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DESIGN DEVELOPMENT AND EVALUATION OF TRANSDERMAL DRUG DELIVERY SYSTEM OF EPROSARTAN MESYLATE

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ABSTRACT

Objective: Transdermal drug delivery systems are polymeric patches containing dissolved or dispersed drugs that deliver therapeutic agents at a constant rate to the human body. Eprosartan mesylate, a BCS class II drug with low solubility and high permeability has oral bioavailability of only 13% which is limited due to its poor aqueous solubility. Hence the objective was to develop transdermal patch of Eprosartan mesylate to enhance its bioavailability and to develop sustained release formulation of EPM to deliver drug for 24 hrs. **Method:** Matrix type transdermal patches containing Eprosartan mesylate were prepared by solvent casting method with different quantities of hydrophilic (Hydroxy propyl methyl cellulose) and hydrophobic (Eudragit RS 100) polymeric system and Dibutylphthalate and dimethyl sulfoxide as plasticizer and penetration enhancer respectively. All the patches were optimized according to 3^2 factorial design. The transdermal patches were evaluated for their physicochemical properties such as thickness, weight uniformity, percent moisture absorption, percent moisture loss, folding endurance, tensile strength, percent elongation, water vapour permeation, surface pH, drug content uniformity, *in vitro* permeation study, skin irritation test and stability study. **Result:** FTIR and DSC studies were carried out which indicated no interaction between drug and polymers. In vitro drug permeation study reveals F8 formulation batch as optimized formulation with 94.00% drug release for 24 hrs. Permeation flux and permeability coefficient were found to be maximum for optimized batch. Drug release kinetics were found to follow first order release mechanism. Skin irritation studies on rat skin revealed patch to be non-irritant. The formulation was found to be stable for period of three months. **Conclusion:** Eprosartan can thus be formulated in the form of transdermal patch for effective delivery of drug via transdermal route and enhance bioavailability of the same.

KEYWORDS: Transdermal patches, transdermal drug delivery system, Eprosartan mesylate, HPMC E15, Eudragit RS100.

INTRODUCTION

Transdermal route of administration is recognized as one of the potential route for the local and systemic delivery of drugs. In comparison to conventional pharmaceutical dosage forms, TDDS offers many advantages, such as elimination of first pass metabolism, sustained drug delivery, reduced frequency of administration, reduced side effects and improved patient compliance[1]. Transdermal drug delivery systems (TDDS), also known as patches are the dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin. Transdermal delivery not only provides controlled, constant administration of the drug, but also allows continuous input of drug with short biological half-lives and eliminates pulsed entry into systemic circulation, which often causes undesirable side effects[2].

Eprosartan mesylate (EPM) is an angiotensin receptor blocker (ARB) which antagonizes angiotensin II by blocking the angiotensin type 1 (AT1) receptor. Angiotensin II is a potent vasoconstrictor, the primary vasoactive hormone of the renin-angiotensin system and an important component in the pathophysiology of hypertension. It also stimulates aldosterone secretion by the adrenal cortex. Eprosartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor found in many tissues (e.g., vascular smooth muscle, adrenal gland). EPM has pH dependent solubility, slightly soluble in aqueous solutions of pH 3-5 and hence shows limited bioavailability after oral administration. The oral bioavailability of EPM is only 13% and plasma half-life of 5-9 hrs which results in dosing frequency of twice a day in order to maintain the therapeutic plasma concentration. Thus, there is a strong

clinical need and market potential for a dosage form that will deliver EPM in a modified manner thereby increasing its bioavailability and resulting in better patient compliance. Therefore present study focused on development of matrix type transdermal patches of Eprosartan mesylate to ensure satisfactory drug release and prolong duration of action.

MATERIALS AND METHODS

Materials

Eprosartan mesylate was obtained as a gift sample from Mylan pharmaceuticals ltd, Sinnar, Nashik, India. HPMC and Eudragit RS100 were obtained from colorcon Asia Pvt Ltd. All chemicals and solvents used were of analytical grade.

Methods

Investigation of physicochemical compatibility of drug and polymer.

A physicochemical compatibility between EPM and polymers used in patch was studied by using Differential Scanning Calorimetry (DSC-60, Shimadzu, Japan) and Fourier Transform Infrared Spectroscopy (BRUKER Eco-ATR).

The powdered sample (3 mg) was hermetically sealed in aluminum pans and heated at a constant rate of 10 °C/min, over a temperature range of 30–300 °C with nitrogen flow rate of 30 ml/min. Thermograms of the samples were obtained using differential scanning calorimetry (DSC-60, Shimadzu, Japan). Thermal analysis data were recorded with Shimadzu software programs. Indium standard was used to calibrate the DSC temperature and enthalpy scale.

Compatibility study was carried out using Fourier transform infrared spectrophotometer (BRUKER Eco-ATR). FTIR study was carried on pure drug. Physical mixture of drug and polymers was prepared and kept for 1 month at 40^0C. The infrared absorption spectrum of Eprosartan Mesylate and physical mixture of drug and polymers was recorded over the wave number 4000 to 400 cm^{-1}

Formulation of transdermal patches[14,21]

All the formulations were prepared by solvent casting method. For each formulation calculated quantity of HPMC and Eudragit RS 100 were accurately weighed in 100 ml glass beaker containing solvent mixture Dichloromethane: methanol (1:1) & mixed well. After clear solution was obtained, required quantity of EPM was added and mixed until dissolved. Dibutylphthalate and DMSO were added as plasticizer and penetration enhancer respectively and mixed well. After bubble free solution was obtained the solution was poured onto glass petri dish containing aluminum foil as backing layer which was then allowed to stand for 24 hrs for solvent evaporation with inverted funnel over it to control the rate of evaporation.

Table 1: Composition of formulations of transdermal patch of EPM

Formulation code	Drug (mg)	HPMCE15 (mg)	Eudragit RS100(mg)	DBP (ml) (30%w/w of polymer weight)	DMSO (ml)	Dichloromethane: methanol (1:1)
F1	100	100	100	0.057	0.06	12
F2	100	150	100	0.072	0.06	12
F3	100	200	100	0.086	0.06	12
F4	100	100	150	0.072	0.06	12
F5	100	150	150	0.086	0.06	12
F6	100	200	150	0.100	0.06	12
F7	100	100	200	0.086	0.06	12
F8	100	150	200	0.100	0.06	12
F9	100	200	200	0.115	0.06	12

Characterization of transdermal patches

Evaluation of patches determines the behavior of dosage form such as when applied onto application site.

Visual appearance

All the prepared patches were visually inspected for color, clarity, flexibility and smoothness.

Thickness uniformity[3,4]

The thickness of patches was measured by using digital Vernier caliper at three different points and average thickness was calculated (n=3).

Uniformity of weight[3,4]

The patches were uniformly weighed on electronic analytical balance in replicates of three, the average weight of patch was calculated. (n=3).

Folding endurance[5,6]

Folding endurance was measured manually for prepared films. A strip of film (5x1cm) was cut and repeatedly folded at same place till it broke. The number of times the film would be folded at same place without breaking gave the value of folding endurance (n=3).

Percentage moisture absorption[4,7,8]

The films were weighed accurately and placed in desiccator containing 100ml of saturated solution of KCl which maintains 80-90% RH. After three days, the films were taken out and weighed. The study was performed at room temperature (n=3).The moisture loss was calculated using formula:

$$\% \text{ moisture absorption} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100$$

Percentage moisture loss[4,7,8]

The prepared films were weighed individually and kept in desiccator containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed. (n=3) The moisture loss was calculated using the formula:

$$\% \text{ moisture loss} = \frac{\text{Initial weight-Final weight}}{\text{Final weight}} \times 100$$

Water vapor permeation rate[9,10]

Glass vials of equal diameter were used as transmission cell. These were washed thoroughly and dried in an oven to a constant weight. About 1g of anhydrous calcium chloride was placed in the cell and the respective polymer films were fixed over brim. The cells were accurately weighed and kept in closed desiccator containing saturated solution of KCl to maintain humidity 84%. The cells were taken out and weighed after 6, 12, 24, 36, 48, 78 hrs of storage (n=3).

Amount of water vapour transmitted was found using formula:

$$\text{WVPR} = \frac{\text{Final weight-Initial weight}}{\text{Time}} \times \text{area}$$

Usually expressed as grams of moisture gained/h/cm^2.

Percentage flatness[11,12]

Three longitudinal strips were cut out from each film: 1 from center, 1 from left side and 1 from right side. The length of each strip was measured and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness.

$$\% \text{ constriction} = \frac{L_1-L_2}{L_1} \times 100$$

L_1 = Initial length of each strip

L_2 = final length of each strip

Tensile strength and % Elongation[13,14]

Films were evaluated for tensile strength and % elongation using an apparatus assembled in the laboratory. Patch of dimension 1 x 5 cm were attached to a support that was inextensible but flexible and this support was in turn held between two clamps separated by a distance of 4 cm. Clamps were designed to secure the patch without crushing it during the test. These were supported on a metal base. One of the clamps was fixed; the other one was movable and weights could be added to the movable clamp. During measurement, the films were pulled by the movable clamp with the addition of weights. The strength and elongation were measured when the films broke and tensile strength and % elongation were calculated using the following formulae (n=3).

$$\text{Tensile strength} = \frac{\text{Tensile load at break}}{\text{Cross sectional area}}$$

$$\% \text{ Elongation} = \frac{\text{Maximum length recorded at break-Original length}}{\text{Original length}} \times 100$$

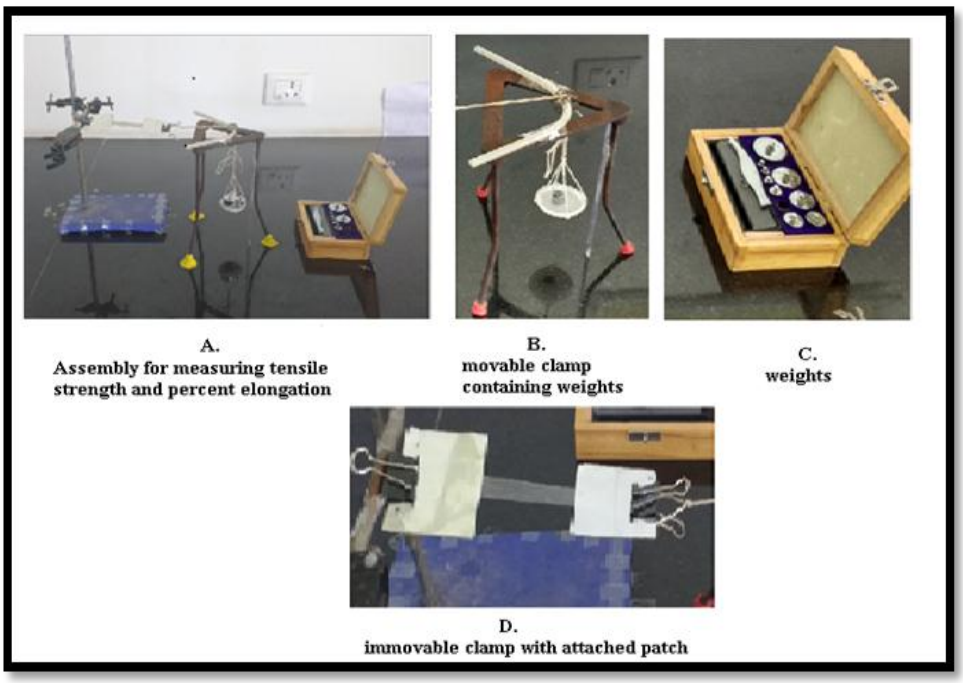

Fig 1: Laboratory fabricated apparatus for measuring tensile strength and % elongation.

Drug content determination[15,16,17]

The patch 1cm^2 was cut and added to a beaker containing 100ml of phosphate buffer pH 7.4. The medium was stirred with Teflon coated magnetic bead for 5hrs. The contents were filtered using Whatman filter paper and the filtrate was analyzed by UV Spectrophotometer at 292 nm for the drug content against blank solution.

Surface pH[15,17,18]

Since acidic or alkaline pH may leads to irritation to the skin surface. The surface pH of the patch was calculated in order to investigate any side effects in vivo. A combined pH electrode was used for this purpose. The patch to be tested was placed in a nesseler cylinder and was slightly moistened with 0.5 ml distilled water for 1 hour. The pH was measured by bringing the electrode in contact with the surface of the patch and allowing equilibrating for 1 min. The study was performed on three patches of each formulation and mean ±SD was calculated.

***In vitro* permeation study**[7,11]

An *in vitro* permeation study was carried out using franz diffusion cell. Full thickness abdominal skin of male wistar rat weighing 200-250g was used. Hair from abdominal region was removed carefully using an electric clipper; the dermal side of the skin was thoroughly cleaned with distilled water to remove any adhering tissue or blood vessels, equilibrate for an hour in phosphate buffer pH 7.4 before starting the experiment and was placed on a magnetic stirrer with a small magnetic needle for uniform distribution of diffusant. The temperature of cell was maintained at 32+ 0.5^{0C} using a thermostatically controlled heater. The isolated rat skin piece was mounted between the compartments of the diffusion cell with the epidermis facing upward into the donor compartment. Sample volume of 5ml was removed from the receptor compartment at regular intervals.

Permeation data analysis[19]

The flux (µg/cm^2 /hr) of EPM was calculated from the slope of the plot of the cumulative amount of EPM permeated per cm^2 of skin at steady state against time using linear regression analysis. The steady state permeability coefficient (K_p) of the drug through rat epidermis was calculated by using following equation:

$Kp = J/C$

Where J is the flux and C is the concentration of EPM in the patch.

Skin irritation studies[15,17]

The patches were tested for its potential to cause skin irritant / sensitization in rats. The rats were shaved carefully avoiding peripheral damage and the patch was applied onto the nude skin using an adhesive. The rats were divided into five groups.

Group 1: control (no treatment), **group 2**: optimized formulation, **group 3**: standard irritant i.e. 0.8% v/v aqueous formalin solution, **group 4**: marketed preparation, **group 5**: optimized formulation without drug. On the previous day of the experiment, the hair on back side of rat was removed. The animals were applied with new patch/formalin solution each day upto 7 days and finally the application sites were graded according to a scoring scale by the same investigator (Table 2). Ethical clearance for the handling of experimental animals was obtained from the Institutional Animal Ethical Committee (IAEC). (RGSCOP/M.Pharm/IAEC/2015-2016/021665/7).

Table 2: Draize Scoring method[17,20]

S.No.	Skin reaction		Score assigned
	(A)Erythema and eschar formation	(B) Edema formation	
1	No erythema	No edema	0
2	Very slight erythema	Very slight edema	1
3	Well defined erythema	Slight edema	2
4	Moderate to severe erythema	Moderate edema	3
5	Severe erythema	Severe edema	4

Kinetics models for diffusion and dissolution study[20,21,22]

To study the release kinetics, data obtained from *in vitro* drug release were study with Zero order, First order, Higuchi mode and Korsmeyer-Peppas model.

Stability studies[3,21,24,25]

Stability study of optimized formulation (F8) was assessed at 40±2 ^{0}C/ 75±5% RH as per ICH guidelines. The drug content of patches were determined at 0,1,2,3 months of study.

RESULT AND DISCUSSION

Investigation of physicochemical compatibility of drug and polymer

DSC thermogram of Eprosartan mesylate mixture with excipients were determined to study any interaction between drug and polymers. The DSC thermogram of drug-polymer mixture reveal a sharp melting point at 241^{0}C which indicates no interaction between drug and polymers. Thus the drug polymer mixture was found to be compatible and sharp peak indicates no conversion of crystalline form to another form.

The data obtained from the IR spectra showed no evidence of the interaction between the drug and the polymer studies. All the major characteristic peaks of the drug were present in the drug-polymer combinations subsequent spectra. FTIR spectrum of EPM with excipients physical mixture is shown in figure 2.

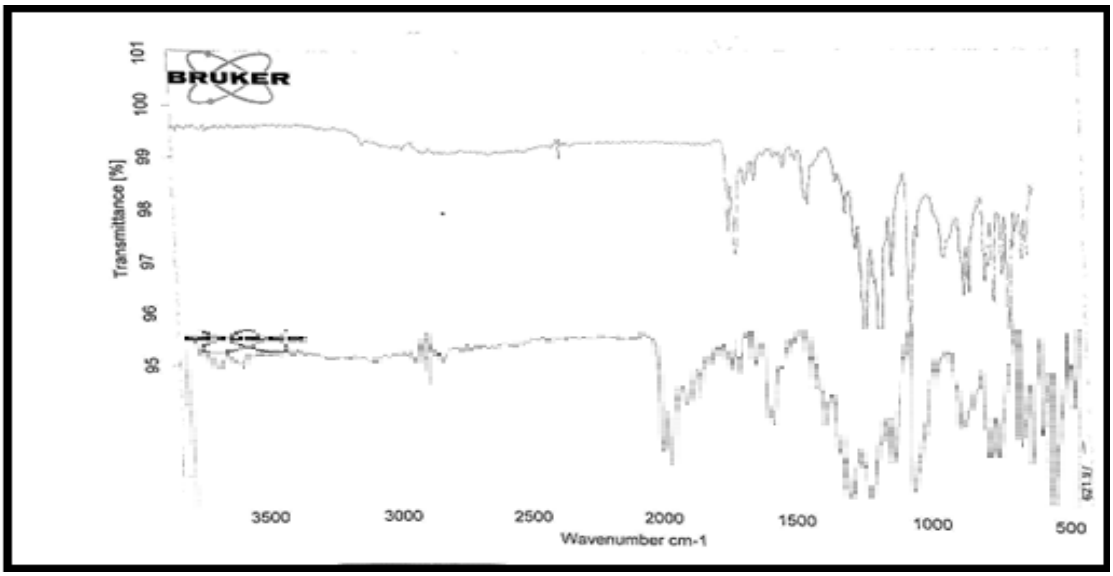

Fig 2: FTIR spectrum of EPM and EPM with excipients

Characterization of patches

Table 3: Evaluation of Eprosartan Mesylate Transdermal Patch

Sr.no	Weight uniformity (mg)	Thickness (mm)	% Moisture absorption (%)	% Moisture loss (%)	Water vapour permeation rate (%)
1	0.381 ± 0.0144	0.11 ± 0.005	3.25 ± 0.25	2.503 ± 0.040	0.027 ± 0.0207
2	0.417 ± 0.0085	0.12 ± 0.01	3.64 ± 0.05	2.965 ± 0.064	0.011 ± 0.0037
3	0.444 ± 0.0217	0.14 ± 0.005	4.18 ± 0.04	3.563 ± 0.062	0.0096 ± 0.0023
4	0.33 ± 0.0105	0.11 ± 0.01	3.18 ± 0.09	2.462 ± 0.068	0.011 ± 0.0038
5	0.280 ± 0.0182	0.14 ± 0.015	3.93 ± 0.08	2.765 ± 0.060	0.0097 ± 0.0036
6	0.429 ± 0.0086	0.16 ± 0.005	4.50 ± 0.01	3.370 ± 0.049	0.0073 ± 0.0056
7	0.274 ± 0.0088	0.10 ± 0.005	2.79 ± 0.12	1.864 ± 0.058	0.010 ± 0.0037
8	0.407 ± 0.0083	0.14 ± 0.005	3.75 ± 0.06	2.508 ± 0.047	0.085 ± 0.0036
9	0.477 ± 0.0165	0.16 ± 0.020	4.15 ± 0.02	2.983 ± 0.044	0.073 ± 0.058

Table 4: Evaluation of EPM transdermal patches

Sr.no	Folding endurance	Tensile strength	%elongation at break	Drug content	Surface pH
1	116 ± 3.4	0.31 ± 0.014	29.17 ± 1.44	97.13 ± 0.179	5.5
2	112 ± 2.4	0.29 ± 0.014	26.67 ± 1.44	96.92 ± 0.205	5.6
3	103 ± 4.1	0.26 ± 0.014	24.16 ± 1.44	97.60 ± 0.071	5.3
4	180 ± 3.0	0.41 ± 0.012	31.67 ± 1.15	98.07 ± 0.045	5.8
5	172 ± 2.6	0.40 ± 0.014	30.83 ± 1.15	98.31 ± 0.085	5.7
6	156 ± 4.16	0.39 ± 0.014	26.67 ± 1.44	96.95 ± 0.091	5.6
7	286 ± 3.78	0.48 ± 0.014	36.67 ± 1.44	97.59 ± 0.140	5.7
8	273 ± 2.64	0.46 ± 0.012	33.33 ± 1.44	98.36 ± 0.080	5.8
9	243 ± 2.6	0.44 ± 0.014	29.16 ± 1.44	97.90 ± 0.200	5.3

All the formulated patches were characterized for physicochemical properties. All the patches appeared transparent, clear, smooth and flexible. The thickness of the drug loaded films F-1 to F-9 formulations were measured with the help of a digital caliper at different strategic locations like four sides and center of the each films. Thickness of a single film varies from 0.11±0.005 to 0.16±0.020 mm. The weight of each patch was taken on electronic analytical balance in replicates of three. Weight of patches ranges from 0.381 ±0.0144 to 0.447 ±0.0165.

Folding endurance studies reflects the influence of concentration of both polymers in the formulation. As

the concentration of Eudragit RS100 is increased, folding endurance increases and at the same time as the concentration of HPMC E15 increase, folding endurance values decreases.

Percent moisture absorption values of patches varied with concentration of both the polymers i.e. HPMC E15 and Eudragit RS100. As the concentration of hydrophilic polymer HPMC E15 is increased, the % moisture absorption values increases. Also as the concentration of hydrophobic polymer Eudragit RS100 is increased moisture absorption decreases. Percent moisture loss values of different batches shows that as the concentration of hydrophilic polymer HPMC E15 is increased, percent moisture loss increases. Also as the concentration of hydrophobic polymer Eudragit RS100 is increased, percent moisture loss decreases. Water vapour permeability of patches depends on concentration of both polymers i.e. HPMC E15 and Eudragit RS100. As the concentration of hydrophobic polymer Eudragit RS100 is increased, its WVP is decreased. Also when concentration of hydrophilic polymer HPMC increases its WVP decreases. % constriction was determined where 0 % constriction represents 100% flatness. Percent flatness of all 9 formulations were found to be 100%.

Mechanical properties of the patches are evaluated using tensile strength apparatus developed in laboratory. From the results it clears that when the concentration of the polymer Eudragit RS100 increases, the tensile strength of the film also increases. The formulation F7 shows the maximum tensile strength. Presence of dibutyl-phthalate as a plasticizer imparts the flexibility to the polymers. Tensile strength measures the ability of the film to withstand rupture. The Formulation F7 shows the maximum strength 0.48± 0.014.This might be due to formation of strong hydrogen bonds between polymer and plasticizer thereby imparting flexibility to withstand rupture.

Percent elongation values of different batches shows that as the concentration of Eudragit RS 100 increases, percent elongation value increases.

Drug content of different formulations were determined. Drug content values shows that there was uniform distribution of drug throughout the sample. The drug content values were shown in table 4. The surface pH of the patch was calculated in order to investigate any side effects in vivo. A combined pH electrode was used for this purpose. The study was performed on three patches of each formulation and mean ± SD was calculated. Surface pH values of patches were found between 5-6 which meets the actual skin pH.

In-vitro drug permeation study of all 9 batches was carried out. Different formulations shows different rate of drug permeation through rat skin. From all the batches sustained release was observed for batch F8 which showed 94.00 % release in 24 hrs while all other batches did not sustain release of drug for 24 hrs. Skin irritation study suggests formulation to be stable as indicated in table 6 and figure 4. Thus appropriate ratio of both hydrophilic and lipophilic polymers (here 2:3; HPMC E15: Eudragit RS100) plays important role in achieving sustained action of drug.

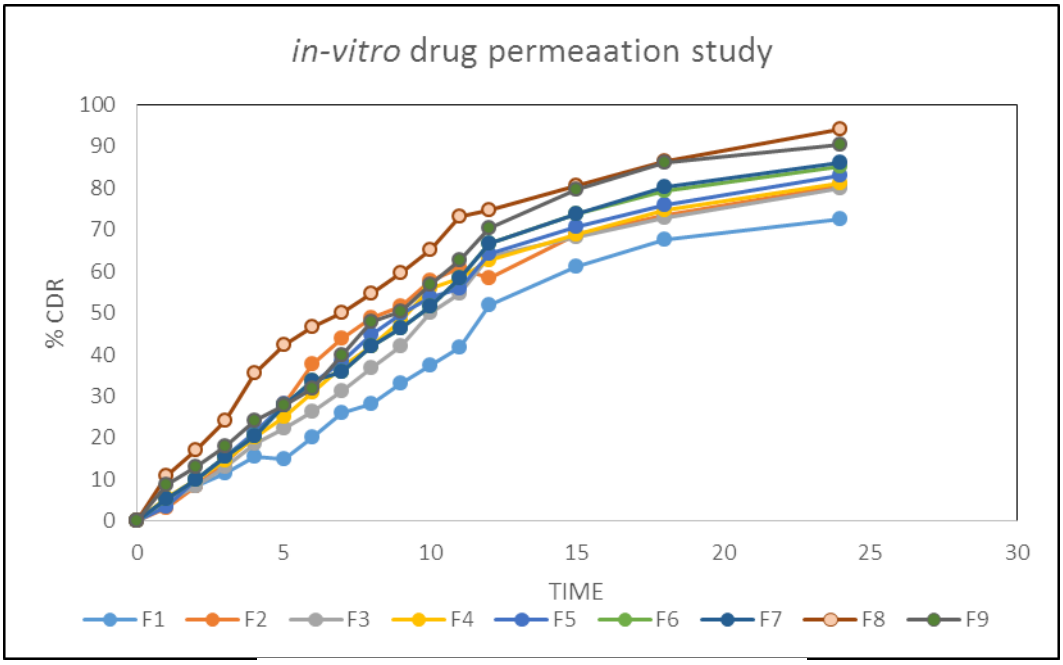

Fig 3*: In vitro* drug release of EPM formulations

Table 5: Permeation data analysis of EPM

Batch no.	Variables levels in actual values		Q_{24hrs} release (%)	Flux (J) ($\mu g/cm^2$ /hr)	Permeability coefficient (Kp) (cm/hr)
	X1	X2			
F1	100	100	72.52	4.8423	0.6361
F2	150	100	78.41	5.489	0.7214
F3	200	100	80.02	5.4231	0.7135
F4	100	150	81.26	5.4655	0.7180
F5	150	150	83.60	5.518	0.7249
F6	200	150	85.66	5.7157	0.7508
F7	100	200	86.16	5.7591	0.7565
F8	150	200	94.00	5.9269	0.7785
F9	200	200	90.28	5.6306	0.7397

Where XI= concentration of HPMC E15
X2= concentration of Eudragit RS100.

Table 6: Evaluation table for Skin irritation test according to Draize scoring method

Rat No.	Control group		Formulation group		Formalin group	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1.	0	0	0	0	4	2
2.	0	0	0	0	4	2
3.	0	0	0	0	4	2

Erythema scale: **0**- none; **1**-slight; **2**- well defined; **3**-moderate; and **4**- scar formation
Oedema scale: **0**- none; **1**- slight; **2**- well defined; **3**- moderate; and **4**- severe.

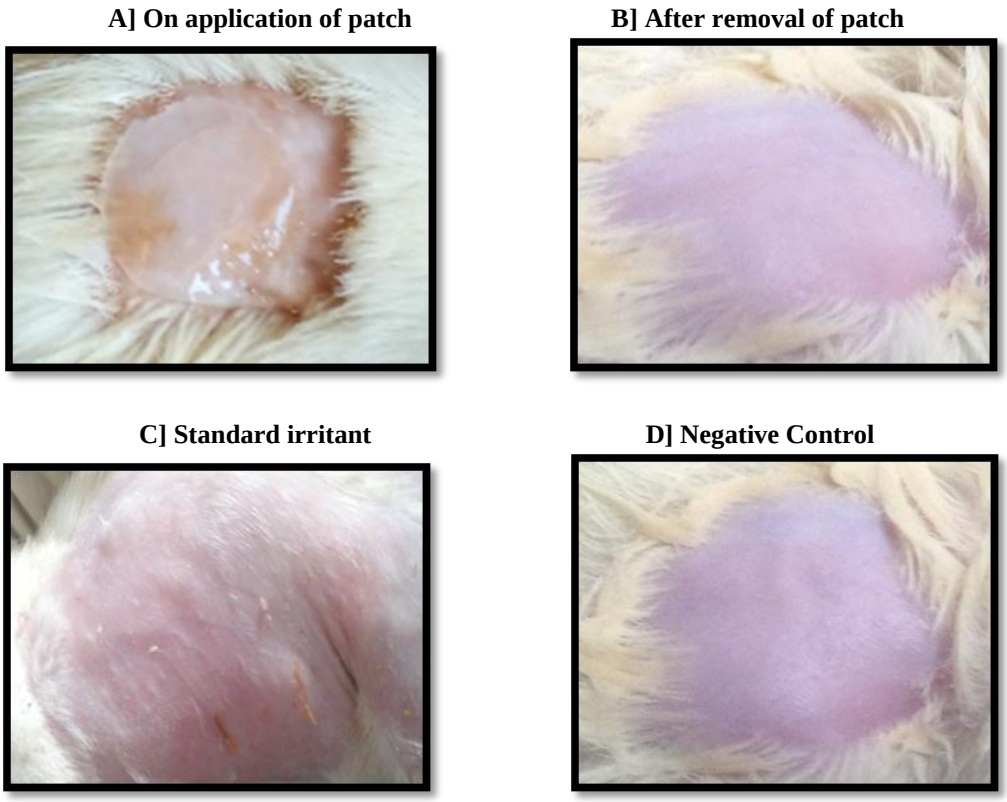

Fig 4: Photographs of skin irritation test.

Kinetics study
Kinetics study was performed to study the mechanism of drug release and drug was found to follow first order release kinetics from transdermal patch.

Table 7: kinetics study of all EPM formulations

Batch / R^2 value	F1	F2	F3	F4	F5	F6	F7	F8	F9
1^{st} order	0.9678	0.9465	0.9735	0.9815	0.9917	0.9821	0.9804	09939	0.9819

Stability studies

Results of stability studies showed that there is no significant change in content uniformity and drug release for optimized formulation after elevated temperature and humidity conditions during stability studies. Thus it can be proved from the stability studies that the prepared formulation is stable and not affected by humidity and temperature conditions.

CONCLUSION

Thin, transparent, flexible matrix type transdermal patch of Eprosartan mesylate were prepared using HPMC E15 and Eudragit RS100 polymers with Dibutylphthalate and dimethylsulfoxide as plasticizer and penetration enhancer respectively. The release rate was sustained with increase in Eudragit RS 100 polymer. The prepared patches showed good uniformity with regard to drug content and drug release. The drug release kinetics of all fabricated patches followed first order kinetics. In conclusion, the present data confirm the feasibility of developing Eprosartan mesylate transdermal patch. Further studies have shown promising results, hence there is a scope for further pharmacokinetic and pharmacodynamic evaluation.

ABBREVIATIONS

TDDS- transdermal Drug Delivery System, EPM- Eprosartan Mesylate, ARB- angiotensin receptor blockers, DSC- differential scanning calorimetry, HPMC- Hydroxy propyl methyl cellulose, DMSO- dimethylsulfoxide.

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