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FORMULATION OF TRANSDERMAL PATCH BY FACTORIAL DESIGN IN MANAGEMENT OF HYPERTENSION: *IN VITRO*, *EX VIVO* AND *IN VIVO* SKIN IRRITATION EVALUATION

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Abstract

An attempt was made to formulate and evaluate the trandolapril transdermal drug delivery system. The matrix type films were prepared by 3^2 factorial design using solvent casting technique with polymers like Eudragit RL 100 and HPMC K15M. Di-butyl phthalate and DMSO were used as plasticizer and penetration enhancer. The prepared films were evaluated for physicochemical characteristics. The drug excepient Compatibility was determined by Fourier transform infrared spectroscopy and Differential scanning calorimetry. *In vitro* permeation studies were performed using Franz diffusion cell. *Ex vivo* studies were performed using skin of albino rats. The results revealed that there is no interaction between drug and selected polymers. Drug content varied from 96.43 ± 0.38 - 99.08 ± 0.8%. Moisture content and moisture uptake were increased for the patches containing higher amount of HPMC due to its hydrophilic nature. It was found that the formulation release followed first order release kinetics with non-fickian anomalous diffusion of drug release mechanism.

Key words: Trandolapril, Eudragit RL 100, HPMC, Transdermal matrix films

Introduction

Tremendous efforts have been focussed on the development of new drug delivery systems [1]. Transdermal drug delivery system was introduced to overcome difficulties faced by the oral drug delivery systems. Transdermal patches are mainly used to deliver a specific dose of drug through the skin into blood stream and this was first approved by FDA in 1981. Transdermal delivery mainly provide sustained release of drug and useful for drugs with short half life [2].These also improves patient compliance and interruption or termination of treatment when necessary [3]. Trandolapril (TLP) the esterified prodrug of the active metabolite trandolaprilate is a nonsulfhydryl angiotensin-converting enzyme (ACE) inhibitor. It is approved for the management of hypertension and for use in stable patients who have evidence of left ventricular systolic dysfunction or symptoms of chronic heart failure within the first two days after acute myocardial infarction. TLP undergoes extensive first-pass metabolism leading to poor bioavailability of 9.5% [4]. It is converted by esterases into the diacid metabolite, trandolaprilate (bioavailability 40-60%), which is approximately, eight times more active as an inhibitor of ACE

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activity. Peak plasma concentrations (C_{max}) of TLP were 1.68 to 1.88 ng/mL and were achieved with in 0.5 to 1.5 h when a single oral 2 mg of TLP was administered to healthy subjects [5, 6]. It possess ideal characteristics such as low molecular weight of 430.54, logarithmic partition coefficient in octanol/water is 1.28 [7], smaller dose (1-4 mg), short elimination half life (0.7 h), and poor oral bioavailability (9.5%) for formulation as transdermal patches. Polymers used should also precede consistent and effective delivery of the drug throughout the products intended shelf life and should be GRAS. Naturally occurring penetration enhancers like essential oils, terpenes, d-limonene, eucalyptus oil, peppermint oil, and turpentine oil are also clinically acceptable enhancers [8, 9]. In the present study DMSO was used as penetration enhancer and di-butyl phthalate as plasticizer. The objective of the present study is an attempt to develop and evaluate the TLP transdermal system for physicochemical characteristics, compatibility studies and for *in vitro* release and *ex vivo* permeation studies.

Materials and methods

Materials

TLP was generous gift sample from Hetero Drugs Pvt. Ltd. (Hyderabad, India). Eudragit RL 100 was a gift sample from Natco, India, Hyderabad. Hydroxypropyl methylcellulose 15 cps, DMSO, disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride were purchased from SD Fine Chemicals, Pvt. Ltd (Mumbai, India).

Experimental method

Preparation of the Transdermal films

Matrix type patches loaded with TLP were prepared by solvent casting method. The transdermal films containing Eudragit RL 100 and HPMC with 2 mg of drug per patch size of 2.25 cm^2 of TLP wt/wt with plasticizer (Dibutyl phthalate) and DMSO 5% as penetration enhancer were prepared. Hydrophilic ingredients were dissolved in distilled water and the hyphophobic ingredients were dissolved in ethanol and were then mixed and stirred on a cyclomixer to get homogenous mixture. The resulting solution was poured into a Teflon petridish of area 18.066 cm^2 containing 16 mg of the drug. An inverted funnel was placed over the petridish to prevent the fast evaporation of the solvent after a period 24h the dried medicated transdermal film were placed in a plastic seal bag and stored in desiccators [11, 12].

Factorial design

The formulation compositions are optimized by factorial design following 3^2 factorial analysis design. This experiment using factorial design allows one to examine simultaneously the effects of multiple independent variables and their degree of interaction. The formulae were developed as 9 sets varying the variables (polymers) following 3^2 full factorial design (3 levels) using Design expert®. (Table 1, 2, 3).

Table No. 01: Formulation and design of 3^2 full factorial experiment design layout

Trial	Variable level in coded formulation	
	(X1)	(X2)
1	-1	-1
2	-1	0
3	-1	+1
4	0	-1
5	0	0
6	0	+1
7	+1	-1
8	+1	0
9	+1	+1

Table No. 02: Values of amounts of variables in 3^2 full factorial design

Coded values	Actual values (X1=Eudragits RL100)	Actual values (X2 =HPMC-15cps)
-1	350	350
0	450	450
+1	550	550

Analytical method

Accurately 50 mg of drug was weighed and placed in a 50 mL volumetric flask and dissolved in phosphate buffer of pH 7.4 and volume adjusted to 50 mL. The Standard solution of drug was subsequently diluted with phosphate buffer to obtain a series of dilutions containing 5, 10, 15, and 20 µg/mL of the solutions. The absorbance of the above dilutions was measured in double beam (Jasco) UV spectrophotometer at 224 nm with a quartz cell of 10 mm path length against phosphate buffer. A graph was plotted by taking concentration of TLP µg/mL on x-axis and absorbance on y-axis. The concentration of drug was calculated using the linear regression equation of the calibration curve.

Table No. 03: Factorial design (3^2) Summary with responses

Design Summary									
Study Type	Factorial		Runs	9					
Initial Design	Full Factorial		Blocks	No Blocks					
Center Points	0								
Design Model	2FI								
Factor	**Name**	**Units**	**Type**	**Low**	**High**				
A	Eudragit RL100	mg	Cat	350	550	Levels:	3		
B	HPMC K15	mg	Cat	350	550	Levels:	3		
Response	**Name**	**Units**	**Obs**	**Analysis**	**Min**	**Max**	**Mean**	**Std. Dv.**	**Ratio**
Y1	Q 24	%	9	Factorial	44.33	73.6	55.076	9.2442	1.660
Y2	Q 8	%	9	Factorial	15.5	38.43	27.735	6.9483	2.479
Y3	RRC 1st Order		9	Factorial	0.0104	0.023	0.0146	0.0039	2.211

Evaluation of physicochemical properties of the patches

Thickness and Weight variation

The thickness of the patches was checked at six different points of the film using a micrometer. For weight variation three films each of 1 cm^2 from each batch were weighted individually and the average weight was calculated.

Folding Endurance

The folding endurance was measured manually as per the method reported [10]. A strip of the film was cut and repeatedly folded at the same place until the film breaks. The more thin the film the more will be the flexibility of the film.

Other mechanical properties like tensile strength (TS), elastic modulus (EM) and elongation at break (E/B) were evaluated by using tensile strength apparatus (Ultra Test, Mecmesin, UK) equipped with 25 kg load cell. Film strip with dimensions 60mmX10mm and free from air bubbles or physical imperfections were held between two clamps positioned at a distance of 3 cm. during measurement, the top clamp at a rate of 2 mm/s pulled the strips to a distance till the film broke. The force and elongation were measured when the film broke. The mechanical properties were calculated according to the below formulae [14]. These properties were evaluated to confirm the physical quality of the patch which denotes patch/film durability and physical stability.

Mechanical properties

$$\text{Tensile strength (kg.mm}^{-2}) = \frac{\text{Force at break (kg)}}{\text{Initial cross sectional area of the sample (mm}^2)}$$

$$\text{Elongation at break (\% mm}^{-2}) = \frac{\text{Increase in length (mm)}}{\text{Original length (mm)}} X \frac{100}{\text{Cross sectional area}}$$

$$\text{Elastic modulus} = \frac{\text{Force at corresponding strain (kg)}}{\text{Cross sectional area (mm}^2\text{)}} \text{ X } \frac{100}{\text{Corresponding strain}}$$

$$\text{Strain} = \frac{\text{Tensile strength}}{\text{Elastic modulus}}$$

Drug content

The drug content was determined by taking a patch (2.25 cm^2) cut it into small pieces and added to a beaker containing 100 mL of PBS (pH 7.4). The film was completely dissolved by cyclomixer. The contents were filtered using Whattman filter paper and the drug content in the filtrate was estimated using a UV spectrophotometer at 244 nm using the reference solution consisting of placebo film.

Percentage of Moisture Content

The dried films were weighed individually and kept in a desiccator containing activated silica. The films were weighed regularly until a constant weight was obtained. The percentage of moisture content was calculated as the difference between initial and final weight with respect to the final weight [17].

Percentage of Moisture Uptake

The dried films were weighed and placed in desiccators containing 200 mL of saturated solution of potassium chloride (84% relative humidity) at room temperature. The percentage moisture uptake of the films was calculated as the difference between final and initial weight with respect to the initial weight [15].

$$\% \text{ Moisture Uptake} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \text{X} 100$$

Skin irritation test

The test was performed on a healthy albino rat weighing around 175 g. Aqueous solution of formalin 0.8% was used as the standard irritant. Placebo polymer patch of 2.25 cm^2 were used as the test patch. Standard irritant was applied on the left dorsal surface of each rat and placebo patch was applied on the right dorsal surface of the albino rat [Ethical Committee Reg. No: 1548/PO/a/11/CPCSEA]. The patches were removed after period of 24 h with the help of an alcohol swab. The skin was examined for erythema or edema [2, 13, 16].

Drug-polymer compatibility studies

Fourier Transforms Infrared Spectroscopy (FTIR) and Differential scanning calorimetry (DSC) were used to know the compatibility between the drug and polymers used in the patches, by KBr pellet method in the wavelength region between 4000 to 400 cm^{-1} the FTIR and DSC was performed for the pure drug, polymers and the physical mixtures of drug and the polymers used and were compared.

***In vitro* drug release studies**

In vitro drug release studies were performed by using a Franz diffusion apparatus. The dialysis membrane was mounted between the donor and receptor compartment of the diffusion cell [18]. The prepared transdermal film of 2.25 cm^2 was placed on the membrane which was previously soaked in phosphate buffer. The receptor compartment was filled with PBS pH 7.4. The total assembly was placed on a hot plate magnetic stirrer and stirred by maintaining the temperature at 32 ± 0.5°C. 5 mL of the samples were withdrawn at regular interval and replaced with an equal volume of PBS. The drug content was estimated spectrophotometrically at 244 nm.

***Ex vivo* skin permeation studies**

The studies were performed by taking the rat skin as the membrane. Young albino rats weighing between 200 g to 250 g were taken and scarified by excess of chloroform inhalation. The abdominal hairs were removed and the abdominal skin was carefully separated from the body with the dermis part remaining intact. Subcutaneous tissue was surgically removed. The inner part of the skin was washed with distilled water thoroughly to separate the adhering fat. The full thickness of skin obtained was placed in normal saline solution and stored at 4 ± 1°C until used for the experiment. The drug permeation from the transdermal patch through the skin was determined using Franz diffusion cell where the contents of donor and receptor was separated by placing the excised skin and the transdermal patch of 2.25 cm^2 containing the drug

was placed in the donor compartment containing the stratum corneum side of the skin. The Franz diffusion cell was placed on a magnetic stirrer and stirred by placing Teflon coated bead at 500 rpm. Samples were withdrawn periodically and replaced with PBS to maintain skin condition. The drug content was estimated spectrophotometrically at a wave length of 244 nm.

Results

Trandolapril transdermal patches were prepared and evaluated for various physical and release parameters. FTIR and DSC studies results revealed the drug excepient compatibility In IR spectra of trandolapril pure drug many number of peaks were found prominently at various wave numbers indicating the presence of functional groups & substitutions like peaks at 1650cm^{-1}, wave number due to C-C stretching in aliphatic chain, prominent peaks at 1736cm^{-1}, 1280cm^{-1}, 1458 cm^{-1} and 1193 cm^{-1} due to C=O stretching, C-O stretching, C-O-H stretching, and C-O-C stretching in aliphatic chain respectively indicating presence of carboxylic group and keto group in the structure. Broad peaks appeared between 3448 cm^{-1}, 2943cm^{-1} wave number are due to C=C stretching in aromatic structure. Peaks appeared at 3270cm^{-1} and 1366cm^{-1} were because of C-H stretching aromatic and definite in CH_2 aliphatic respectively. A more intense peak was found at 3280cm^{-1} because of N-H stretching indicating presence of amino group in the structure & peak at 1433cm^{-1} wave number also indicates the presence of C-N stretching. All these peaks were appeared unchanged in IR spectra of combinations like trandolapril + EU + HPMC. (Figure 4).

Table No. 04: Physical properties of prepared trandolapril transdermal patches

Formulation code	Weight (mg/2.25 cm^2)	Thickness (mm)	Folding Endurance	Drug content (%)	Moisture uptake	Moisture content
TF1	57.14±0.96	1.16±0.06	137±3.60	99.08±0.8	8.57±0.59	4.39±0.46
TF2	54.41±1.02	1.13±0.05	165±5.68	97.54±1.09	16.80±2.55	2.72±0.50
TF3	46.4±1.8	1.01±0.05	222±3.21	97.85±0.91	18.92±0.34	4.55±0.38
TF4	45.40±1.04	0.99±0.05	142±7.09	97.33±0.77	9.98±0.30	2.12±0.30
TF5	61.55±1.37	0.96±0.18	124±8.71	98.10±0.84	18.36±0.95	2.4±0.24
TF6	57.64±1.17	0.96±0.04	175±6.80	97.52±0.55	15.69±0.38	4.25±0.18
TF7	58.11±30.62	1.03±0.05	129±7.23	98.73±0.36	15.22±0.75	3.41±0.22
TF8	52.67±1.11	1.02±0.04	132±3.78	96.43±0.38	17.94±0.53	1.24±0.21
TF9	41.86±0.79	1.03±0.044	135±4.04	97.54±0.55	17.48±0.66	2.62±0.23

Table No. 05: Release kinetic data of transdermal patches formulations (TF_1-TF_9)

Formulation	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Peppas	
				n	(R^2)
TF_1	0.9185	0.9723	0.8409	0.5341	0.9133
TF_2	0.9242	0.9744	0.9807	0.6557	0.9887
TF_3	0.990	0.9815	0.982	0.6329	0.988
TF_4	0.9666	0.9924	0.9579	0.6215	0.9849
TF_5	0.9607	0.992	0.9608	0.5791	0.9842
TF_6	0.9451	0.9917	0.9753	0.5875	0.9815
TF_7	0.8662	0.9286	0.943	0.7814	0.9856
TF_8	0.9677	0.9977	0.9498	0.6363	0.9723
TF_9	0.973	0.9967	0.9515	0.7474	0.9878

The melting point of pure trandolapril was found at 127.91^{o}C & followed exothermic type of reaction where onset was started 123.26^{o}C & ends with 131.21^{o}C (Figure 3). The glass transition lag was found around 7.95oC & the same exothermic type of reactions was found in all combinations like trandolapril + EU + HPMC. No change was found in melting point as well as glass transition lag but special peaks were found indicating melting point of eudragit at 98.22^{o}C, HPMC at 110.21^{o}C, & the influence of excepients were found only in changing on's & end's sets of melting point peaks of drug by absorbing heat but not by interactions. Finally, DSC data states that the crystallinity of

pure drug found unchanged & stable, and indirectly determines the compositions are compatible.

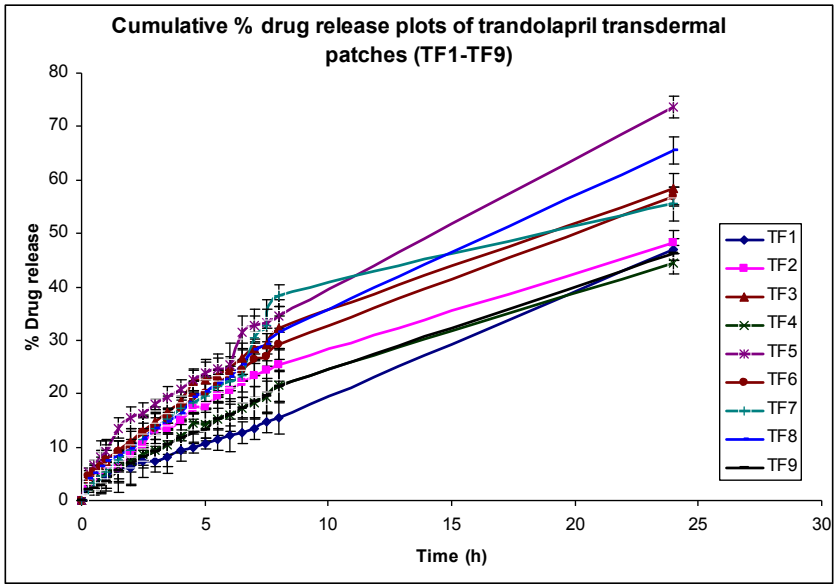

Fig. 01: *In vitro* drug release plots of formulations TF_1 – TF_9

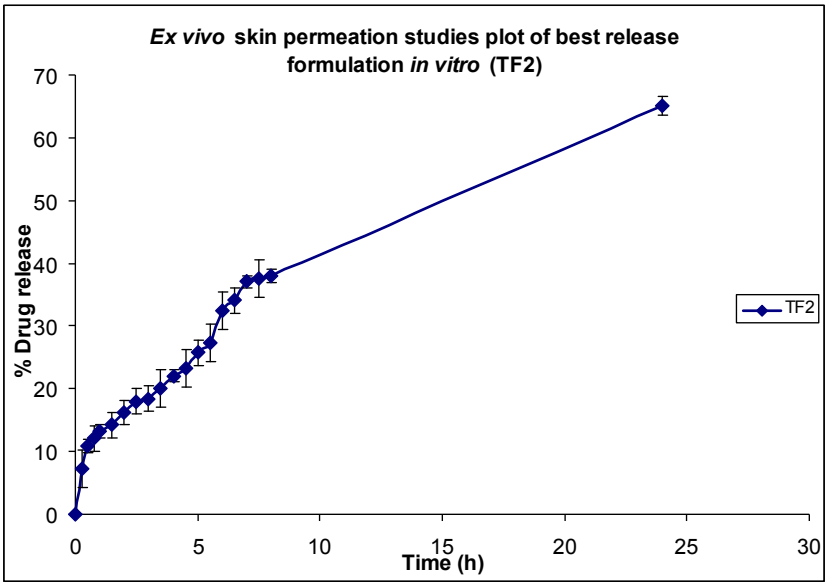

Fig. 02: *Ex vivo* drug skin permeation studies plot of formulation TF_2

Average weight of the patch with an area of 2.25 cm^2 was found least in formulation TF_9 around 41.86 ± 0.79 and highest in TF_5 61.55 ± 1.37 mg. Thickness was found more in formulation TF_1 1.16 ± 0.06 and less in TF_6 0.96 ± 0.04 mm. Folding endurance was found to be more with TF_3 formulation 222 ± 3.21 and less in TF_5 124 ± 8.71, % Drug content was found highest in TF_1 99.08 ± 0.8% and least in TF_8 around 96.43 ± 0.38. Moisture uptake and moisture content was found more in TF_1 and TF_3 around 18.92 ± 0.34% and 4.55 ± 0.38% respectively and less in TF_3 and TF_8 around 8.57 ± 0.59% and 1.24 ± 0.21% respectively. Highest values of tensile strength and elastic modulus was found to be in formulation TF_1 around 1.93 ± 0.156 kg/m^2 and 3.66 ± 0.335 kg/mm^2 respectively with strain value of 0.527 and least values of above were found in formulations TF_7 and TF_5 around 0.96 ± 0.51 kg/m^2 and 0.56 ± 0.115 kg/mm^2 respectively. Elongation at break was found more in TF_2 around 161.3 ± 2.46 and less in formulation TF_7 around 74.5 ± 4.016 (Table 4).

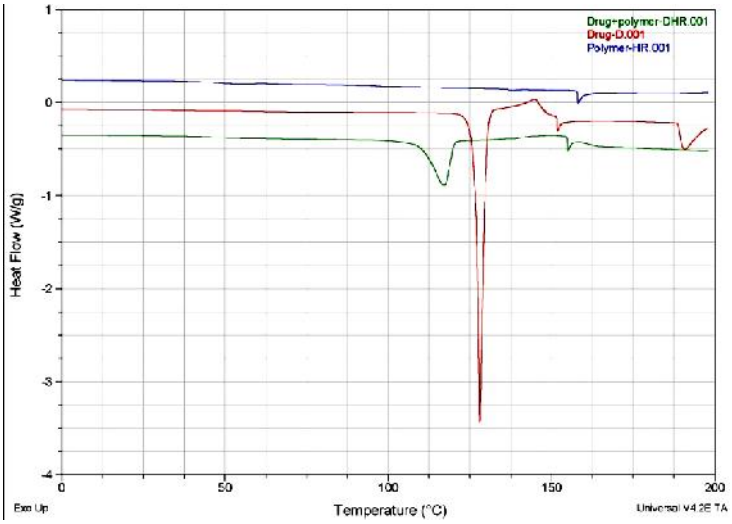

Fig. 03: DSC overlay thermograms of pure drug trandolapril, polymer blend and drug with polymer.

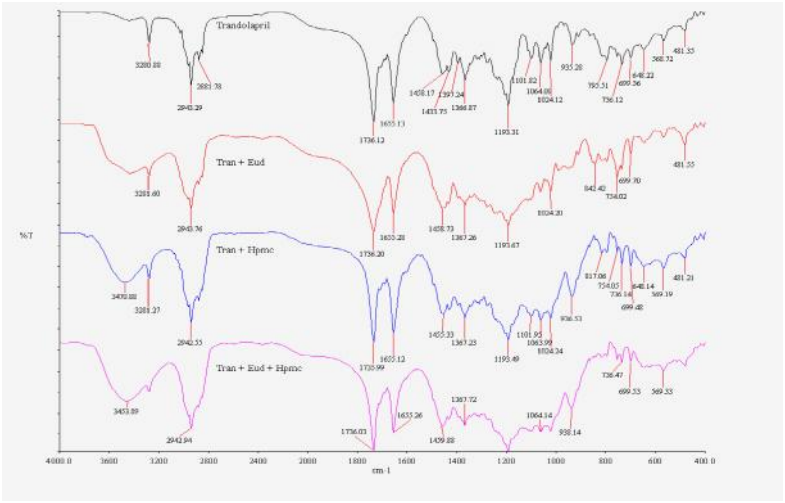

Fig. 04: FTIR overlay spectra of trandolapril pure drug, polymers and pure drug with polymers

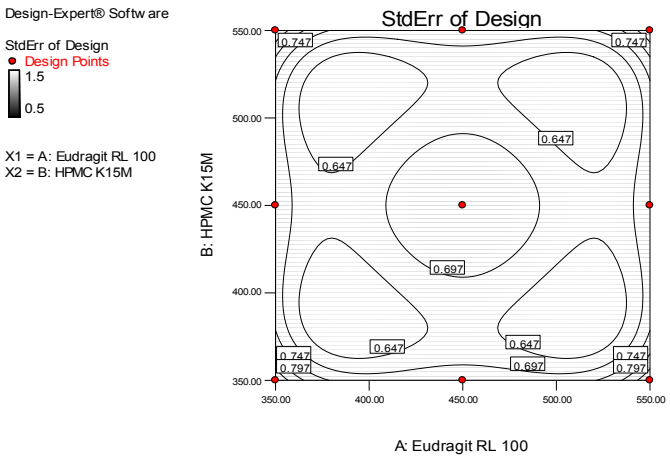

Fig. 05: Standard error of design counter plot of 3^2 full factorial design

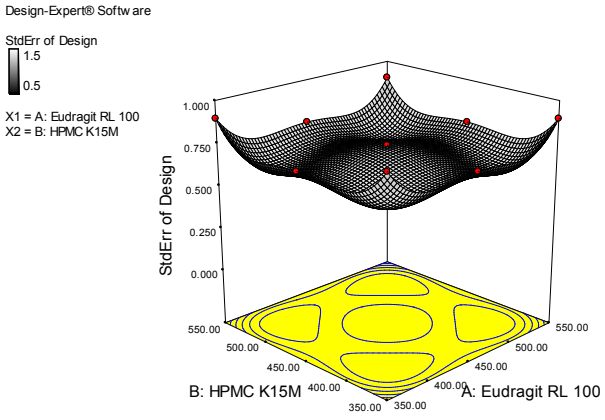

Fig. 06: Standard error of design 3D plot of 3^2 full factorial design

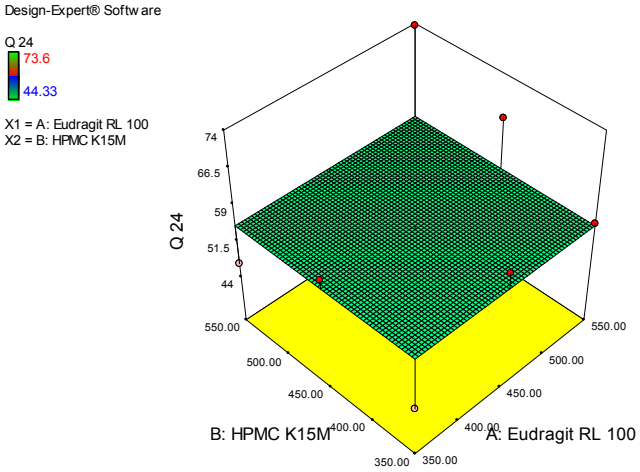

Fig. 07: 3D plot of Q24 % drug release influenced by HPMC and Eudragit combinations

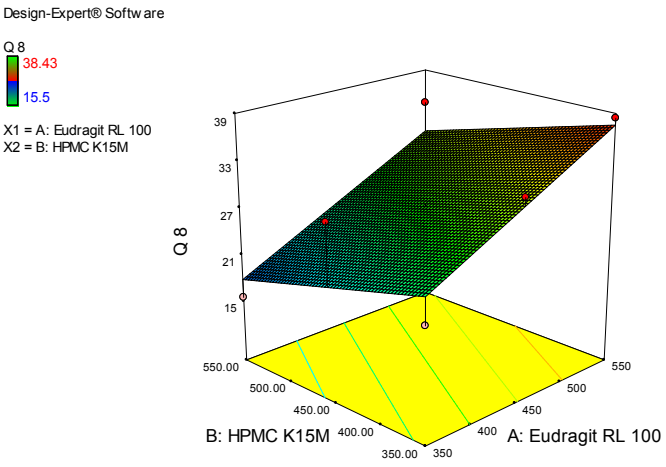

Fig. 08: 3D plot of Q8 % drug release influenced by HPMC and Eudragit combinations

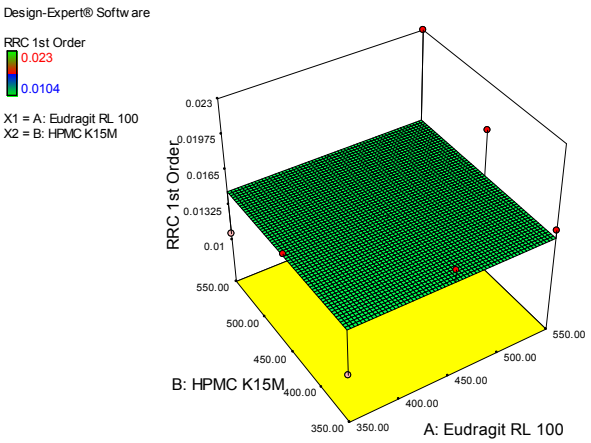

Fig. 09: 3D plot of release rate constant (first order) influenced by HPMC and Eudragit combinations

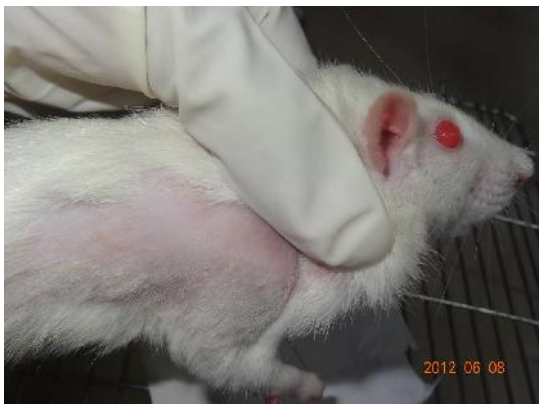

Fig. 10: Picture depicting the skin irritation test in rat by the formulation TF_4

Fig. 11: Picture depicting the skin irritation test in rat by the standard (phenolic compound)

In *In vitro* drug release studies the more release retardation was found around 44.33 ± 2% in formulation TF_4 up to 24 h where as less retardation was observed around 73.60 ± 2.1% in formulation TF_5 after 24 h. All formulations followed first order non fickian release kinetics with anomalous transport mechanism (Figure 1) (Table 5, 6). *Ex vivo* characterization studies carried by selecting formulation as per *in vitro* drug release results, with an intention to develop 48 h release plots formulation TF_2 was selected to perform *Ex vivo* permeation studies (Figure 2). Release was found more as compared to *in vitro* results where 65.15±1.5% was release with in 24 h. Skin irritation test in rats proved that the formulations are found to be less irritant as

compared to standard with less erythema (Figure 10, 11). The *in vitro* data is used to calculate various other dependable responses like Q_{24}, Q_8 and first order release rate constant. The data was plotted as surface response graph and analysed statistically where the *p* – value was found significant (less that 0.050) (Figure 4, 5, 6, 7, 8).

Table No. 06: Release of drug at 24 h, 8 h and release rate constant (first order)

Formulation	Q24 (%)	Q8 (%)	Release rate constant (*Ko*) (first order)
TF_1	46.89	15.50	0.0106
TF_2	48.37	25.33	0.0119
TF_3	58.33	32.27	0.0158
TF_4	44.33	21.50	0.0104
TF_5	73.60	34.53	0.0230
TF_6	56.87	29.33	0.0149
TF_7	55.50	38.43	0.0154
TF_8	65.47	31.50	0.0189
TF_9	46.33	21.23	0.0112

Discussion

All physical parameters evaluation results were found in acceptable range. Folding endurance was found more with formulations with high percentage of eudragit than HPMC. Tensile strength was found more in formulations with high percentage of eudragit where as the elongation and elastic modulus were found more in formulations containing high percentage of HPMC than Eudragit. The drug release from the formulations was sustained over an extended period of time i.e. up to 48 h. The study states the release depended on the polymers ratio. Formulations prepared using HPMC showed better sustained action and formulation TF_4 is confirmed as best formulation as it released maximum drug upto 48 h.

Conclusion

The development of the 48 h releasing transdermal films by solvent casting could be adaptable in laboratory as well as in industry since it is simple and reproducible. In conclusion, HPMC and EU formulations could be used for better sustained action. However further *in vivo* studies are needed to optimize for sustained action in human beings for better bioavailability, efficacy thus safety.

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