml solution. Further diluted with the same to get 10 µg /ml solution and scanned for maximum absorbance (λ_{max}) in a Shimadzu U.V. spectrophotometer (double beam) between a U.V ranges from 200 to 400 nm against distilled water as blank.

Calibration curve of Bisoprolol fumarate

Procedure

The above prepared clear stock solution of drug was subsequently diluted with distilled water to get 2 µg,4 µg, 6 µg, 8 µg and 10 µg of drug per ml of the final solution.

Then the absorbance of these dilute solutions was measured at a λ_{max} of 224 nm by using double beam U. V. spectrophotometer against a blank of distilled water. The results obtained were tabulated and are given in Table 1 a plot of absorbance versus concentration is shown in Figure 1. The analytical method so developed was validated for linearity, accuracy and precision.

Table. 1: Spectrophotometric data of Bisoprolol fumarate at 224 nm.

Concentration (µg /ml)	Absorbance (nm)
0.00	0
2.00	0.083
4.00	0.163
6.00	0.238
8.00	0.326
10.00	0.413

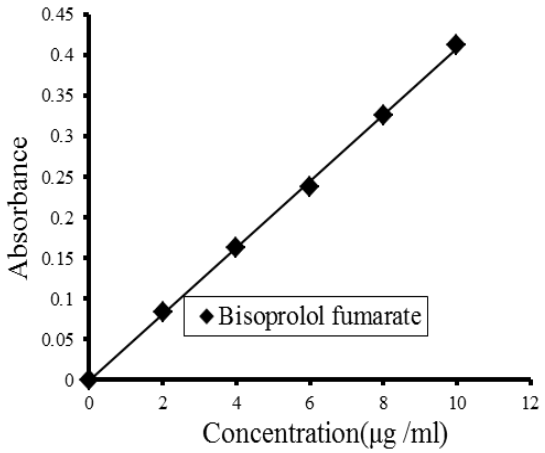

Figure. 1: Calibration curve of Bisoprolol fumarate.

Thickness: The thickness of the films of Eudragit RL 100 and also of the rat's abdominal skin was determined using a micrometer (Mitutoyo, Japan). Average of five readings was considered, then mean thickness, standard deviation and percentage coefficient of variation was computed and is reported.

Drug content

One gm of gel containing Bisoprolol fumarate was placed in a volumetric flask containing 10 ml of distilled water and kept aside with constant shaking for 24 h to extract the total drug present in the gel. The solution was than centrifuged at 2000 rpm for 5 min and subsequently filtered to remove any particles. Later, the absorbance of solution was measured after suitable dilution at 224 nm against drug devoid distilled water as blank. Average of triplicate readings was taken. The content of the drug was calculated using a standard graph.

Water vapour transmission studies (WVT)[9]

One gm of calcium chloride was accurately weighed and placed in a previously dried empty vials having equal diameter. The polymer films were pasted over the brim with the help of an adhesive, and then the vials were weighed and placed over a mesh in desiccators, containing 200 ml of saturated sodium bromide and saturated potassium chloride solutions. The desiccators were tightly closed and the humidity inside the desiccators was measured by using a hygrometer and was found to be 56% relative humidity (RH) and 84% relative humidity (RH) respectively. The vials were weighed at the end of every first day, second day, third day upto seven consecutive days. The average of triplicate readings was taken. The results were tabulated and a graph of cumulative amount water vapour transmitted Vs time was plotted.

Scanning electron microscopy of rate controlling membrane (30 µm): The morphology of 30 µm thick of Eudragit RL100 was studied in a scanning electron microscope (LEICA S-430, UK) at 2 KV and a magnification between x1000 to x5000.

Permeation studies using hairless abdominal rat skin

Preparation of skin[10]

The abdominal skin of excised hairless rat skin was separated along the epidermal junction and was heated for 50 seconds with a stream of 60^{o}C water. The heat-treated skin was cleared of subcutaneous fatty substance and kept in normal saline solution to flatten and smooth. This step caused the layer to unwrinkled. This skin was mounted on to the donor cell of the Keshary-Chien cell.

In vitro permeation

The gel was placed in the donor compartment so that, the epidermis faces the donor compartment. The receptor compartment was filled with solvent. A teflon coated magnetic bead was placed in the receptor compartment and the whole assembly was placed on a magnetic stirrer at a temperature of 37^{0}C ± 0.5^{0}C and the receptor fluid

was stirred at 50 rpm. Throughout the work, samples of 1 ml were withdrawn at regular intervals of time 1, 2, 3 h and so on. These were suitably diluted and the absorbance measured at their respective wavelength maxima. The volume of the receptor compartment was maintained constant by replacing equal volume of solvent. Similarly, a drug devoid gel of same composition was taken and simultaneously diffusion was carried out in a separate cell. Average of triplicate readings was taken.

Hypersensitivity studies: Hypersensitivity reactions were tested by patch testing method upon rabbit skin for the formulations. The rabbits were divided into two groups each having six animals. The ventral surface of rabbits was depilated. The test gels were applied on to the depilated area of the animal with a backing laminate of aluminum foil. These rabbits were kept under observation for 7 days and observed any of the following symptoms. Flushing (redness of the skin) Papules and wheals. Erythema, vesicles and marked oedema.

Stability studies[11,12]

The stability experiments were conducted acording to ICH guidelines to investigate the influence of temperature and relative humidity on the drug content in different formulations. The formulations were exposed to temperature maintained at 40°C / 75% RH in a hot air oven. The sample was removed from the oven and was analyzed for drug content. Further periodically, *in vitro* diffusion studies were carried out and were compared with unconstrained diffusion profile. Average of triplicate readings was taken. Data were analyzed.

Method of preparation of transdermal reservoir gels containing Bisoprolol fumarate

Xanthan reservoir gels: An accurately weighted quantity 0.75 gm of Xanthan gum (7.5% w/w) was soaked in distilled water (10 ml) for 4 hours. After swelling of the gel, drug solution in distilled water 5 mg/gm of drug bisoprolol fumarate was incorporated into Xanthan gum gel separately with continuous mixing in a blender.

Almond reservoir gels: An accurately weighted quantity 0.75 gm of Almond gum (7.5% w/w) was soaked in distilled water (10 ml) for 4 hours. After swelling of the gel, drug solution in distilled water 5 mg /gm of drug Bisoprolol fumarate was incorporated into Almond gum gel separately with continuous mixing in a blender.

Fabrication of rate controlling membranes

For the development of rate controlling membranes, Eudragit RL100 was used. Eudragit was dissolved in acetone and placed in a magnetic stirrer with continuous stirring for 20 min.By controlling the volumetric flow rate of the polymer matrix (Eudragit RL 100), rate controlling membranes of thickness i.e. 30 µm were casted within a teflon ring (4 cm) placed on a mercury substrate, allowed to uniformly dry for 2 h at 40 °C, by inverting a funnel over the film, in hot air oven.

Table. 2: Formula for different transdermal reservoir systems containing Bisoprolol fumarate.

Ingredients	BX (mg)	BA (mg)	BXE (mg)	BAE (mg)
Bisoprolol fumarate	5	5	5	5
Xanthangum (7.5% w/w)	1000	-	1000	-
Almond gum (7.5% w/w)	-	1000	-	1000

*The above formulae are for preparing 1 gm of reservoir gel.

Steady state flux of drug[13]

The fick's law states that the amount of a substance 'dq' passing through a unit cross section 'S', of a barrier in unit time't' is called as flux 'J'. It can be mathematically expressed by the following equation.

J = dq. 1 / S.dt. Therefore, dq /S = J. dt.

The cumulative amount of drugs (dq) permeated per unit skin surface area (S) was plotted against time, and the slope of the linear portion of the plot was estimated as the steady state flux (J_{ss}).

The *in vitro* results obtained during permeation studies were corrected for concentration using Hayton-Chen equation.[14]

$C'_n = C_n (V_t / V_t - V_s) (C'_{n-1} / C_{n-1})$

Where,

C'n = Corrected drug concentration of 'n'th sample,

C_n= Actual drug concentration of 'n'th sample

V_t= Volume of receptor fluid

V_s= Volume of sample fluid

Permeability coefficient of drug[15]

The permeability coefficient of the drugs was calculated by "Potts and Guy equation"

Log Kp = - 2.7+ 0.71 log $K_{o/w}$ – 0.0061 X M. W.

Where, Log K_p = Permeability coefficient.

M. W. = Molecular Weight.

$K_{o/w}$ = Partition Coefficient.

Determination of Diffusivity (D)[16]

The diffusivity can be determined by equation

$J = C_0 {}^*K {}^*D/L = C_0 {}^*P_m$

D = J*L/C_0 *K Where,

J = flux (µg/cm^2/h)

C_0 = drug concentration in the donor Compartment

K = partition coefficient

L = thickness of the skin

P_m = permeability coefficient.

Statistics[17-19]

The *in vitro* data was subjected to regression analysis by least square method. The standard deviation was calculated and reported. *In vivo* data was analyzed by

ANOVA. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Thickness of the skin and the film of Eudragit RL100 were found to be 274.92 ± 8.33 µm (n= 3) and 30.65 ± 0.89 µm (n=3) respectively. Melting point of Bisoprolol fumarate was found to be 100 ^{o}C. The solubility of Bisoprolol fumarate in distilled water was found to be 0.958 mg /ml, in methanol 0.829 mg /ml, in ethanol 0.809 mg /ml, in isopropyl alcohol 0.617 mg /ml. Similarly, The n-octanol**:** water partition coefficient of Bisoprolol fumarate was found to be 0.177 and log K_p computed using Pott's and Guy equation was found to be -4.152 which is within the range of requirements for TTS patch. Drug content in the gels was determined and reported in the Table 3. The study of water vapour transmission of Eudragit RL 100 of 30 µm at 56% and 84% R.H reveals that both the films transmit water vapour when exposed to 56% R.H. and 84% R.H in the Table 4. The amount of water vapor transmitted is shown in figure 2. Data were subjected to regression analysis in the Table 5. Scanning electron photomicrographs of Eudragit RL 100 (30 µm) reveal the presence of regular and uniform pores in the films as shown in the figure 3.

Table. 3: Average thickness and drug content of different formulations used in this study (n=3).

Formulation code	Thickness of skin (µm)			Thickness of Eudragit RL 100 30 µm			Drug content (mg /gm)
	Mean	SD	%CV	Mean	SD	%CV	
BX	269	11.4	4.23	-	-	-	4.41
BA	230	8.36	3.63	-	-	-	4.63
BXE	284	5.47	1.92	30.6	1.83	5.85	4.41
BAE	272	8.36	3.07	32.5	1.32	4.06	4.63

Table. 5: Slope and Regressional values of Eudragit RL100 film used in WVT study.

R.H	Film	Slope	r
56%	Eudragit RL100	0.049	0.999
84%	Eudragit RL100	0.057	0.999

Table. 4: Cumulative amount of water vapour transmitted through the films of Eudragit RL 100, 30 µm, at 56 % R.H and 84 % R.H (n=3).

Time (days)	WVT at 56 % R.H (gms)	WVT at 84 % R.H (gms)
	Eudragit RL 100	Eudragit RL 100
0	0	0
1	0.047	0.057
2	0.097	0.114
3	0.147	0.171
4	0.197	0.228
5	0.247	0.285
6	0.297	0.342
7	0.347	0.399

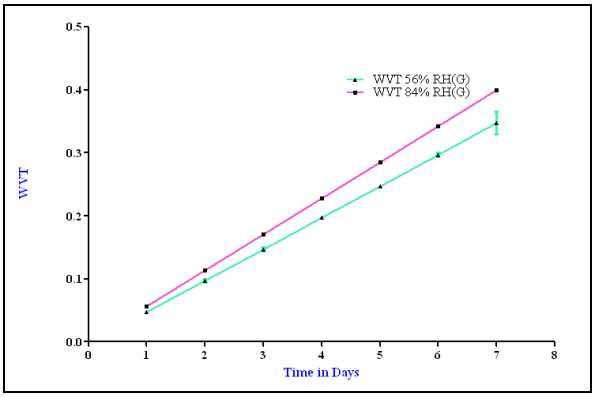

Figure 2: Cumulative amount of water vapour transmitted at 56 % R.H and 84% R.H through Eudragit RL 100 films.

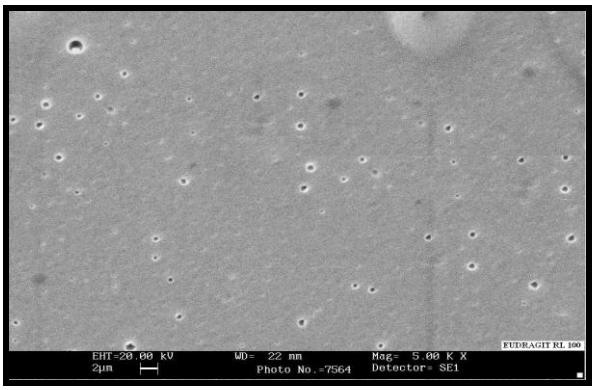

Figure 3: SEM photomicrograph of 30 µm Eudragit RL100 film.

In vitro permeation study of Bisoprolol fumarate in various gels or in membrane moderated systems of Eudragit RL100, across depilated rat's abdominal skin

The drug chosen in this investigation was Bisoprolol fumarate**.**The process of drug release in most controlled-release devices including transdermal patch is governed by diffusion and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymer chains.The basic *in vitro* data were corrected using Hayton-Chen equation to remove any sample induced bias during the *in vitro* diffusion across intact skin. Also the data was subjected to regression analysis by least squares method. The permeation study was conducted for 12 h. The total amount of the drug left at the end of the study in the receptor compartment and bound in the matrix of the skin and or gel was determined. The total amount of the drug in hydrated skin and in the remaining gel and film was found to be the initial amount taken in the donor compartment ±

0.006 to 0.26 mg at the end of the study. The *In vitro* data was analyzed by ANOVA and a p value of < 0.05 was considered significant.When cumulative amount of drug permeated (mg /ml) of all the formulations were plotted, as shown in figure 4. Flux (J) was obtained from the slope of the curves of dq x 1/s versus time 't' and are shown in figure 4 Correlation coefficient 'r' were found to be high and the values of flux (J), 'r', permeability coefficient (K_p), diffusivity (D), were derived from the *in vitro* data and are shown in Table 6.

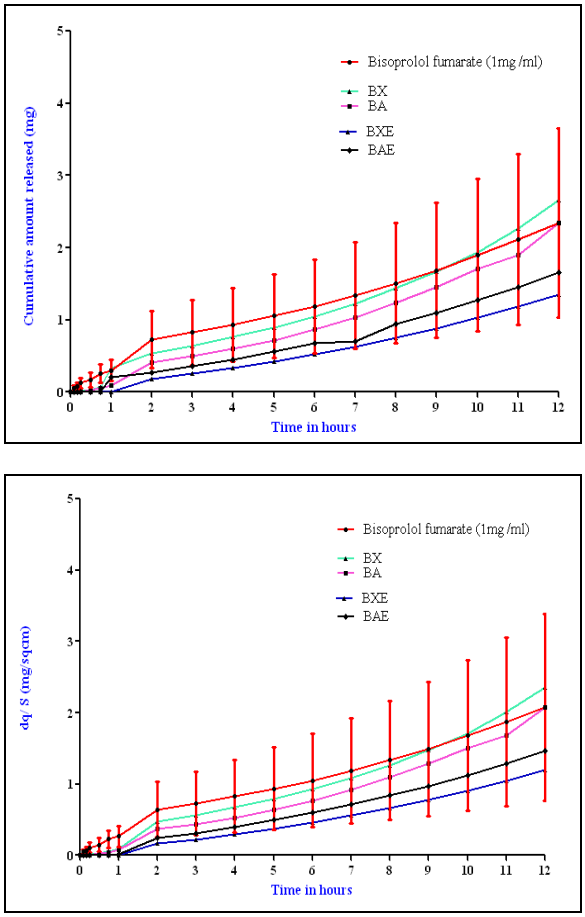

Figure 4: In vitro permeation and In vitro flux of Bisoprolol fumarate from Xanthan gum and Almond gum reservoir across depilated rat's skin

Table 6: Various kinetic parameters derived from *in vitro* permeation study of TTS formulations.

Formulations	' r'	Flux's mg/sq.cm/ h	Kp	D
BX	0.990	0.140	0.031	0.049
BA	0.990	0.130	0.028	0.042
BXE	0.993	0.130	0.029	0.045
BAE	0.995	0.120	0.025	0.039

In *vitro data* obtained was corrected to remove any sampling induced bias in concentration- time profiles, using Hayton-Chen equation.The basic *in vitro* study was conducted by preparing a 1 mg /ml solution of drug and the data obtained was plotted as dq/S versus time (h). From slope of the curve flux of Bisoprolol fumarate 0.170 mg / cm^2 / h was obtained.

In vitro permeation studies showed that, Bisoprolol fumarate permeate through the skin and also from the gels, xanthan gum gel and almond gum gel, (BX and BA). During permeation, a significant amount of Bisoprolol fumarate in the receptor fluid from xanthan gum gel was found at 0.166 h and significant amount of Bisoprolol fumarate in the receptor fluid from almond gum gel was found at 0.166 h. The flux of BX and BA found to be 0.140 mg /cm^2 /h and 0.130 mg /cm^2 /h respectively. Bisoprolol fumarate permeates greater from almond gum gel compared to xanthan gum gel and lowest flux of the series was found to be from xanthan gum gel. Diffusivities were found to be 0.049 and 0.042 respectively and permeability coefficients 'K_p' were found to be 0.031 and 0.028 respectively.

The flux of membrane-moderated systems of Eudragit RL100, BXE and BAE, was found to be 0.130 mg /cm^2 /h and 0.120 mg /cm^2 /h respectively. Significant amounts of Bisoprolol fumarate in the receptor fluid from BXE was found at 0.75 h, from BAE at 0.5 h. Diffusivities were found to be 0.045 and 0.039. Permeability coefficients 'K_p' were found to be 0.029 and 0.025 respectively.

Stability of the formulations BX and BA was conducted at 40 ºC / 75 % R.H. for ninty days. At the end of each day drug content was estimated, and the data obtained are reported in Table.7. It was observed that percentage reduction in drug content was not significantly altered. Further, the diffusion profiles of reservoir gels were also studied periodically. The data obtained were compared and statistically analysed by ANOVA, at $p < 0.05$, with the unconstrained diffusion profiles.

Table 7: Stability studies according to ICH guideline at 40 ^{0}C /75 % R.H.

Time in days	Formulations	
	BX(mg)	BA(mg)
	DC*±S.D	DC*±S.D
0	4.41±0.002	4-.63±0.002
1	4.40±0.002	4.63±0.004
2	4.40±0.001	4.6±0.003
3	4.4±0.007	4.61±0.001
7	4.4±0.001	4.61±0.001
15	4.34±0.001	4.58±0.004
30	4.29±0.001	4.54±0.002
45	4.21±0.003	4.53±0.004
90	4.20±0.001	4.44±0.002

*DC=Average of triplicate readings.

Hypersensitivity reactions test was conducted on depilated rabbit's skin for seven days. Every day at regular intervals, the skin of the rabbit was observed for any of the symptoms, flushing, or erythema or oedema or papules or wheals. The observations were tabulated and are given in Table 8.

Table 8: Hypersensitivity reactions of Eudragit RL100.

Film	EudragitRL100		
Time in Days	**A**	**B**	**C**
1	-ve	-ve	-ve
2	-ve	-ve	-ve
3	-ve	-ve	-ve
4	-ve	-ve	-ve
5	-ve	-ve	-ve
6	-ve	-ve	-ve
7	-ve	-ve	-ve

CONCLUSION

In this investigation a sincere effort was made to study the feasibility of drug release from xanthan gum and almond gum reservoir gels. Bisoprolol fumarate is successful antihypertensive drug used in the treatment of hypertension and has lower bioavailability in systemic circulation. They are available commercially only as conventional dosage form. Therefore, in this work the feasibility of permeation of these drugs across intact skin, permeation through membrane moderated systems of Eudragit RL 100 were studied and were compared. The study is important in order to understand the drug release kinetics across intact skin in therapeutic concentrations and subsequently to control the delivery of the drugs into the receptor.The drug was characterized for their λ_{max}, melting point, solubility in various solvents, n-octanol-water partition coefficient. The films were characterized for thickness, water vapour transmission and surface morphology using SEM.*In vitro* diffusion studies were carried out in Keshary-Chien diffusion cells at 50 rpm and at 37 ± 0.5^0C. The data was subjected to linear regression by least squares method and were graphed. The data was also subjected to ANOVA, a value of $p < 0.05$ was considered statistically significant. Slope values were obtained from the graphs so plotted and were used to ascertain the drug release kinetics. Various parameters like flux, permeability coefficient and diffusivity were computed.The reservoir patches were tested for any hyper sensitivity reactions by "Patch testing" method on depilated skin of rabbits. Further ICH stability studies at 40^oC temperature and 75% relative humidity was conducted and reported.

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