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Research Article

EFFECT OF VARIOUS PERMEATION ENHANCERS ON PROPRANOLOL HYDROCHLORIDE FORMULATED PATCHES

*GURU SHARAN[1], BIPLAB KUMAR DEY[2], K. NAGARAJAN[1], SUJIT DAS[2], S. VIJAYA KUMAR[2], V. DINESH[1],

[1]Dept of Pharmaceutics, IIMT College of Medical Sciences, Meerut, UP, [2]Dept. of Pharmaceutics, Himalayan Pharmacy Institute, Sikkim

Email: guru11d@gmail.com

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ABSTRACT

Propranolol hydrochloride is a non-selective beta blocker, mainly used in the management of various cardiovascular disorders. It reduces the oxygen requirement of the heart at any level of effort by blocking catecholamine induced increase in the heart rate, systolic blood pressure, the velocity and extent of myocardial contraction. Our present work comprises the formulation and evaluation of propranolol hydrochloride transdermal patches for sustained or extended period of time. Drug loaded patches were prepared using various biocompatible polymers like (EC + PVP) & (AC + HPMC). Drug loaded patches were formulated by using solvent casting and evaporation technique. By using, these technique patches obtained were almost flat, having very less weight variation, and optimum flexibility suggesting the reproducibility of the formulation technique. Patches were smooth and of desirable consistency. Patches were evaluated under various parameters such as in vitro skin permeation studies which were carried out by using excised skin of hairless albino rats. The formulation containing oleic acid as permeation enhancer showed the better permeation in comparison to the other enhancers. Further study was conducted on healthy adult male rabbits for 24 hours. Our article reports that, no any trace of edema, erythema or any skin irritation on site of application of the patch was observed. Hence, we conclude that, formulations are non irritable to the skin tissue and it can be safer for therapeutic use.

Keywords: Acrycoat L-100 (AC), Ethyl cellulose (EC), Polyvinyl Pyrrolidone (PVP),

INTRODUCTION

Transdermal delivery has many advantages over conventional modes of drug administration, as because it avoids hepatic first-pass metabolism, potentially decreases side effects and improves patient compliance. Propranolol hydrochloride is a non selective Beta-blocker used in almost most of cardiovascular ailments. It blocks the action of epinephrine on both β1- and β2-adrenergic receptors. It has little intrinsic sympathomimetic activity (ISA) but has strong membrane stabilizing activity (only at high blood concentrations, eg overdosage). When access to beta-adrenergic receptor sites is blocked by propranolol, the chronotropic, inotropic, and vasodilator responses to beta-adrenergic stimulation are decreased proportionately. Propranolol may reduce the oxygen requirement of the heart by blocking catecholamine induced increases in the heart rate, systolic blood pressure, and the velocity and extent of myocardial contraction.[1]

Propranolol has a variable bioavailability due to extensive first-pass metabolism. The short half-life being 3–4 hours, make its frequent dosing essential for maintaining effective plasma drug concentration (10–100 ng/ml) for required pharmacological or therapeutic response. Also the suitability of propranolol hydrochloride with respect to dose, solubility, molecular weight to get incorporated into a matrix type transdermal delivery system prompted selection of drug propranolol hydrochloride for the study.[2]

MATERIALS AND METHODS

Propranolol HCl was obtained as a gift sample from Sun Pharmaceuticals Ltd, Baroda. Polyvinyl pyrrolidone K-30 (PVP K-30), Thomas Baker, Mumbai; Hydroxypropyl methyl cellulose K4M (HPMC K4M), Loba Chemicals, Mumbai; Ethyl cellulose LR (EC), SD Fine Chemicals Limited, Mumbai; Acrycoat S-100, Corel Pharma-chem,, Ahmadabad; Polyethylene glycol 400 (PEG 400), Loba Chemicals, Mumbai; Dimethyl sulphoxide (DMSO), Burgoyne Mumbai ; Oleic acid; SD Fine Chemicals Limited; Mumbai.

Preparation of backing membrane

A 4% (w/v) solution of polyvinyl alcohol (PVA) in distilled water was prepared using mechanical stirrer. Then 2 ml of the solution was poured in both side open glass moulds, having specific diameter (2.8 cm), one side of which is previously covered by aluminum foil. It was placed in dryer at 60º C ± 2º C for drying over a period of 6 hours. After 6 hours moulds were removed from dryer and air dried for 24 hours[3]

Formulation of drug loaded transdermal patches

Matrix type transdermal patches of propranolol hydrochloride were prepared by using two different sets of polymer composition, one set containing EC and PVP K30 and other set containing acrycoat-S100 and HPMC-K4M indifferent ratios as shown in the Table 1 & 2, by solvent evaporation technique in cylindrical both side opened glass moulds. The bottom of the mould was wrapped with aluminium foil on which the backing membrane was cast by pouring 4 % (w/v) PVA solution followed by drying at 60° C for 6 hours.[4] The two polymers were weighed in requisite ratio and they were then dissolved in ethanol as a solvent. Dibutyl phthalate 30 % (w/w) of polymer composition was used as a plasticizer. The drug was added 20 % (w/w) of the total weight of polymer, in the homogeneous dispersion, by slow stirring with a magnetic stirrer. The uniform dispersion (2 ml each) was casted on the PVA backing membrane casted earlier and dried at 40° C for 6 hours. After drying patches were removed from the mold, wrapped with aluminium foil and kept in desiccators until they were used for further study. All the patches obtained from this composition were smooth, elastic and were easily removed from glass moulds.[5]

Formulation of drug loaded medicated transdermal patches with permeation enhancer[6, 7]

The two polymers were weighed in requisite ratio and they were then dissolved in ethanol as a solvent. Dibutyl phthalate 30 % (w/w) of polymer composition was used as a plasticizer. The drug was added 20 % (w/w) of the total weight of polymer, in the homogeneous dispersion, by slow stirring with a magnetic stirrer. Then different permeation enhancers (PEG 400, DMSO and oleic acid) 15 % (w/w) of the total polymer was added individually. The uniform dispersion (2 ml each) was casted on the PVA backing membrane casted earlier and dried at 40°C for 6 hours. After drying patches were removed from the mould, wrapped with aluminium foil and kept in desiccators until they were used for *in vitro* skin permeation study.

Table 1: Formulation of drug loaded transdermal patches using EC and PVP (K30)

S. No.	Formulation code	Ratio of EC: PVP	Total weight of EC and PVP	Solvent (Ethanol)	Plasticizer (% w/w) of total polymer	Drug (% w/w) of total polymer
1.	ECP-1	5:1	500 mg	10 ml	30	20
2.	ECP-2	4:1	500 mg	10 ml	30	20
3.	ECP-3	3:1	500 mg	10 ml	30	20
4.	ECP-4	2:1	500 mg	10 ml	30	20
5.	ECP-5	1:5	500 mg	10 ml	30	20
6.	ECP-6	1:4	500 mg	10 ml	30	20
7.	ECP-7	1:3	500 mg	10 ml	30	20
8.	ECP-8	1:2	500 mg	10 ml	30	20

Table 2: Formulation of drug loaded transdermal patches using Acrycoat-S100 and HPMC-K4M

S.No.	Formulation code	Ratio of Acrycoat : HPMC	Total weight of Acrycoat and HPMC	Solvent (Ethanol)	Plasticizer (% w/w) of total polymer	Drug (% w/w) of total polymer
1.	ACH-1	5:1	500 mg	10 ml	30	20
2.	ACH-2	4:1	500 mg	10 ml	30	20
3.	ACH-3	3:1	500 mg	10 ml	30	20
4.	ACH-4	2:1	500 mg	10 ml	30	20
5.	ACH-5	1:4	500 mg	10 ml	30	20
6.	ACH-6	1:5	500 mg	10 ml	30	20
7.	ACH-7	1:3	500 mg	10 ml	30	20
8.	ACH-8	1:2	500 mg	10 ml	30	20

EVALUATION OF DRUG LOADED TRANSDERMAL PATCHES

A) Uniformity of thickness

For measuring thickness uniformity of the transdermal patches, micrometer (Mitutoyo) with least count of 0-0.01 mm was used. The thickness of the patch at five different points was measured and the average of five readings with the standard deviation was calculated. The procedure was followed for all the formulation batches.

C) Folding endurance

The folding endurance was measured manually for the prepared patches. The patches were repeatedly folded at the same place till it broke. The number of times the patches could be folded at the same place without breaking gave the exact value of folding endurance.

D) Percent moisture content (% MC)

The patches were weighed individually and kept in desiccators containing 10 gm of calcium chloride as desiccant at 37°C for 24 hour. The patches were weighed again and again individually until it showed a constant weight. The final weight was noted when there was no further change in the weight of individual patch. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight.

$$\% \text{ MC} = \frac{(X-Y)}{Y} \times 100$$

Where, X = initial weight,Y = final weight

E) Percentage moisture uptake (% MU)

The patches were weighed accurately and placed in desiccators where a humidity condition of 75 % RH was maintained by using saturated solution of sodium chloride. The patches were taken out periodically and weighed for a period of 72hours. The percentage of moisture uptake was calculated as difference between final and initial weight of the patch with respect to initial weight.

$$\% \text{ MU} = \frac{(X-Y)}{Y} \times 100$$

Where X = initial weight, Y = final weight

F) Water vapour transmission (WVT) rate

For this study glass vials of equal diameter (1.4 mm) were used as transmission cells. These cells were washed thoroughly and dried in a woven. The transdermal patch of known thickness was fixed over the edge of the glass vial containing 3 gm of fused calcium chloride as a desiccant by using an adhesive. Then the transmission cells were weighed accurately and initial weight was recorded. The cells were then kept in a desiccator containing saturated solution of potassium chloride (200 ml). The humidity inside the desiccator was to be 80-90 % RH. The cells were taken out periodically and weighed for a period of 72 hours. The experiment was performed and values were calculated by the method of Ragavendra et al., using the formula

$$\text{WVT rate} = \text{WL}/ \text{S}$$

where; W = water vapour transmitted in gm., L = thickness of the transdermal patch in cm., S = exposed surface area in cm2

G) Percent flatness study:

Longitudinal strips were cut out from each transdermal patch, one from the centre and two from the either side. The length of each strip was measured and the variation in the length because of non-uniform in flatness was measured by determining % constriction, considering 0 % constriction is equivalent to 100 % flatness.

$$\% \text{ constriction} = \frac{l_1 - l_2}{l_2} \times 100$$

Where, l_1 = initial length of each strip.

l_2 = final length of each strip.

H) Mechanical properties

The mechanical properties of the polymeric transdermal patch are related to the deformation of the material by applied force.

Tensile strength[11]

The tensile strength measurement was made using an instrument assembled in the laboratory and following the method used by Sadhna *et al*. The films were fixed individually to the assembly. The required weights to break the films were noted. Tensile strength was calculated by using the following formula.

$$\text{Tensile strength} = (\text{break force}/a \times b) \times (1+L/I)$$

Where, a, b, L and I are the width, thickness, length and elongation of the films.

I) Weight variation study[12]

This test provides a means for measuring uniformity in terms of the weight within a batch as well as batch to batch. The weight of each patch was taken using single pan balance with sensitivity of 0.001 mg.

J) Drug content study[13]

This test provides the means for measuring the amount of drug that is actually present in each transdermal patch formulations. Transdermal patches were taken individually, crushed and taken in a 100 ml volumetric flask. The volume was made up to 100 ml with distilled water and kept for 48 hours at room temperature with occasional shaking. After 48 hours, samples are withdrawn, suitably diluted and analyzed using spectrophotometer at 290 nm for the actual amount of drug present in the patches.

K) *In vitro* permeation studies using dialysis membrane[14]

In vitro permeation studies were carried out using modified Keshary – Chien diffusion cell. The dialysis sac was previously soaked for 24 hours in phosphate buffer *(pH 7.4)*. The patches were adhered to the barrier membrane (dialysis membrane) and the sac is tied firmly to the donor compartment of the Keshary – Chien diffusion cell, the receptor compartment of which is filled with 100 ml phosphate buffer *(pH 7.4)*. The donor compartment is lowered to the receptor compartment in such a way that the dialysis sac just touches the media of the receptor compartment. The total setup was placed on a thermostatically controlled magnetic stirrer set at 37 ± 2°C. The content of the diffusion cell was stirred using a teflon coated bead at a constant speed (100 rpm). Samples were withdrawn (1 ml) at predetermined time intervals and replaced with same amount of phosphate buffer *(pH 7.4)* to maintain the sink condition. After suitable dilution, the samples were analyzed for drug content using UV spectrophotometer at λ_{max} 290 nm. The permeation study was carried out for 24 hours.

L) *In vitro* skin permeation study using albino mice skin: [15]

In vitro skin permeation study was performed taking albino mice skin. Young albino mice weighed between (200 gm - 250 gm) were taken and sacrificed by excess chloroform inhalation. The abdominal hairs were removed with marketed hair removers. The abdominal skin was carefully separated from the body, with the dermis part remaining intact. Subcutaneous tissues were surgically removed. The inner part of the skin was washed with distilled water thoroughly to separate the adhering fat. The skin, so obtained, was examined microscopically for the presence of any possible damage. The full thickness skin thus obtained was kept in normal saline solution and stored at 4 ± 1°C until used for the experiment.

The drug permeation from the transdermal patches through the skin was determined using modified Keshary – Chien diffusion cell. The contents of the donor and receptor compartments were separated by placing the excised skin in between two compartments. The skin was mounted in such a way that the stratum corneum side of the skin continuously remained in an intimate contact with the transdermal patch in the donor compartment. The receptor compartment contained 100ml phosphate buffer *(pH 7.4)* at 37 ± 2°C. The content of the diffusion cell was stirred using a teflon coated bead at a constant speed (100 rpm). Samples were withdrawn (1 ml) at predetermined time intervals and replaced with same amount of phosphate buffer *(pH 7.4)* to maintain the sink condition. After suitable dilution the samples were analyzed for drug content using UV spectrophotometer at λ_{max} 290 nm. The permeation study was carried out for 24 hours.

Data analysis[16]

Cumulative amounts of drug that permeated through excised mice skin were plotted as a function of time. Steady state flux was expressed as the slope of the linear portion of the resulting permeation profile (J_{ss}, mg cm^{-2} h^{-1}) by regression analysis. The permeability coefficient (K_p) was calculated using the following equation:

$$K_p = \frac{J_{ss}}{C_v}$$

Where J_{ss} is the steady state flux and C_v is the initial concentration of drug in the donor compartment. The penetration enhancing effect of the permeation enhancer was calculated in terms of enhancement ratio flux (ER_{flux}) using the following equation:

$$ER_{flux} = \frac{\text{Drug flux with permeation enhancer}}{\text{Drug flux without permeation enhancer}}$$

M) Scanning electron microscopy (SEM)

The surface morphologies of the transdermal patch were investigated by using scanning electron microscope, model Jeol JSM-5200, Japan, at 15 kV. Prior to examination, samples were gold coated to make them electrically conductive.

L) Skin irritation test[17]

Skin irritation test was performed on healthy adult male New Zealand rabbits (Oryctolagus caniculus) weighing between 2 - 3.5 kg. Adhesive tape USP was used as control patch. The transdermal films of 3.1644 cm^2 were used as test samples. The test was conducted on unbraided skin of rabbits. The control patch was placed on left dorsal surface of the rabbits. The film formulations were removed after 24 hours with the help of an alcohol swab and the skin was examined for erythema/edema.

M) Drug-polymer interaction study[18, 19]

Drug polymer interaction was studied by FTIR spectroscopy (Fourier Transforms Infrared Spectroscopy). The spectra were recorded for propranolol hydrochloride with polymer mixture. Drug- polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for propranolol hydrochloride, physical mixture of polymers and physical mixture of drug with polymers using FTIR – spectrophotometer (FTIR 8400S; SHIMADZU, Japan) from KBr pellets. The scanning range was 400-4000cm^{-1}

Table 3: Thickness uniformity, folding endurance, percentage moisture content (% MC), percentage moisture uptake (% MU) of drug loaded transdermal patches.

Sl No.	Formulation code	Thickness ±SD** (cm)	Folding endurance ±SD**	% MC (w/w)	% MU (w/w)
1	ECP-1	0.002024±0.00028	182±5.6	1.212	1.378
2	ECP-2	0.001958±0.00010	195±7.4	2.047	1.826
3	ECP-3	0.001768±0.00023	172±9.2	2.977	2.758
4	ECP-4	0.001736±0.00012	191±8.6	3.215	3.019
5	ECP-5	0.001684±0.00019	200±7.8	5.349	4.676
6	ECP-6	0.001802±0.00021	176±9.6	4.345	4.058
7	ECP-7	0.001860±0.00028	187±8.2	4.348	3.832
8	ECP-8	0.001924±0.00011	198±6.3	4.177	3.864
9	ACH-1	0.002036±0.00016	188±8.7	1.828	1.924
10	ACH-2	0.001982±0.00014	190±9.8	2.096	2.173
11	ACH-3	0.001936±0.00012	197±6.3	2.312	2.544
12	ACH-4	0.001826±0.00022	178±8.7	2.846	2.968
13	ACH-5	0.001667±0.00018	198±9.7	5.344	4.497
14	ACH-6	0.001692±0.00016	204±7.5	5.137	4.153
15	ACH-7	0.001748±0.00012	174±8.3	4.864	3.988
16	ACH-8	0.001748±0.00013	200±7.2	4.327	3.676

**SD = Standard Deviation

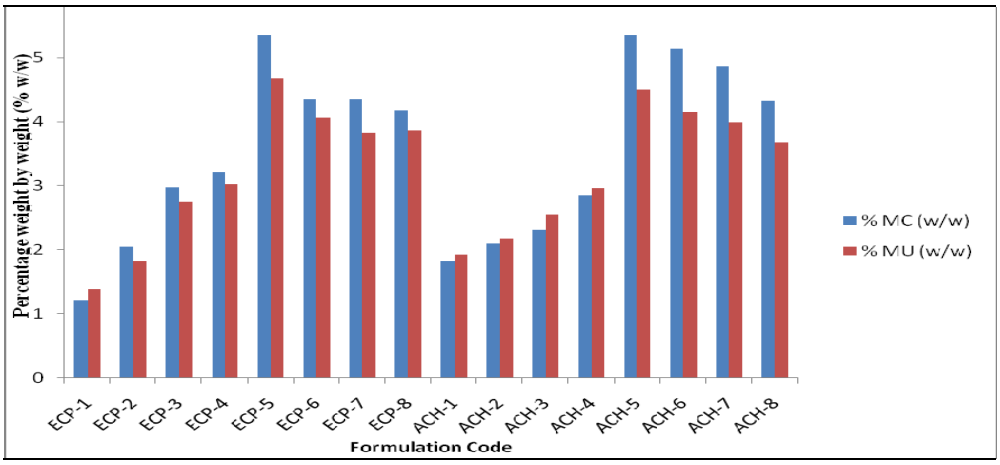


Fig. 1: Comparative percentage moisture content & moisture uptake profile of all formulations.

Table 4: Water vapour transmission rate (WVTR), Percent flatness, Tensile strength, Weight variation and drug content of drug loaded transdermal patches of EC and PVP-K 30.

Sl No.	Formulation Code	WVTR (gm/cm/h)	% Flatness*	Tensile strength (gm/cm²)	Weight in gm ± S.D.**	% Drug content
1	ECP-1	1.01022×10^{-4}	100	267.39	0.144 ± 0.0043	99.90
2	ECP-2	1.24632×10^{-4}	100	254.28	0.137 ± 0.0035	99.48
3	ECP-3	1.27458×10^{-4}	100	238.57	0.131 ± 0.0055	98.84
4	ECP-4	1.30712×10^{-4}	100	229.75	0.126 ± 0.0038	99.78
5	ECP-5	2.39641×10^{-4}	100	197.43	0.132 ± 0.0028	99.68
6	ECP-6	1.57724×10^{-4}	100	208.64	0.124 ± 0.0033	98.63
7	ECP-7	1.32472×10^{-4}	100	214.33	0.133 ± 0.0040	99.54
8	ECP-8	1.03894×10^{-4}	100	226.91	0.141 ± 0.0012	98.37
9	ACH-1	0.98354×10^{-4}	100	274.67	0.140 ± 0.0026	99.88
10	ACH-2	1.05976×10^{-4}	100	259.25	0.138 ± 0.0049	98.86
11	ACH-3	1.10684×10^{-4}	100	248.48	0.133 ± 0.0040	99.32
12	ACH-4	1.20688×10^{-4}	100	236.02	0.131 ± 0.0042	98.77
13	ACH-5	1.95409×10^{-4}	100	203.41	0.128 ± 0.0040	99.64
14	ACH-6	1.83427×10^{-4}	100	211.38	0.130 ± 0.0050	98.68
15	ACH-7	1.45534×10^{-4}	100	219.29	0.124 ± 0.0033	99.36
16	ACH-8	1.78533×10^{-4}	100	230.73	0.135 ± 0.0043	99.57

* Average of three determinations, **S.D = Standard Deviation

Table 5: Percentage cumulative drug release profile of transdermal patches composed of ethyl cellulose (EC) and polyvinyl pyrrolidone (PVP-K30)

Time	% Cumulative drug release/cm² from different transdermal patches composed of EC and PVP K30							
(hour)	ECP-1	ECP-2	ECP-3	ECP-4	ECP-5	ECP-6	ECP-7	ECP-8
1	2.18	8.55	7.79	10.49	21.59	27.42	25.78	21.68
2	8.12	17.66	9.67	13.63	30.67	37.23	37.09	25.47
3	9.03	22.59	12.27	16.00	47.78	43.89	43.51	35.16
4	10.45	25.08	17.18	20.84	55.18	49.31	48.59	41.53
5	13.58	27.15	19.71	25.28	62.22	56.62	54.71	49.87
6	17.08	28.93	23.20	27.48	65.13	63.22	58.40	51.48
7	21.44	30.97	26.76	29.81	69.44	67.80	62.21	55.20
8	22.80	32.74	30.32	32.99	71.63	70.02	66.60	61.89
9	26.46	33.95	31.92	36.23	74.53	72.66	69.91	63.69
10	28.89	35.68	35.08	40.01	78.30	77.29	72.59	66.30
11	30.97	36.61	38.90	43.28	79.07	80.65	75.25	70.11
12	34.40	38.60	42.48	50.11	84.65	83.36	79.01	72.58
13	36.80	41.21	47.72	55.23	86.93	85.20	80.62	81.71
14	38.89	43.57	52.50	61.61	89.82	87.26	88.20	88.82
15	40.67	45.64	57.81	63.65	92.21	89.90	91.16	92.75
16	41.33	47.98	62.87	66.80	96.23	92.30	94.37	94.96
17	43.04	51.15	66.23	68.65	98.13	95.52	96.22	96.21
18	45.06	54.66	67.82	70.96	100.17	97.11	97.71	97.12
19	52.64	57.91	69.86	73.59	--	98.59	98.91	97.73
20	53.64	61.14	71.66	75.41	--	99.80	99.55	98.61
21	55.07	62.98	73.16	76.89	--	--	99.84	100.67
22	56.26	65.57	74.92	78.64	--	--	--	--
23	57.16	67.94	76.42	80.40	--	--	--	--
24	58.04	70.06	77.91	81.89	--	--	--	--

Table 6: Percentage cumulative drug release profile of transdermal patches composed of acrycoat-S100 and hydroxypropyl methyl cellulose (HPMC-K4M)

Time (hour)	% Cumulative drug release/cm² from different transdermal patches composed of acrycoat-S100 and HPMC-K4M							
	ACH-1	ACH-2	ACH-3	ACH-4	ACH-5	ACH-6	ACH-7	ACH-8
1	10.75	15.06	15.82	19.27	33.79	27.13	24.16	22.72
2	14.73	18.50	20.14	25.49	42.80	36.92	30.10	34.94
3	18.26	20.89	23.68	30.28	46.56	43.58	40.11	41.16
4	20.67	26.06	29.45	33.63	50.64	49.84	48.49	47.88
5	22.75	28.61	32.02	37.46	53.93	53.84	52.59	51.33
6	26.09	30.98	34.66	41.60	56.34	55.46	60.87	53.46
7	28.31	33.07	35.92	45.75	58.97	58.91	63.60	60.24
8	32.59	35.71	37.11	49.35	65.50	62.20	65.96	62.87
9	34.78	37.82	38.84	51.80	68.38	66.03	68.04	64.95
10	38.77	40.74	41.73	53.61	72.14	71.29	71.50	67.01
11	42.88	44.82	46.63	57.91	77.91	77.48	75.04	68.52
12	45.33	49.25	49.14	63.49	81.85	80.36	78.58	71.93
13	48.79	51.47	55.40	67.17	85.96	85.28	81.85	73.25
14	51.21	55.78	59.67	70.45	92.02	87.81	83.70	74.70
15	53.55	58.83	64.09	75.40	95.43	91.03	86.03	77.01
16	55.35	63.48	69.91	79.33	99.50	94.30	89.51	79.64
17	57.39	67.67	75.53	80.39	--	95.89	92.49	83.13
18	59.72	68.78	76.97	83.80	--	97.93	95.69	87.49
19	61.24	71.33	79.27	86.24	--	100.02	97.86	92.19
20	62.15	72.86	80.79	88.34	--	--	99.09	94.41
21	64.14	73.79	82.27	90.14	--	--	--	96.47
22	66.75	74.96	83.47	92.20	--	--	--	99.08
23	68.28	76.70	85.76	94.00	--	--	--	--
24	69.48	77.93	87.29	95.50	--	--	--	--

Table 7: *In vitro* drug release kinetics of drug loaded transdermal patches composed of ethyl cellulose (EC) and polyvinyl pyrrolidone (PVP-K30)

Sl No.	Formulation code	(Zero order model) Correlation coefficient (r^2)	(Higuchi model) Correlation coefficient (r^2)
1	ECP-1	0.989	0.981
2	ECP-2	0.981	0.957
3	ECP-3	0.985	0.966
4	ECP-4	0.981	0.972
5	ECP-5	0.911	0.975
6	ECP-6	0.941	0.991
7	ECP-7	0.959	0.992
8	ECP-8	0.963	0.984
9	ACH-1	0.984	0.987
10	ACH-2	0.983	0.972
11	ACH-3	0.982	0.954
12	ACH-4	0.977	0.984
13	ACH-5	0.991	0.965
14	ACH-6	0.979	0.990
15	ACH-7	0.953	0.991
16	ACH-8	0.962	0.987

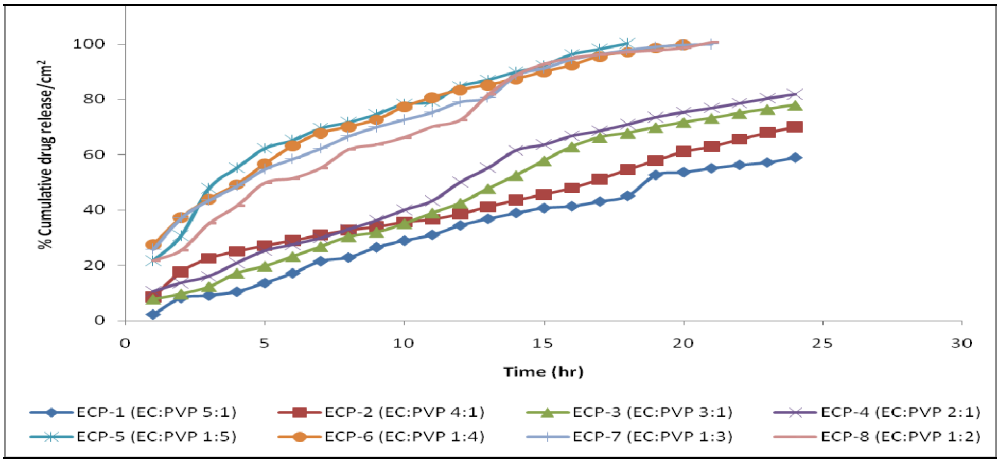


Fig. 2: *In-vitro* Percentage cumulative drug release profile of formulations ECP-1 to ECP-8 using dialysis membrane.

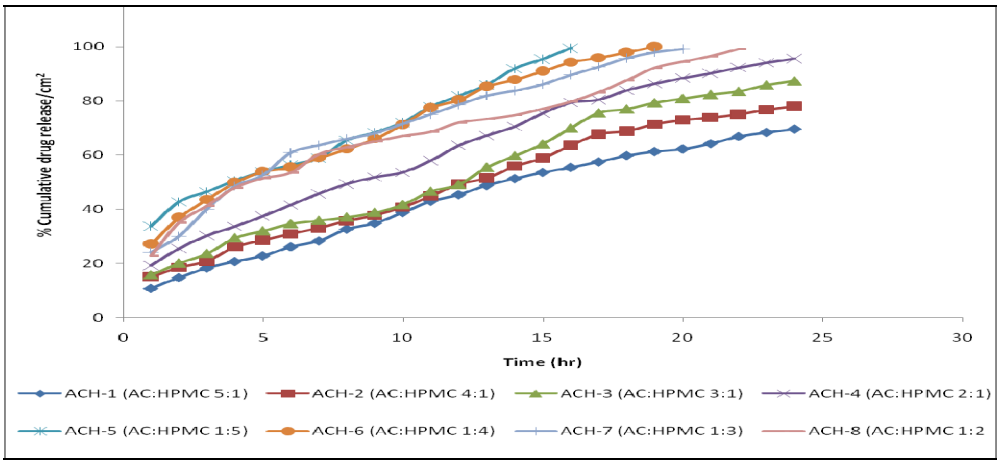


Fig. 3: *In-vitro* Percentage cumulative drug release profile of formulations ACH-1 to ACH-8 using dialysis membrane.

Table 8: *In vitro* skin permeation profile of drug loaded transdermal patches ECP-1 (EC: PVP 5:1) and ACH-1 (acrycoat-S100: HPMC-K4M 5:1) with and without different permeation enhancers

Time (hour)	% Cumulative drug release/cm²							
	ECP-1				ACH-1			
	Without P.E*.	PEG-400	DMSO	Oleic acid	Without P.E*.	PEG-400	DMSO	Oleic acid
1	2.46	6.60	10.75	7.99	4.12	9.29	8.26	11.03
2	5.93	10.61	14.73	16.50	5.48	13.63	12.96	15.03
3	7.36	14.98	19.36	26.40	8.03	14.87	17.60	18.27
4	9.24	18.00	26.00	32.23	10.40	19.92	23.96	25.65
5	10.47	23.98	28.32	36.44	13.58	23.54	27.93	28.03
6	13.02	28.20	33.97	41.92	15.42	24.58	30.09	32.02
7	14.28	32.03	37.63	44.18	17.74	32.37	35.18	40.28
8	18.22	35.06	43.09	46.02	18.70	34.51	38.53	45.47
9	20.95	38.40	45.35	47.45	20.69	35.16	40.65	47.98
10	22.76	43.14	50.17	55.27	22.75	38.79	43.26	55.58
11	27.29	47.29	57.38	62.94	24.25	39.84	47.29	62.40
12	28.94	50.29	60.29	66.14	26.55	42.38	53.53	66.39
13	34.01	54.06	65.42	69.91	30.28	45.83	57.84	71.31
14	36.25	58.15	69.05	74.00	31.88	47.16	63.35	74.92
15	38.31	60.32	72.85	75.90	34.45	52.49	69.48	79.27
16	40.64	62.66	78.32	80.15	41.25	57.51	73.43	82.01
17	41.33	64.45	80.30	83.72	44.14	62.23	79.19	84.38
18	42.47	66.77	82.34	85.04	45.96	69.97	83.40	86.45
19	44.47	69.94	83.29	89.53	48.82	78.44	85.85	90.72
20	45.14	72.07	86.38	92.01	50.65	82.81	87.09	92.91
21	47.39	73.57	87.40	92.98	52.69	85.26	89.37	95.24
22	48.63	75.31	88.28	95.52	54.47	86.23	91.44	97.04
23	48.98	76.52	89.99	96.23	55.40	86.55	93.22	98.25
24	49.27	78.25	92.08	97.92	56.01	86.85	94.98	99.14

P.E* = Permeation enhancer

Table 9: *In vitro* skin permeation steady state flux (J_{ss}), permeability coefficient (K_p) enhancement ratio of flux (ER_{flux}) and Kinetics followed by transdermal patches ECP-1 and ACH-1 without and with different permeation enhancers

F. Code	Permeation enhancer	Steady state flux (J_{ss}) mg $cm^{-2}h^{-1}$	Permeability coefficient (K_p)	Enhancement ratios of flux (ER_{flux})	(Zero order model) Correlation coefficient (r^2)	(Higuchi model) Correlation coefficient (r^2)
ECP-1	Without P.E	0.072	0.02218	-	0.977	0.971
	PEG-400	0.103	0.03173	1.43055	0.976	0.991
	DMSO	0.115	0.03542	1.59722	0.972	0.986
	Oleic Acid	0.122	0.03758	1.69444	0.970	0.992
ACH-1	Without P.E	0.079	0.02433	-	0.989	0.948
	PEG-400	0.116	0.03573	1.46835	0.977	0.929
	DMSO	0.128	0.03943	1.62025	0.988	0.973
	Oleic Acid	0.133	0.04097	1.68354	0.972	0.984

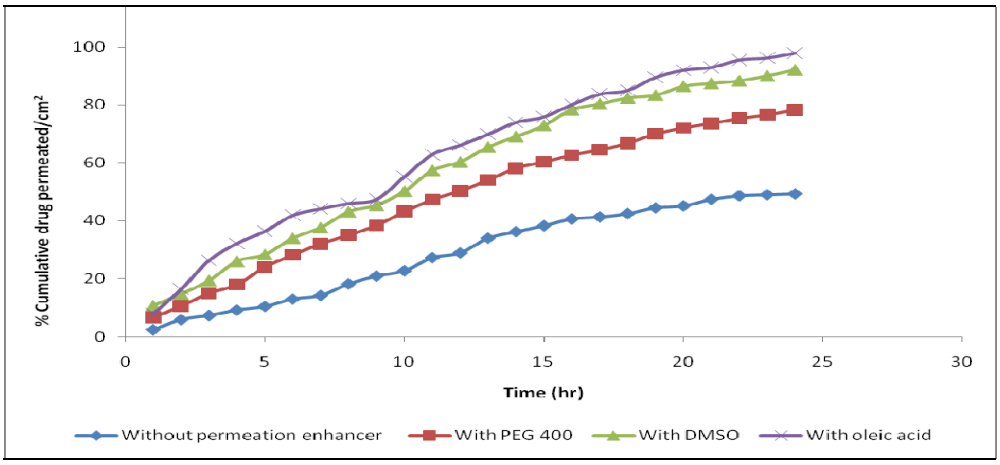


Fig. 4: Comparative skin permeation profile of transdermal patch ECP-1 (EC: PVP 5:1) with and without different permeation enhancers.

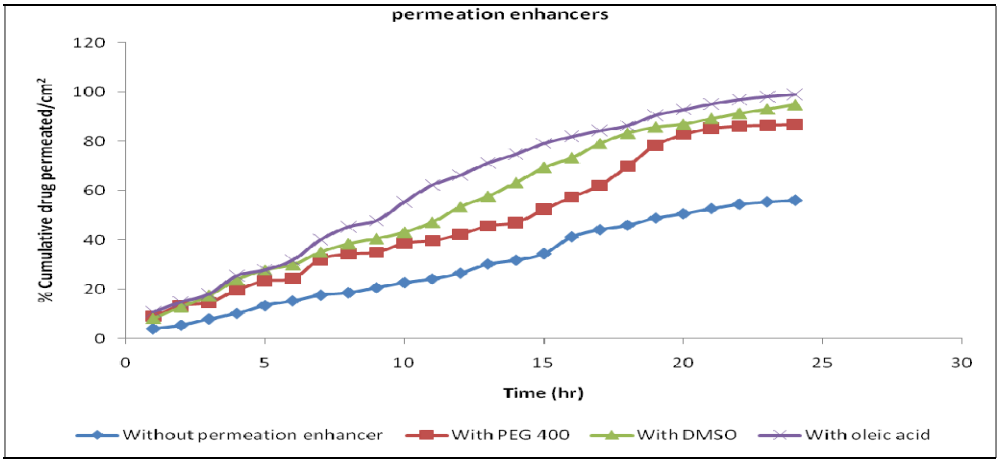


Fig. 5: Comparative skin permeation profile of transdermal patch ACH-1 (AC: HPMC 5:1) with and without different permeation enhancers.

Table 10: Results of skin irritation study for transdermal patch ECP-1 and ACH-1 having oleic acid as permeation enhancer, performed on healthy adult male New Zealand rabbits (Orytolagus caniculus)

Sl No.	Formulation code	Observation
1	ECP-1 with oleic acid as permeation enhancer	No any trace of edema, erthema or any skin irritation was observed.
2	ACH-1 with oleic acid as permeation enhancer	No any trace of edema, erthema or any skin irritation was observed.

RESULTS AND DISCUSSION

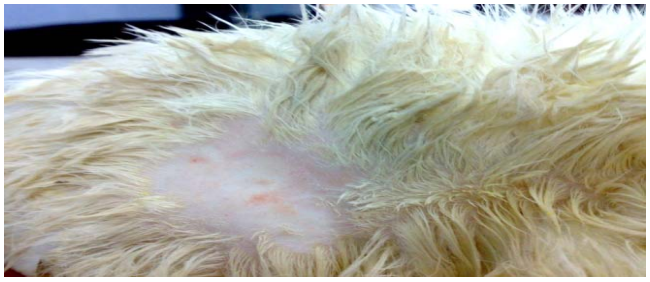
In the present study transdermal patches of propranolol hydrochloride were prepared by solvent casting and solvent evaporation technique employing glass moulds of known diameter. The monolithic transdermal patches of propranolol hydrochloride were formed using various polymers, which were ethyl cellulose (EC) with polyvinyl pyrrolidone (PVP-K30) in different combinations and acrycoat-S100 with hydroxypropyl methyl cellulose (HPMC-K4M) in different combinations.

The patches prepared with acrycoat-S100 and HPMC-K4M (ACH-1 to ACH-8) were found to be comparatively more clear, transparent, flexible, smooth and were removed easily from the mould, than the patches prepared with ethyl cellulose and polyvinyl pyrrolidone (PVP-K30) (ECP-1 to ECP-8).

The thickness of the formulated patches ECP-1 to ECP-8 was found to be in between 0.001667 to 0.002036 cm (Table 3). A low standard deviation value in the patch thickness measurement confirms uniformity of the patches prepared by solvent casting and solvent evaporation technique. The folding endurance value of all the patches was found satisfactory which ensures that patches prepared using plasticizer dibutyl phthalate (DBP) (30 % w/w of polymer) were having optimum flexibility and were not brittle (Table 3).

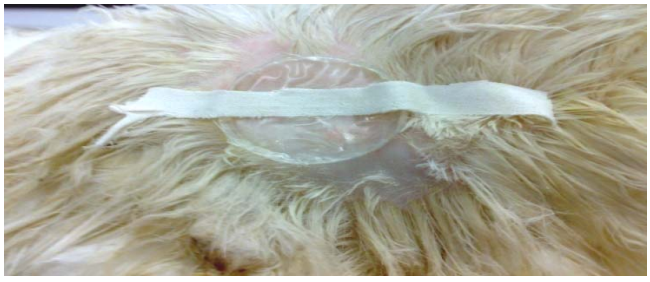
The percent moisture content (% w/w) of the formulated patches were found to be in between 1.212 to 5.349 (% w/w) (Table 3, Figure 1) It was observed that with increase in hydrophilic polymer concentration the moisture content was also increasing. But little moisture content is desirable so as the patches would not become dry and brittle. So formulation ECP-1 and ACH-1 can be considered as desired formulations with respect to moisture content.

The percent moisture uptake (% w/w) of the formulated patches prepared with ethyl cellulose and PVP-K30 in different ratios were found in between 1.378 to 4.676 (Table 3, Figure 1) and 1.924 to 4.497 (% w/w) were found for the formulations prepared with acrycoat-S100 and HPMC-K4M in different ratios (Table 3, Figure 1).

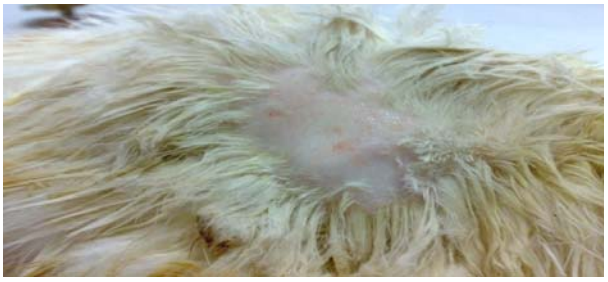


A) Before application of the transdermal patch

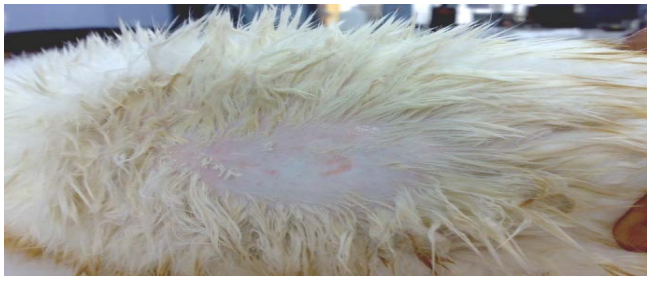
B) After application of control (adhesive tape USP)



C) After application of transdermal patch ECP-1 containing oleic acid



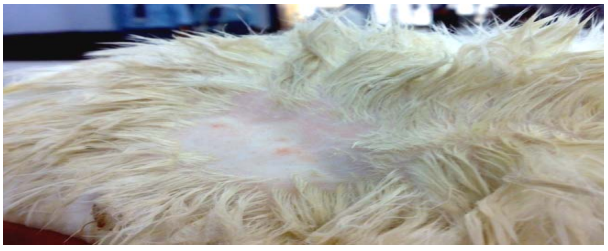
D) After removal of the patch at 24 hours of patch application



E) Before application of transdermal patch

F) After application of control (adhesive tape USP).

G)After application of transdermal patch ACH-1 containing oleic acid



H) After removal of the patch at 24 hours of patch application

Fig. 6: Photographs of skin irritation study for transdermal patch ECP-1 (Figure: A to D) and ACH-1 (Figure: E to H) having oleic acid as permeation enhancer which was performed on healthy adult male New Zealand rabbits (Orytolagus caniculus)

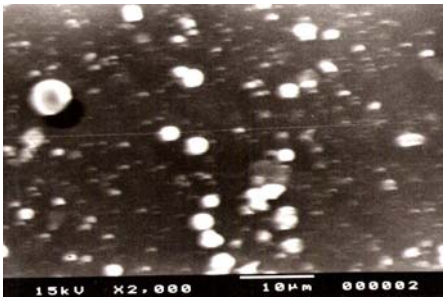


Fig. 7: Scanning electron microscopic photograph of drug loaded transdermal patch ECP-1. (EC:PVP 5:1) before in vitro skin permeation

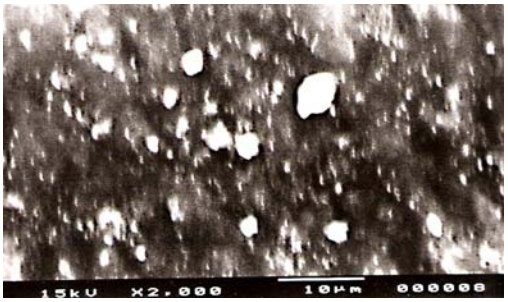


Fig. 8: Scanning electron microscopic photograph of drug loaded transdermal patch ECP-1 (EC:PVP 5:1) after in vitro skin permeation

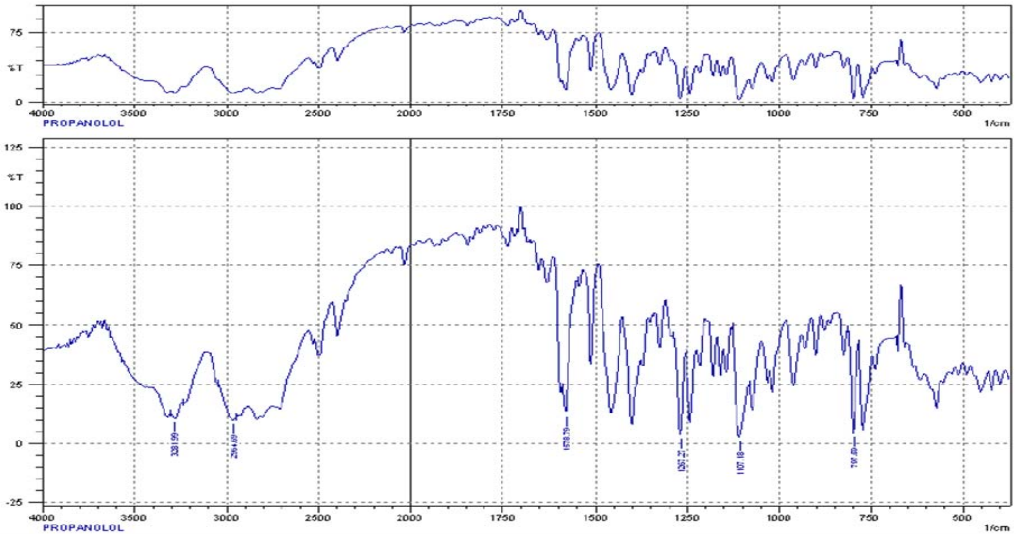


Fig. 9: FTIR spectrum of propranolol hydrochloride

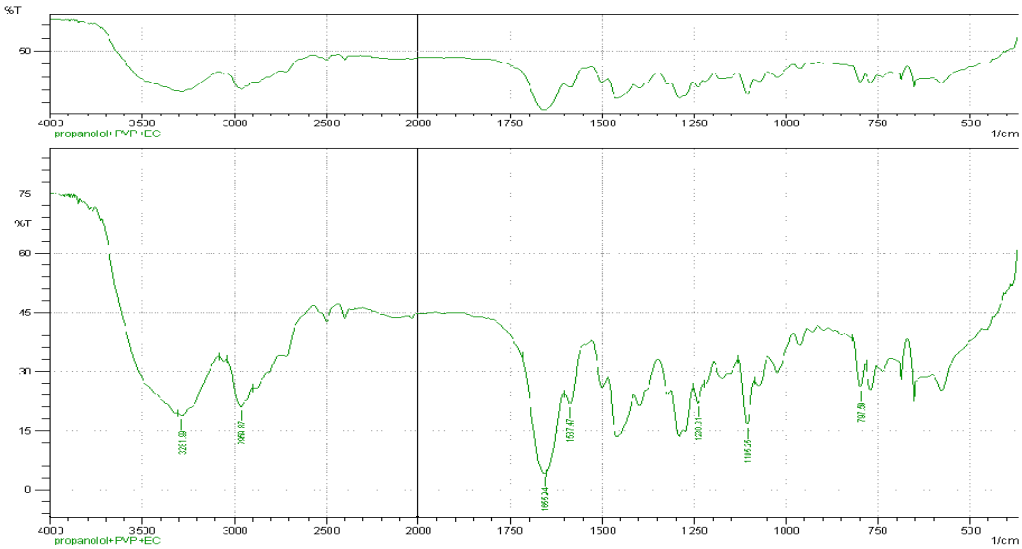


Fig. 10: FTIR spectrum of propranolol hydrochloride, ethyl cellulose and polyvinyl pyrrolidone.

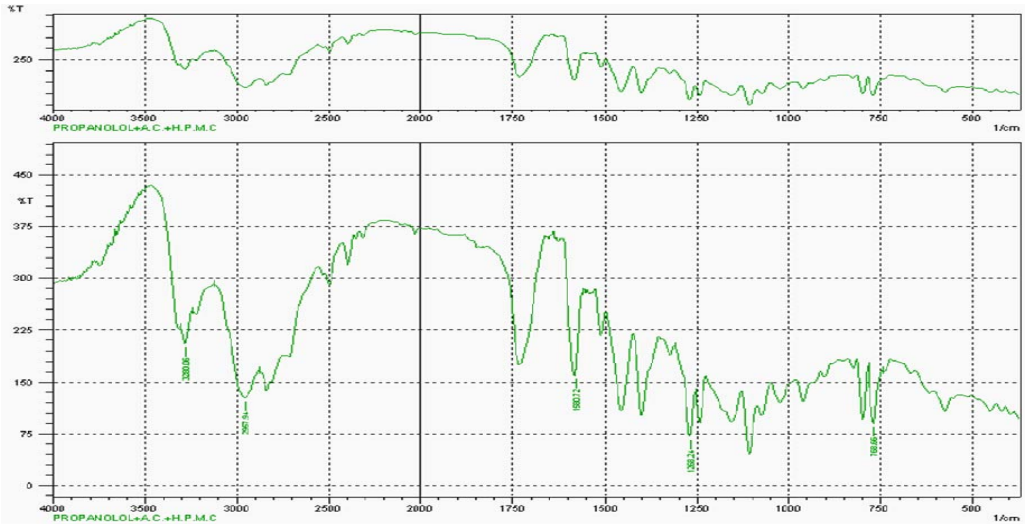


Fig. 11: FTIR spectrum of propranolol hydrochloride, acrycoat-S100 and hydroxypropyl methyl cellulose.

In moisture uptake study it was observed that moisture uptake value increases with gradual increase in concentration of hydrophilic polymer like PVP-K30 and HPMC-K4M. But it was seen in some formulations (ECP-5, ECP-6, and ECP-7) containing PVP-K30, in more concentration showed more moisture uptake than compared with formulations where concentration of HPMC-K4M increases. This may be due to hygroscopic nature of PVP-K30. Also high moisture uptake value may lead to microbial contamination of the formulation. Formulations ECP-1, ECP-2, ECP-3, ECP-4 and ACH-1, ACH -2, ACH -3, ACH -4 are acceptable in this respect.

The water vapour transmission through the different patch formulations prepared taking ethyl cellulose with PVP-K30 and acrycoat-S100 with HPMC-K4M in different compositions showed that the patches were permeable to water and the patches showed uniform flatness without any observed constriction (Table 4). The uniformity in flatness of the prepared patches indicates that the formulation by solvent casting and solvent evaporation technique is reproducible and the formulation can maintain satisfactory surface smoothness.

The weight of the drug loaded patches prepared with ethyl cellulose and PVP-K30 in different proportions were found to be in between 0.124 ± 0.0033 gm and 0.144 ± 0.0043gm and the weight of drug loaded patches of acrycoat-S 100 and HPMC-K4M was found to be in between 0.124 ± 0.0035 gm and 0.141 ± 0.0026 gm (Table 4). The low value of standard deviation shows that there is negligible variation in weight. The percentage drug content of all the formulations was found in between 98.37 % to 99.90 % (Table 4). The drug content of all the formulations was found satisfactory.

The tensile strength was determined for all the patches by *Sadhna et al.* method. The tensile strength of the patches was found in between 197.43 gm/cm^2 to 274.67 gm/cm^2 (Table 4). It was observed that with increase in concentration of hydrophilic polymers (PVP and HPMC) the tensile strength of the patches decreases gradually.

The *in vitro* permeation of drug from the formulated patches were carried out in modified Keshary-Chein diffusion cell through dialysis membrane using 100 ml phosphate buffer (pH 7.4) as diffusion media for a period of 24 hours. It was observed that the patches prepared with hydrophilic polymer in a higher concentration like formulations ECP-5, ECP-6, ECP-7, ECP-8 where concentration of PVP-K30 is in gradual increasing order and ACH-5, ACH-6, ACH-7, ACH-8 where concentration of HPMC-K4M is in gradual increasing order, the release was very quick and the patches releases more than 98 % of the loaded drug far before 24 hrs and drug release is not in a controlled manner. But in case of the patches prepared with hydrophobic polymer, ethyl cellulose concentration in a gradual increasing order like formulation ECP-4, ECP-3, ECP-2, ECP-1 and ACH-4, ACH-3, ACH-2, ACH-1 showed a controlled release of the loaded drug over an extended period of 24 hours. (Table 5 and Table 6)

The data obtained from the *in vitro* permeation study of the prepared patches were fitted to various kinetic models to determine the kinetics of drug release. The main models were Zero order model and Higuchi model (Table 5). A comparative % Cumulative drug release per cm^2 vs. time profile was plotted for all the formulated patches (Figure 2 & Figure 3). A burst release was observed for all the patch formulations in drug release study. But it was more prominent in patches where concentration of hydrophilic polymer is in gradual increasing and hydrophobic polymer decreasing i.e. ECP-5 and ACH-5 with highest and fastest releasing within 16-18 hours having no control on release of drug. This may be due to rapid dissolution of the surface hydrophilic drug and the rapid leaching of hydrophilic fraction of the film former which results in the formation of pores and thus leads to the decrease of mean diffusional path length of the drug molecules to permeate in to the dissolution media and hence higher permeation rates. [20]

On the basis of the data obtained from *in vitro* diffusion study, and regression values obtained after fitting these data in various kinetic models formulations ECP-1 and ACH-1 were chosen for further skin permeation study as the formulations showed a most prolonged and controlled release over a period of 24 hours under study.

The skin permeation study was carried out using albino mice skin in modified Keshary – Chein diffusion cell taking 100 ml phosphate buffer (pH7.4) as diffusion media. It was observed that the permeation of drug through the skin is lesser than dialysis sac. The percentage cumulative drug permeation per cm^2 of the patch formulations at 24 hours were ECP-1; 49.27 % and ACH-1; 56.01% (Table 8).

A skin permeation enhancement study was carried out for the above formulations by incorporating chemical permeation enhancers PEG 400, DMSO, and oleic acid at a concentration of 15 % w/w of the total polymer to each selected formulations individually. It was found that incorporation of PEG-400 in the patch formulations slightly increased the permeation of drug through the skin. In case of PEG 400 as the permeation enhancer the maximum percentage cumulative drug permeated per cm^2 of the formulations through the skin at the end of 24 hrs were found for ECP-1; 78.25 % and ACH-1; 86.85 % (Table 8). DMSO and oleic acid markedly enhanced the permeation rate of ECP-1 and ACH-1 transdermal formulation. In case of DMSO the maximum percentage cumulative drug release at the end of 24 hours for ECH-1 was 92.08 % for ACH-1 was 94.8 % (Table 8). Similarly for oleic acid as permeation enhancer the maximum percentage cumulative drug release in case of ECP-1 was 97.92 % and for ACH-1 was 99.14 % (Table 8). The steady state flux rate, permeability coefficient, and enhancement ratio flux was calculated for all these three permeation enhancers which were found maximum for oleic acid and minimum for PEG 400 (Table 9).

Among the various chemical permeation enhancers used, oleic acid showed the highest enhancing effect. The mechanism of action of various chemical permeation enhancers may be attributed to their activity on lipophilic matrix and/or hydrophilic protein gel in stratum corneum, which act through interaction with intercellular lipids, leading to disruption of their organization and increasing their fluidity. Some of them may also interact with intercellular protein, keratin denaturation.[21]

The data obtained from *in vitro* skin permeation studies were fitted into various kinetic models mainly Zero order and Higuchi model in order to view the release pattern of formulation ECP-1 and ACH-1 with and without various permeation enhancers. Regression values obtained from each models were within the range of these models and satisfactory, so as all followed zero order as well as Higuchi model kinetics (Table 9).

A 24 hour skin irritation study was conducted for formulation ECP-1 and ACH-1 on healthy adult male New Zealand rabbits (Orytolagus caniculus). There was no any trace of edema, erythema or any skin irritation on site of application of the patch hence these formulations are non irritable to the skin tissue and can be considered as the safe therapeutic transdermal patches (Table 10, figure 6).

The scanning electron microscopic examination of drug loaded transdermal patches ECP-1 ratio shows good film formation superficially. After skin permeation study the drug release from the patches can be evidenced by pore formation in the film (Figure 7 and 8).

FT-IR (Fourier Transform Infrared) spectroscopy study of the pure drug (propranolol hydrochloride) alone and the combination of drug with various polymers (ethyl cellulose, PVP-K30, acrycoat-S100, and HPMC-K4M) under study was carried out (Figure 9 to 11). The FT-IR spectrum of propranolol hydrochloride revealed the presence of peaks at 2965.1 cm^{-1} due to the presence of a secondary amine group, peaks at 3283.7 cm^{-1} due to the hydroxyl group (secondary), the aryl alkyl ether displayed a stretching band at 1268 cm^{-1} and the peak at 797.9 cm^{-1} was due to a-substituted naphthalene. Major frequencies of functional groups of pure drug remained unchanged in presence different polymers under study; hence there is no major interaction between the drug and polymers used in the study.

CONCLUSION

The present work comprised the formulation and evaluation of propranolol hydrochloride transdermal patches for sustained or extended period of time. It was observed that basic physicochemical properties of all the patches were satisfactory except for a few. It was observed that basic physicochemical properties as well as diffusion profile of all the formulated patches entirely depends on the composition of excipients incorporated in it. Basically the polymer composition has a direct influence on physicochemical properties as well as diffusion profile.

Further *in-vitro* skin permeation studies suggested the permeation enhancing ability follows the order: without permeation enhancer < PEG 400 < DMSO < oleic acid at the concentration of 15 % w/w of total polymer weight for propranolol hydrochloride transdermal patches.

Hence it may be concluded that formulation of propranolol hydrochloride transdermal patches with suitable and optimum concentration of excipients in form of polymers, plasticizers, permeation enhancers etc. may be used to get the effective rate of release of drug from the patches. In this new era of sustained release dosage form, prolonged delivery of propranolol hydrochloride may be possible by these transdermal drug delivery systems, after having further studies like – *in vivo* studies, stability studies etc.

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