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Effect of Various Polymers on Carvedilol Transdermal Films: *In-vitro* Permeation Studies

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ABSTRACT

Transdermal films of carvedilol were prepared by the solvent evaporation technique with combination of different polymers such as EC: PVP K-30, EC: HPMC K-15M, EC: Carbopol-934. During the optimization of various formulations, polymer ratios of 6:4 and 8:2 amongst the all combinations of various polymers ratio were taking into consideration to develop and evaluation of tansdermal films. It was optimized on the basis of film's uniformity, folding endurance and transparency. Total six formulations were selected (A-1 to A-6) for further evaluation. The prepared patches possessed satisfactory physicochemical characteristics. Thickness, mass and drug content were uniform in prepared batches. In vitro permeation studies were performed using a Franz diffusion cell across hairless albino rat skin. On the basis of physicochemical evaluations and In-vitro drug permeation study, A-3 and A-5 were chosen as the best films and among the both preparation, only A-5 was found to have maximum permeation, maximum steady state flux and maximum permeability coefficient and kinetic model proved that permeation of drug was followed the first order rate. The patches were seemingly free of potentially hazardous skin irritation. In-vitro drug permeation study, the percent of drug permeated was found to maximum 95.44 and 90.12% from A-3 and A-5 film respectively. Based on physicochemical and in-vitro release experiments, A-3 and A5 chosen as the best films among both only A-5 was found to have maximum release, maximum steady state flux and maximum permeability coefficient.

Key words: Transdermal film, Carvedilol, Ethyl cellulose, PVP K-30, HPMC K15M., Carbopol 934.

INTRODUCTION

Carvedilol is widely used for the therapeutic management of hypertension, congestive heart failure. Carvedilol is a novel, multiple-action cardiovascular drug that is currently approved in many countries for the treatment of hypertension. The reduction in blood pressure of carvedilol results primarily from beta-adrenoceptor blockage and vasodilatation. These actions as well as

several other carvedilol activities are associated with cardio protection in animal models that occurs to a degree that is greater than that observed with other drugs. The multiple actions of carvedilol may also provide the underlying rationale for the use of the drug in the treatment of coronary artery disease and congestive heart failure [1]. Carvedilol is well absorbed from the gastrointestinal tract but is subject to considerable first-pass metabolism in the liver; its absolute bioavailability is about 25%. It has a half-life of 2.2 ± 0.3 h; longer half-lives of about 6 h have been measured at lower concentrations [2, 3].

Carvedilol was chosen as the model candidate for this study since it possesses near ideal characteristics that a drug must have in formulating a transdermal drug delivery system: low molecular mass, high lipid solubility, effective in low plasma concentration as well as a high degree of first-pass metabolism. It also means multiple daily administrations with subsequent lack of patient compliance. The aim of this study was to develop and evaluate transdermal patches of carvedilol so as to prevent its first-pass metabolism and achieve controlled release. It is drug of choice for hypertension but it has several drawbacks such as short biological half life, readily metabolized in liver primarily by cytochrome P450 isoenzyme, CYP 206 and CYP 209 and has a lower oral bioavailability. These factors in addition to its low molecular weight (406.05), low melting point (117°C), high lipid solubility and effective in low plasma concentration necessitates the formulation of sustained release transdermal drug delivery system for carvedilol [4-7].

Polymers such as Ethyl cellulose, PVP K-30, HPMC K15M and Carbopol 934 were selected on the basis of their adhering property and non toxicity to prepare Carvedilol transdermal films. Transdermal drug delivery system is a most suitable system for a long-term treatment or for a multi dose treatment because different transdermal patches are prepared for a long period of time in a suitable dose proving treatment from a day to even up to seven days. In this study Carvedilol transdermal films were prepared with combination of different polymers such as EC: PVP K-30, EC: HPMC K-15M, EC: Carbopol-934. Formulations were further evaluated on the basis of their physicochemical properties, in-vitro drug permeation study and surface pH of the film. For physicochemical evaluation it was found that thickness, weight variation, moisture content, drug content, folding endurance and flatness of the prepared transdermal films. In-vitro drug permeation study, the percent of drug permeated was found and based on physicochemical and in-vitro release experiments, chosen the best films among all the formulations and found which one having maximum release, steady state flux and permeability coefficient. The main objective of this current study was to develop a potentially competitive product by optimizing screening of various formulations variables to provide the delivery of the drug at a controlled rate across the skin.

MATERIALS AND METHODS

Carvedilol, HPMC K4 M, PVP K30, Carbopol-934 were obtained as gift sample from Zydus Cadila, Ahmedabad. Ethyl cellulose, PEG 200, PVP and n-Octanol were procured from Cental Drug House (P) Ltd. Mumbai. Methanol, potassium dihydrogen phosphate, sodium chloride, chloroform were procured from Ranbaxy fine chemicals, India.

Optimization of Placebo Polymeric Films

Preparation and optimization of placebo polymeric film was mandatory to prepare finally drug-loaded polymeric film. Optimization of placebo polymeric films were done by making total five trials that were performed consequently for each polymer combination i.e. EC:PVP K-30; EC:HPMC K15 and EC: Carbopol-934 respectively. In all of the following trials (Table I)

optimization almost based on the total polymers weight and different polymer ratios whereas rest parameters were remained constant during optimization processes.

Same surface area of Petri dishes were used i.e. 46.57cm^2 to prepare all polymeric films. Backing membranes of 4% (w/v) polyvinyl alcohol (PVA) solution (5 ml) were dried at 60°C for 6 h in hot air oven and placebo polymeric films were dried at 40°C for 6 h in hot air oven.

Preparation of Transdermal Films

Drug free film was prepared by using the solvent evaporation method. The bottom Petri dish was wrapped with aluminum foil on which the backing membrane was cast by pouring 4 %(w/v) polyvinyl alcohol (PVA) solution (5 ml) prepared but dissolving in double distilled water followed by drying at 60°C for 6 hr in hot air oven. After the drying backing membrane, different polymers were mixed in chloroform containing 30 % (w/w) PEG-200 of total polymer composition and 5ml of the polymer solution was poured in the Petri dish and an inverted funnel was placed on the Petri dish to facilitate the evaporation of solvent at controlled manner over a drying period of 6 hr at 40°C.The film was retrieved by cutting with surgical knife and kept in the desiccators for further evaluation. All of the parameters optimized during preparation of placebo polymeric film were remaining same for drug loaded polymeric film except amount of the drug.

Drug loaded polymeric film was prepared in similar manner except that 16 mg (2.5 % w/w of polymer composition) of carvedilol dissolved in 5ml chloroform and it was added in the polymer solution containing plasticizer. Different formulations were prepared by using each polymer ratio of 6:4 and 8:2 of EC: PVP K-30; EC: HPMC K15 and EC: Carbopol-934 (Table II).

Evaluation of Transdermal Film

Thickness

The thickness of the each patch was measured using screw gauge at different positions of the patch and the average was calculated [8].

Weight variation

Weight variation was studied by individually weighing 10 randomly selected patches (46.57 cm^2). Such determination was performed for each formulation [9,10].

Folding endurance

Folding endurance was determined by repeatedly folding one film at the same place till it broke. The number of times the film could be folded at the same place without breaking/ cracking gave the value of folding endurance [11].

Moisture content

The patches were weighed individually and kept in a desiccators containing activated silica at room temperature for 24 h. Then, the final weight was noted when there was no further change in the weight of the individual patch. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight [10].

Drug content uniformity

Three longitudinal strips were collected by cutting off three zones from each film: one from the centre, one from the left side and one from the right side. Films of 0.64 cm^2 areas from each zone were dissolved in 200 ml of methanol and the volume was made up to 100 ml with same solvent and placed on electronic shaker for 1h to dissolve completely films in methanol. The solutions

were filtered through a 0.45 µm membrane, diluted suitably and absorbance were noted at 242 nm in a double beam UV-Visible spectrophotometer (Model-1700, Shimadzu, Japan) against a blank that was prepared using a drug-free patch treated similarly after that drug content was calculated [11] and tabulated in Table III.

Flatness study

Three longitudinal strips were collected by cutting off three zones from each film: one from the centre, one from the left side and one from the 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 [10, 12].

$$\% \text{ Constriction} = [(l_1-l_2) / l_2] \times 100$$

Where, l_1= initial length of each strip

l_2= final length of each strip

Fourier Transformed Infrared Spectroscopy

The drug loaded transdermal film was analyzed by FT-IR to confirm the chemical interaction between the drug and polymer using the thin film. IR spectrums were scanned on a model, RX-I FT-IR system, Perkin Elmer, USA in the range of 450- 4000cm^{-1} [13].

Differential Scanning Calorimetry

The physicochemical compatibility between Carvedilol and polymers used in the patches was further studied by using differential scanning calorimetry (DSC Q10 V9.4 Build 287, TA Instruments, USA). In DSC analysis, the samples were weighed (2 mg), hermetically sealed in flat-bottom aluminum pans, and heated over a temperature range of 50 to 150°C and 50 to 250°C in an atmosphere of nitrogen (50 mL/min) at a constant increasing rate of 10°C/min. The Thermograms (Fig. 2) obtained for Carvedilol, polymers and formulations of Carvedilol with polymers were compared [11].

X-ray diffraction studies

X-ray diffraction studies (Fig. 3) were carried on physical mixture and drug containing transdermal film using the XRD technique with model X'Pert-Pro diffractometer system PANalytical, The Netherlands. XRD studies were performed on the samples by expose them to Cu K-α-1 radiation (45kV, 40mA) and scanned from 2 to 50° 2θ, at a step size of 0.0170 2θ and a step time of 20.0271 s [14-16].

Surface pH of the Film

Transdermal films were allowed to swell for 2 h at 37˚C on the surface of an agar plate, prepared by dissolving 2% (w/v) agar in worm isotonic phosphate buffer of pH 5.5 under stirring and then pouring the solution into a Petri dish till gelling at room temperature. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch. After 90 s the color developed. The mean of six reading was recorded and tabulated in Table III.

***In-vitro* Permeation Studies**

In vitro skin permeation studies were performed by using a Franz diffusion cell with a receptor compartment capacity of 50 ml. The excised rat abdominal skin was mounted between the donor and receptor compartment of the diffusion cell. The formulated films were placed over the skin and covered with paraffin film [16-18]. The receptor compartment of diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer and the

solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37±0.5°C. The 2 ml aliquots were withdrawal at different time intervals (0, 30, 60, 90, 120, 150 and 24 h) and analyzed the drug content by UV-Visible spectrophotometer (Model-1700, Shimadzu, Japan) at 242 nm. The receptor phase was replenished with an equal volume of phosphate buffer (37°C) at each sample withdrawal, the cumulative amount of drug permeated per square centimeter of patches were plotted against time. Percent drug permeated and log % DRP was calculated and tabulated in Table IV.

Data Analysis

For each experiment skins from at least three rats were used. Concentration of Carvedilol sample were analyzed, and the cumulative amount permeated (µg/cm^2) was plotted against time. The steady state fluxes "J" (mcg.cm^{-2}.hr^{-1}) was determined from the slope of the linear portion of the graph and tabulated in Table VI**.**

Permeability Coefficient "K_p" (cm.hr^{-1}) is expressed as, $K_p = \frac{J}{C_0}$

Where C_0 = concentration of drug in donor phase and J = Flux

The values reported are mean from at least three experiments regarding steady state flux and permeability coefficient and tabulated in Table VI**.** The rate and the mechanism of drug release were analyzed by fitting the diffusion data into zero-order equation, $Q=Q_0\ k_0t$, where Q is the amount of drug released at time t, and k_0 is the release rate. First order equation, $\ln Q=\ln Q_0\ k_1t$, where k_1 is the release rate constant and Higuchi s equation, $Q= k_2t^{1/2}$, where Q is the amount of the drug released at time t and k_2 is the diffusion rate constant. The diffusion data was further analyzed to define the mechanism of release by applying the diffusion data following the empirical equation,

$$M_t / M_\alpha = K.t^n$$

Where, M_t / M_α, is the fractional release of drug, M_t is the amount released at time t, M_α is the total amount of drug contained in the transdermal film, t is the release time, K is a kinetic constant and is the diffusional release exponent indicative of the release mechanism of drug release from the formulations during diffusion process [12, 19-20].

Scanning Electron Microscopy (SEM)

Sample, for the SEM was prepared by sprinkling the film on one side of a double adhesive stub. The stub was then coated with gold under vacuum (Fine Coat, in sputter, EC-1100). The films were then observed under the scanning electron microscope (JEOL, JSM-6360 Scanning Electron Microscope, Japan) at 15Kv. The samples include blank film (without drug), film before and after carrying out the permeation studies [13].

Stability Studies

Accelerated stability testing was conducted for 30 days at different temperatures: 4, 45, and 60°C. At specific intervals of time (Day 5, 10, 15, 20, 25, and 30), patches were taken out to assay their drug content, appearance, and texture [21].

RESULTS AND DISCUSSION

Preparation and **optimization of placebo polymeric film** was mandatory to prepare finally drug-loaded polymeric film. Optimization of placebo polymeric films were done by making total five trials that were performed consequently for each polymer combination i.e. EC:PVP K-30; EC:HPMC K15 and EC: Carbopol-934 respectively. In all of the following trials (Table I) optimization almost based on the total polymers weight and different polymer ratios whereas rest parameters were remained constant during optimization processes.

Drug loaded polymeric film was prepared in similar manner which was followed to the optimization process, except that 16 mg (2.5 % w/w of polymer composition) of carvedilol dissolved in 5ml chloroform and it was added in the polymer solution containing plasticizer. Different formulations were prepared by using each polymer ratio of 6:4 and 8:2 of EC: PVP K-30; EC: HPMC K15 and EC: Carbopol-934 (Table II).

The **thickness** for various formulations ranged between 0.09±0.012 to 0.21±0.020 (Table III). The deviation in the thickness was within the limits, as it gets confirmed by low standard deviation for thickness. In case of A-3 and A-4, EC and HPMC K15M containing formulations, it has been seen with increases of HPMC content thickness was increased consistently but which was not observed in case of EC: Carbopol 934 formulations. It may be due to the gel forming properties of Carbopol, where as PVP do not contribute significantly towards thickness building as compared to HPMC and Carbopol.

The **total weight** for various formulations ranged between 839 ± 0.92 to 840.9±1.52 (Table III) The deviation in the weight was within the limits as it get confirmed by low standard deviation for weight (Table III). It was observed that the weight of the patches was increasing gradually with increase of HPMC content. The weight of the patches increased with increase in thickness of the respective patches the thickness of the patches were increased. The individual total weight in mg/46.57 cm^2 was shown in Table III.

Table. I. Compositions of various Trails for Optimization of Placebo Polymeric Films of Different polymeric combination

	Total Polymers Weight	Polymer ratios *	Observation (EC:PVP K-30)	Observation (EC:HPMC K15M)	Observation (EC:Carbopol-934)
1.	390 mg	2:8; 6:4; 7:3, 8:2; 9:1	Uniform film was not found and flaking was observed.	Uniform film was not found.	Uniform film was not found.
2.	580 mg	2:8	Non uniform and thin film was found.	Cracking was observed.	Non uniform film was having clumps of Carbopol.
		6:4	Poor folding endurance and non uniform film was found.	Poor folding endurance and non uniform film was found.	Non uniform film and cracking was observed.
		7:3	Non uniform film was found.	Non uniforms and cracked film was observed.	Non uniform film was found.
		8:2	Poor folding endurance and non uniform film was found.	Poor folding endurance and some black spots were observed.	Poor folding endurance and non uniform film was found.
		9:1	Film was uniform	Film was uniform	Clumps of Carbopol

			but folding endurance was poor.	but folding endurance was poor.	polymer were observed.
3.	780 mg	2:8; 6:4; 7:3, 8:2; 9:1	Uniform film was not found and not well dried.	Film not well dried.	Uniform film was not found and not well dried.
		2:8	Transparent but not uniform film was found.	Transparent but not uniform was found.	Transparent but not uniform was found.
		6:4	Film was not found uniform.	Film was uniform but some black spots were observed.	Film was not found uniform and clumps of Carbopol polymer were observed.
4.	663 mg	7:3	Film was uniform but folding endurance was poor.	Film was uniform but folding endurance was poor.	Film was uniform and transparent but folding endurance was poor.
		8:2	Film was uniform and folding endurance was poor.	Non uniform film, poor folding endurance and film was not well dried.	Film was not well dried.
		9:1	Film was not well dried.	Film was not well dried.	Film was not found uniform and clumps of Carbopol polymers were observed.
		2:8	Film was found uniform but folding endurance was poor.	Film was found uniform and transparent but folding endurance was poor.	Film was found uniform but folding endurance was poor.
		6:4	Film was found uniform and folding endurance was good	Film was found uniform, transparent and having good folding endurance.	Film was found uniform, transparent and having good folding endurance.
5.	624 mg	7:3	Film was uniform and having good folding endurance.	Film was uniform but having poor folding endurance.	Film was uniform but some clumps of polymer were observed.
		8:2	Film was uniform and having good folding endurance.	Film was uniform and having good folding endurance.	Film was found uniform, transparent and having good folding endurance.
		9:1	Film was not found uniform and it was extremely thin.	Extremely thin film was found.	Film was not found uniform and not well dried.

**Polymers ratios considered as written in observation columns*

The values of the **folding endurance** for all formulations are tabulated in Table 3. The A-1 and A-2 films made of EC and PVP K-30 showed a folding endurance of more than 16 and 14 respectively. The A-3 and A-4 films made of EC and HPMC K15M showed folding endurance more than 10 and 8 respectively. In comparison with films made by EC:PVP K-30 and EC:HPMC K15M, The films (A-5 and A-6) made of EC and Carbopol, showed folding endurance more than 50, it means films made of Carbopol was having better folding endurance compared to films containing PVP K-30 and HPMC K15M.

Tab. II: Composition of Different Optimized drug loaded polymeric films

S. No.	Ingredients	Formulations Code					
		A-1	**A-2**	**A-3**	**A-4**	**A-5**	**A-6**
1.	Ethyl cellulose (EC) in mg	374.4	499.2	374.4	499.2	374.4	499.2
2.	Polyvinyl pyrrolidone K-30 (PVP K-30) in mg	249.6	124.8	—	—	—	—
3.	Hydroxypropyl methylcellulose K15M (HPMC K15M) in mg	—	—	249.6	124.8	—	—
4.	Carbopol- 934 in mg	—	—	—	—	249.6	124.8
5.	Carvedilol (2.5% w/w of total polymer composition) in mg	16	16	16	16	16	16
6.	PVA (4% w/v of total polymer composition) in ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
7.	PEG-200 (30% w/v of total polymer composition)	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
8.	Chloroform	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml

Moisture content studies indicated that the increase in the concentration of hydrophilic polymer was directly proportional to the increase in moisture content of the patches. Moisture content of A-1 and A-2 films were found in the range of 3.17±2.1 to 4.21±3.4% (Table III), which shows least moisture content compared to other films. Moisture content of A-3 and A-4 films was found in the range of 4.12±2.9 to 5.06±2.4% which having more moisture content in compared to A-1 and A-2 films due to hydrophilic nature of HPMC K15M in A-3 and A-5 films. The moisture content of A-5 and A-6 films was found in the range of 4.79±2.2 to 5.68±3.4 which having greater moisture content in comparison of other films, due to presence of more hydrophilic nature of Carbopol 934. The moisture content of the prepared formulations was low, which could help the formulations remain stable and reduce brittleness during long term storage. Although the moisture uptake of the formulations was also low, this could protect the formulations from microbial contamination and reduce bulkiness.

For various formulations, the drug content in area of 0.64 cm^2 was between 0.210±0.031 to 0.232±0.021mg (Table III). The drug content of the prepared formulations has shown that the process employed to prepare transdermal films in this study was capable of giving films with a uniform drug content and minimum batch variability.

The **flatness study** showed that all the formulations had the same strip length before and after their cuts, indicating 100% flatness (Table III). Thus, no amount of constriction was observed; all patches had a smooth, flat surface; and that smooth surface could be maintained when the patch was applied to the skin.

Surface pH of the Film

The surface pH of the polymeric patches ranged between 5 to 7 (Table III) which falls with in the pH range of skin i.e. 4.0-6.5. Further, the surface pH data reveals that upon increase in Carbopol concentration the surface pH of the formulation towards acidic media. Fergany *et al.* suggested that excess concentration of Carbopol in the formulation may cause irritation to the skin due to its acidic nature [8, 9]. That is why formulation containing Carbopol, its concentration should be optimized and it was further confirmed by conducting the skin irritation studies.

Tab. III. Physicochemical Characteristics of Transdermal Films

Formulation Code No.	Polymers Used	Ratio of Polymer	Thickness (mm) Mean ± S.D.	Weight (mg) (46.57 cm^2) Mean ± S.D.	Folding Endurance	Moisture Content (%)	Drug Content (mg/0.64cm^2) Mean ± S.D.	Flatness (%)	Surface pH
A-1	EC:PVP K-30	6:4	0.09 ± 0.012	840 ± 0.75	>16	4.21 ± 3.4	0.232 ± 0.021	100	≈ 6-7
A-2	EC:PVP K-30	8:2	0.10 ± 0.013	839 ± 0.92	>14	3.17 ± 2.1	0.219 ± 0.018	100	≈ 6-7
A-3	EC:HPMC K15M	6:4	0.21± 0.014	840.7 ±1.62	>10	5.06 ± 2.4	0.212 ± 0.011	100	≈ 6-7
A-4	EC:HPMC K15M	8:2	0.21± 0.020	840.9 ± 1.52	>8	4.12 ± 2.9	0.210 ± 0.031	100	≈ 6-7
A-5	EC:CP-934	6:4	0.11 ± 0.016	840.5 ± 2.43	>50	5.68 ± 3.4	0.227 ± 0.043	100	≈ 5-6
A-6	EC: CP-934	8:2	0.11 ± 0.014	840.4 ± 1.46	>50	4.79 ± 2.2	0.223 ± 0.025	100	≈ 5-6

In-Vitro Skin Permeation Study

Release of the drug from transdermal patches is controlled by the chemical properties of the drug and delivery form, as well as physiological and physicochemical properties of the biological membrane. *In Vitro* skin permeation experiments are known for their value for studying the rate and mechanism of percutaneous absorption of drugs [10-13]. In this experiment, variable permeation profile of Carvedilol from the different experimental transdermal films (0.64 cm^2) compared with various blends of different polymers, EC, PVP K-30, HPMC K-15M and Carbopol-934. The percent of drug permeated after 24 h of the experiments which was found between the ranges of 69.54% to 95.44% (Table IV). The percent of drug permeated after 24 h was found to be maximum 95.44% and 90.12% from formulation A-5 and A-3 respectively (Table IV).

Tab. IV. *In Vitro* permeation of Carvedilol across the Rat Skin from various Transdermal Films

Time (min)	% Drug Permeated (Mean ± S.D.)					
	A-1	A-2	A-3	A-4	A-5	A-6
0	0.000±0.00	0.000±0.00	0.000±0.00	0.000±0.00	0.000±0.00	0.000±0.00
30	9.191±1.19	8.042±0.64	13.096±0.82	10.821±0.72	14.035±2.34	11.095±0.31
60	16.197±2.18	14.157±1.22	20.038±0.61	17.075±1.14	20.043±1.98	18.482±0.78
90	21.067±0.12	19.777±0.65	26.090±0.32	21.856±0.57	27.011±1.06	23.634±0.70
120	26.217±0.31	26.802±0.26	32.001±0.52	26.901±0.43	33.056±0.54	28.634±0.56
150	29.189±0.75	30.017±0.82	34.014±0.61	30.955±0.72	35.1±0.87	32.099±0.45
360	45.0±0.23	40.877±0.25	50.080±0.85	45.736±0.34	52.00±0.92	47.296±0.99
1440	80.916±1.81	69.546±0.57	90.127±0.87	76.835±0.84	95.441±1.00	81.957±0.88

The process of drug release in most controlled release device is governed by diffusion, and the polymer matrix has a strong influence of the diffusivity as the motion of small molecules is restricted by the three-dimensional network of polymer chains. The alteration of the cross linking and the modification of structural arrangements of polymers by using different blends of polymers already reported. So, different drug permeation profiles from various formulations could be attributable to the varied cross linking networks of polymeric chains of the different blends of polymeric transdermal experimental formulations as tortuosity and diffusion pathway varied, and they thereby have been reported to vary the release of drug and duration of diffusion.
In *in-vitro* skin permeation experiments also, as the concentration of hydrophilic was increased, the amount of drug permeated was increased. This may be a result of the initial rapid dissolution

of the hydrophilic polymers when the patch is in contact with the hydrated skin, which results in accumulation of high amount of drugs on the skin surface and thus leads to the saturation of the skin with drug molecules at all time. The rapid dissolution of the aqueous soluble fraction of the film also leads to the formation of pores, and hence higher release rate.

Permeation studies

To examine the drug permeation kinetics and mechanism, the data were fitted to models representing zero-order; first-order, Higuchi and Koresmeyer-Peppas [11]. Permeation of the drug from a transdermal drug delivery system mainly involves the factor of diffusion. Diffusion is related to the transport of the drugs from a dosage matrix into the *in vitro* study fluid, depending on the concentration. As the gradient varies, the drug is released, and the distance for diffusion becomes increasingly greater. This could be an explanation as to why the drug diffuses at a slower rate as the distance for diffusion increases. The kinetic parameters of drug permeation for different formulations were presented in Table V. In our experiments the in vitro permeation profiles of all formulations did not fit into zero-order (R^2 = 0.8754 to 0.9355) they could be best expressed by the first-order (0.9593 to 0.9977) and Higuchi equation (R^2 = 0.979 to 0.9919) for the permeation of drug from a homogenous-polymer matrix type delivery system that depends mostly on diffusion characteristics. The percent of drug permeated in 24 h was found to be maximum 95.44% and 90.12% from formulations A-5 and A-3 respectively. It has been confirm from the Table 5 that permeation of drug from films followed both first order (R^2 =0.9975 and 0.9977) and Higuchi model (R^2 = 0.9909 and 0.9869). The data was further treated as per the following equation for confirming the Koresmeyer-Peppas model.

$$M_t / M_\alpha = K.t^n$$

Where, M_t / M_α, is the fractional release of drug, M_t is the amount released at time t, M_α is the total amount of drug contained in the transdermal film, t is the release time, K is a kinetic constant and is the diffusional release exponent indicative of the release mechanism [13-15]. For Film A-3 (n=0.489) and A-5(n=0.492) has n value near to 0.5, it means drug permeation followed Fickian diffusion mechanism because if n=0.5 which stands, for Fickian diffusion other films was also followed Fickian diffusion because heir n value was just over the 0.5 value. The permeability coefficients was tabulated in Table 6 and when it was compared to each other, it has been seen only A-5 formulation was the maximum permeability coefficient compared to other formulations and it was the highest level of drug release from the film. Based on the physicochemical and *in vitro* permeation studies, it has been shown A-3 and A-5 was the best film. Among the both these two films only A-5 formulation was found to have maximum drug permeation (Table IV), maximum steady state flux and maximum permeability coefficient (Table VI).

Tab. V. Kinetics Models of *In Vitro* Carvedilol Permeation across Rat Skin from Transdermal Films

Formulation Code No.	Zero-Order		First-Order		Higuchi Model		Koresmeyer-Peppas Model		
	k_0 (mg.min^{-1})	R^2	k_1 (min^{-1})	R^2	k_2 (mg. min$^{-1/2}$)	R^2	n	k_3	R^2
A-1	0.0459	0.9223	0.00115	0.9911	2.1977	0.9919	0.547	0.0211	0.9752
A-2	0.0381	0.8754	0.00069	0.9593	1.9632	0.9664	0.5397	0.0237	0.938
A-3	0.0493	0.9287	0.00161	0.9977	2.4942	0.9869	0.489	0.0307	0.9852
A-4	0.042	0.9029	0.00092	0.9799	2.1583	0.979	0.502	0.0291	0.9799
A-5	0.0526	0.9355	0.00207	0.9975	2.6172	0.9909	0.4922	0.0297	0.9885
A-6	0.045	0.9133	0.00115	0.9895	2.2844	0.9834	0.5041	0.0285	0.9774

Tab. VI. Steady State flux (J_{ss}) and Permeability Coefficient (K_p) data of Various Formulations (A-1 to A-6)

FC	(J_{ss}) Mean ± S.D.	(K_p) Mean ±S.D.
A-1	0.101±0.007	0.431±0.02
A-2	0.101±0.006	0.457±0.004
A-3	0.120±0.002	0.476±0.002
A-4	0.102±0.006	0.472±0.007
A-5	0.210±0.001	0.881±0.003
A-6	0.102±0.004	0.44±0.009

FC-Formulation Code No., J_{ss} -Steady State Flux ($mcg.cm^{-2}.hr^{-1}$), K_p- Permeability Coefficient ($cm.hr^{-1}$) $\times 10^{-3}$.

Scanning Electron Microscopy (SEM)

Photomicrograph (Fig.1) represents the SEM of blank transdermal film, Carvedilol loaded transdermal film before permeation and Carvedilol loaded transdermal film after permeation study, respectively. The scanning electron micrographs of the drug loaded film clearly indicates that Carvedilol is molecularly dissolve in the polymer matrix (Fig. 1). After permeation experiment the film (Fig. 1) shows the presence of pores/channels indicating the drug permeation is diffusion controlled across cellulose membrane and Albino mice epidermis [16].

The possible drug-excipient interaction study was studied by FTIR spectroscopy and DSC of different formulations (A-3 and A-5) [10, 13, 16].

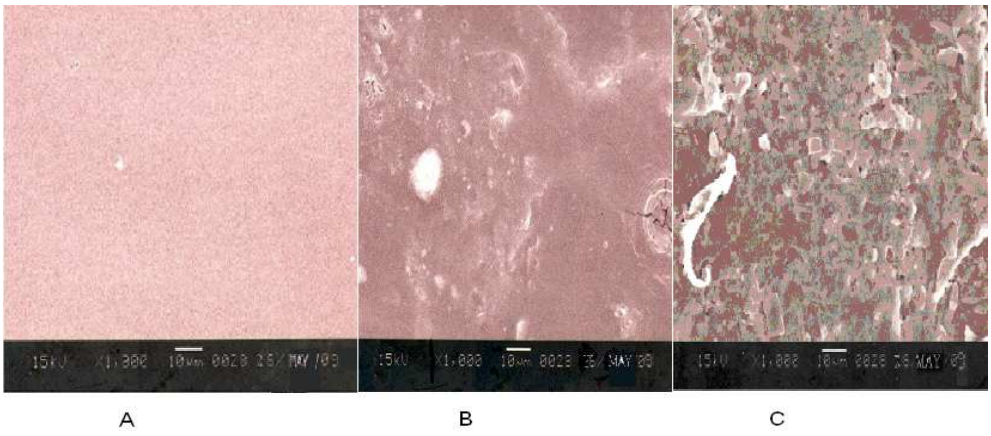

Fig.1: Scanning Electron Micrographs (SEM): A: SEM of Blank Transdermal Film (Without Drug), B: SEM of Carvedilol Loaded Transdermal Film (A-5) before Carrying out the Permeation Study, C: SEM of Carvedilol Loaded Transdermal Film (A-5) after Carrying out the Permeation Study. Drug-Excipient Interaction Studies

Fourier Transform Infrared Spectroscopy

In the IR spectra of formulations of A-3 and A-5 the major peaks responsible for functional groups like –OH, N-H, C-N and C-O-C of Carvedilol slightly altered that may be due to formation of weak hydrogen bonding with polymers and other excipients and that was further confirmed by DSC studies.

Differential Scanning Calorimetry

The DSC analysis (Fig. 2.A) of pure Carvedilol showed a sharp exothermic peak at 120.99°C corresponding to its melting point of 110°C but in case of final formulations (A-3 and A-5) it was changed slightly as shown in Fig.2.B and 2.C, may be due to weak hydrogen bonding with polymers and other excipients. Although in vitro permeation datas we can concluded that it does

not seems to interfere with the drug permeation from the transdermal films and drug was also in a stable form within the films [14].

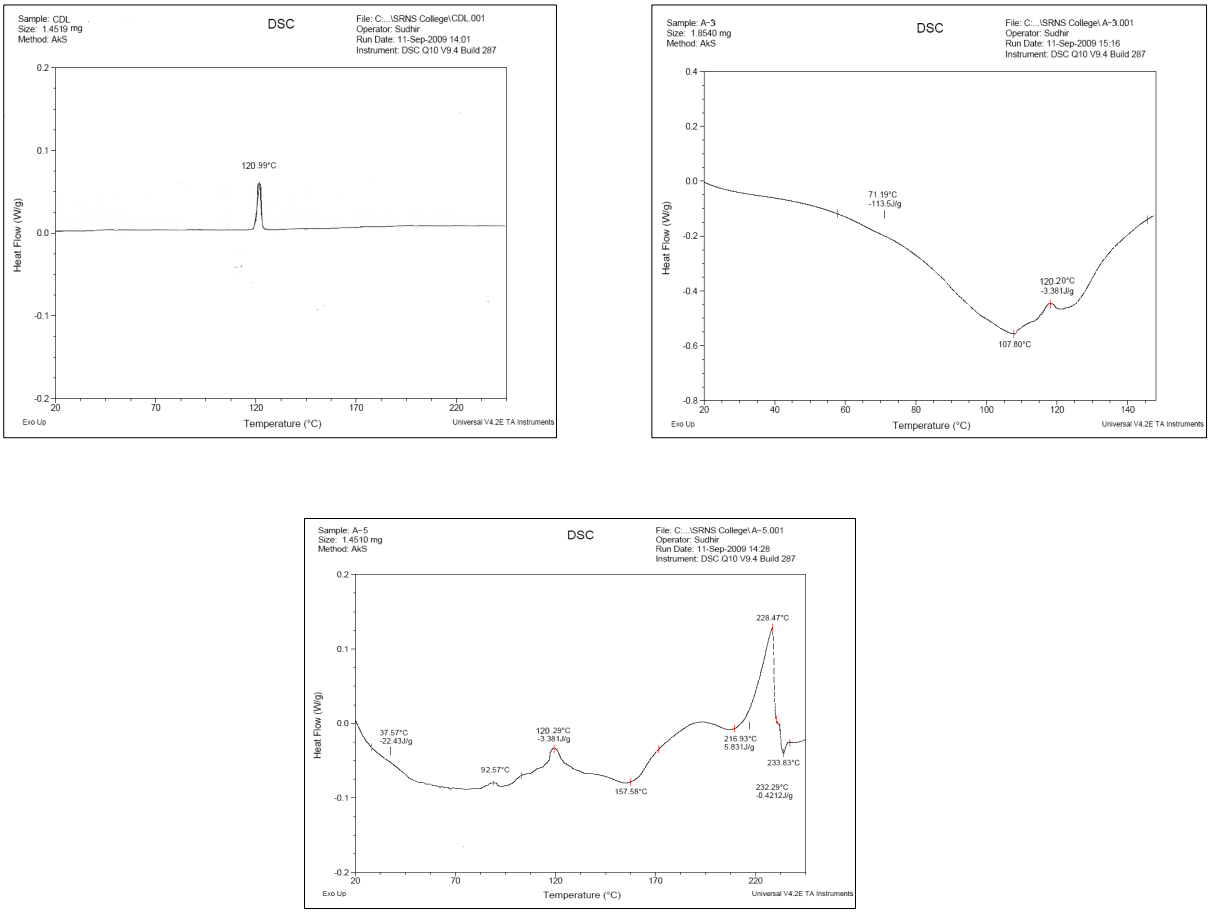

Fig. 2: A: DSC Thermo gram of Carvedilol (CDL), B:.DSC Thermo gram of A-3 Formulation (Drug, EC and HPMC K15 M), C: DSC Thermo gram of A-5 Formulation Composed of CDL, EC and Carbopol-934.

X-Ray Diffraction study

X-ray diffraction study was carried out to reveal the crystalline modifications after the preparation of patches. Results of x-ray diffractograms for A-3 and A-5 formulations were studied and compared in respect of crystalline modifications with physical mixtures of EC-HPMC K15M-CDL (PM-1) and EC-CP 934-CDL (PM-2) respectively which was shown in Fig. 3. According to diffractograms it was concluded that pure CDL which having crystalline form due to more no of peaks which was shown in Fig. 3, but in case of physical mixtures (PM-1 and PM-2) prepared in a same ratio of polymers and drugs , shown 2θ values 32.8854, 41.8848, 43.4429, 48.9334 and 32.9052, 41.8953, 43.4426, 48.9359 receptively but it has not been observed in transdermal films of (A-3 and A-5), may be suppressed due to changes of the polymorphic amorphous form [16-18].

Stability Study of the Best Formulation

An accelerated stability study [18] contains results regarding remaining drug content and physical appearance of best formulation i.e. A-5 which concluded that R.D.C. of A-5 formulation was ranged between 0.227±0.022 to 0.219±0.045 and physical appearance was also good at 4°C and 45 °C up to 30 days except in case of 60 °C. At 60°C the physical appearance was good up to 15 days after that film was found hard, rigid and brittle. From stability data it was calculated

that the shelf life of the A-5 formulation was 328.50 days at 25°C. It is therefore preferable to store the patches in the refrigerator.

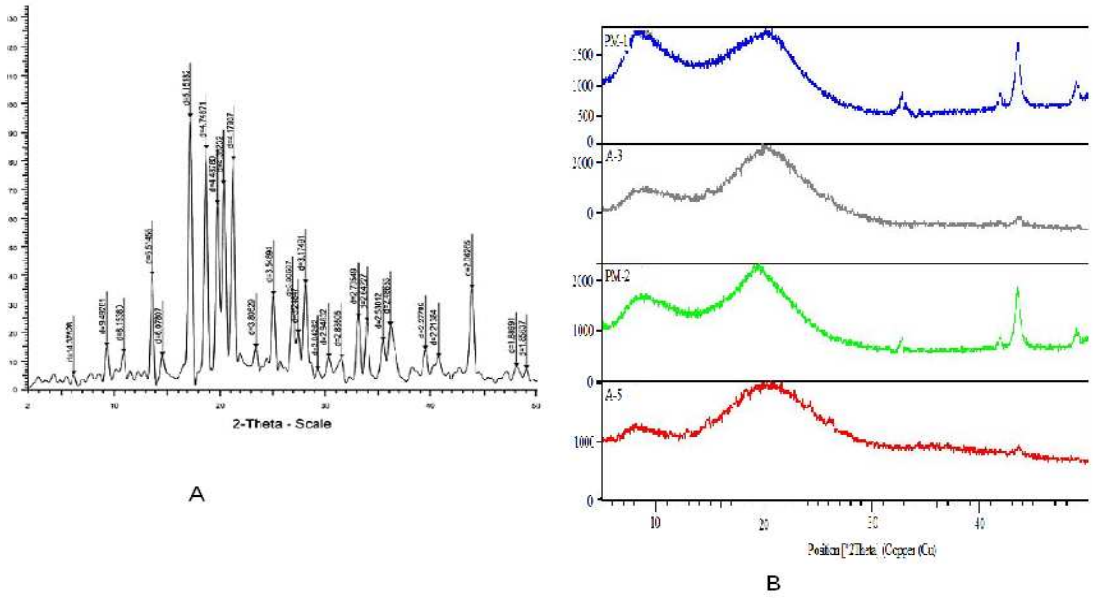

Fig. 3:A. XRD of Carvedilol (CDL), B. XRD of Two Best formulations A-3 (CDL: EC: HPMC K15 M), A-5 (CDL: EC: Carbopol 934) and Corresponding Physical Mixtures PM-1 (CDL: EC: HPMC K15 M), PM-2(CDL: EC: Carbopol- 934).

CONCLUSION

Transdermal drug delivery system is a most suitable system for a long-term treatment or for a multi dose treatment because different transdermal patches are prepared for a long period of time in a suitable dose proving treatment from a day to even up to seven days. Lower molecular weight, good lipid solubility, lower elimination half-life, and lower melting make it an ideal candidate for transdermal formulation. In their work, they have developed a suitable matrix film for furosemide by employing ethyl cellulose (EC) and hydroxypropyl methylcellulose polymer and have studied the effect of EC and HPMC on the physicochemical properties of transdermal film [22]. In this study Carvedilol transdermal films were prepared with combination of different polymers such as EC: PVP K-30, EC: HPMC K-15M, EC: Carbopol-934. Total six formulations were selected (A-1 to A-6) for further evaluation such as physicochemical properties, *in-vitro* drug permeation study and surface pH of the film.

From Physicochemical evaluation it is found that thickness, weight variation, moisture content, drug content, folding endurance and flatness were suitable for maximum stability [18] of the prepared transdermal films. The carvedilol transdermal patch was prepared by utilizing 3^2 full factorial experimental designs by using HPMC K100M and HPMC E5 with mixed system of penetration enhancer containing oleic acid and propylene glycol. The results of in-vitro skin permeation studies performed using freshly excised abdominal skin of rat showed highest permeation rate of carvedilol. The higher permeation responses of the formulations may be attributed to permeation enhancing effect of oleic acid in presence of propylene glycol [23].

In this study i*n-vitro* drug permeation studied, the percent of drug permeated was found to maximum 95.44 and 90.12% from A-3 and A-5 film respectively. Based on physicochemical and *in-vitro* release experiments, A-3 and A5 chosen as the best films among both only A-5 was

found to have maximum release, maximum steady state flux and maximum permeability coefficient. In SEM, after permeation experiment the film shows the presence of pores/channels indicating the permeation is diffusion controlled across cellulose membrane and Albino mice epidermis. In FTIR study, regarding interaction of drug and excipients, it was concluded that Carvedilol slightly altered that may be due to formation of weak hydrogen bonding with polymers and other excipients and that was further confirmed by DSC studies. But in *vitro* permeation datas we can conclude that it does not seems to interfere with the drug permeation from the transdermal films and drug was also in a stable form within the films. In XRD study, according to diffractograms it was concluded that pure CDL which having crystalline form due to more no of peaks which was shown in Fig. 3 but in case of physical mixtures (PM-1 and PM-2) prepared in a same ratio of polymers and drugs , shown 2θ values 32.8854, 41.8848, 43.4429, 48.9334 and 32.9052, 41.8953, 43.4426, 48.9359 receptively but it has not been observed in transdermal films of (A-3 and A-5), may be suppressed due to changes of the polymorphic amorphous form. The stability study concluded that the shelf life of the A-5 formulation was 328.50 days at 25°C. It is therefore preferable to store the film in the refrigerator. In view of the results obtained, Carvedilol films prepared with EC: Carbopol-934 in ratio of 6:4 (w/w) were the best among other polymer combination in similar ratio. This present study is holds promise for the further clinical study and screening of various formulations variables through pilot plant scale up.

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