



FORMULATION DEVELOPMENT AND EVALUATION (INVITRO-IN VIVO STUDY) OF TRANSDERMAL DRUG DELIVERY SYSTEMS WITH ANTIHYPERTENSIVE DRUG.

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ABSTRACT

The present work aim to formulate and characterize transdermal drug delivery systems of carvedilol applying different ratios of polymer HPMCK₁₅M, (PVPK₃₀) Polyvinyl Pyrrolidone and Ethyl Cellulose, & In –Vivo study by monitoring the effect of the TDDS on blood pressure of methyl prednisolone acetate induced hypertensive rats. The blood pressure of rats was measured using a noninvasive rat B.P. instrument based on cuff tail techniques. Formulations F4 & F5 were most effective in the reduction of systolic BP. The developed transdermal patches increase the efficacy of carvedilol for the therapy of hypertension. A significant fall in the rat B.P. was observed in the treatment of hypertensive rats. It was concluded that a single patch application of carvedilol TDDS can effectively control hypertension in rats. Solvent evaporation method was used for the formulation of patches. The patches showed satisfactory folding endurance and tensile strength and it indicated good physical stability. Transdermal permeation of carvedilol through rat abdominal skin was determined by using modified Franz diffusion cells, Based on the drug release and physicochemical values, formulation F4 and F5 were considered as an optimized formulation which shows higher percentage of drug release at 24 hours. The drug-excipients compatibility studies were performed by Infrared spectrophotometer (FTIR). Release kinetics studies revealed that the drug release from formulations F4 and F5 followed Higuchi kinetics. Skin irritation test performed on rats did not show any erythema/edema for transdermal patches.

KEYWORDS: Carvedilol, Transdermal patches, PVPK₃₀, Ethyl cellulose, HPMCK₁₅M, Release kinetics, In-Vivo, Skin irritation test.

INTRODUCTION

Carvedilol is a third generation; nonselective β -blocker that also possesses α_1 adrenergic blocking, calcium antagonist properties, carvedilol blocks both the β_1 and β_2 adrenergic receptors, resulting in improved myocardial function.^[1] The result of this study indicate that carvedilol containing transdermal patch is a promising approach therapy to maximum bioavailability and prolong release of the drug. Hypertension, a cardiovascular diseases account for a large proportion of all deaths and disability worldwide. Global Burden of Disease study reported that there were 5.2 million deaths from cardiovascular diseases in economically developed countries and 9.1 million deaths from the same causes in developing countries. Worldwide prevalence estimates for hypertension may be as much as 1 billion individuals and approximately 7.1 million deaths per year may be attributable to hypertension. Hypertension is directly responsible for 57% of all stroke deaths and 24% of all coronary heart disease deaths in India. Transdermal systems are ideally suited for diseases that demand

chronic treatment. Despite the suitability of TDDS in the treatment of chronic disease like hypertension.^[2]

Carvedilol transdermal patches were prepared using different combination of polymers ethyl cellulose and polyvinyl pyrrolidone, HPMC by solvent evaporation technique. Several physicochemical parameters like moisture content: moisture uptake, film thickness, folding endurance, tensile strength, permeation study, skin irritation study and hypertensive study were evaluated. Penetration depends on ability of drug to penetrate the stratum corneum, enter the systemic circulation and to achieve the therapeutic effect. Drug with log P of ≤ 2 considered as potential candidate for transdermal delivery. There has been increased interest during recent years in the use of chemical enhancer that could modify drug permeation through skin. In the pre-formulation studies λ_{max} , IR, solubility, partition coefficient and the antihypertensive activity of the patches was studied using methyl prednisolone acetate induced hypertensive rat. It was observed that all

optimized formulation was most effective in the reduction of systolic B.P. The hypertension was successfully induced in the normotensive rats by MPA administration as on treating experimental hypertensive rats with carvedilol.^[3]

MATERIALS AND METHODS

Materials

Carvedilol was received as a gift sample from Astra IDL Limited, Bangalore. Polyvinyl Pyrrolidone K₃₀, Polyethylene Glycol 400, obtained from S.D. Fine Chemicals, Mumbai. All other materials and chemicals used were of either pharmaceutical or analytical grade.

Method

Preparation of patches^[4]

The transdermal patches of composition listed in Table were prepared by solvent evaporation technique employing a glass substrate (Bangles wrapped with aluminium foil). Membrane type transdermal patches containing carvedilol prepared by using different ratio of HPMCK₁₅M, PVPK₃₀ and ethyl cellulose. The polymers were dissolved in suitable solvent mixed well by using magnetic stirrer. Carvedilol was added slowly to the polymer solution and mixed thoroughly to obtain uniform solution. PEG-400 and dibutyl phthalate used as a plasticizer. DMSO and tween-80 were used as a penetration enhancer. The polymeric solution was poured into bangles placed in a level, hard rigid surface and dried at room temperature in a dust free environment for 24 hrs. An inverted funnel was placed over the mould to prevent fast evaporation of the solvent. Patches of 2.0 cm diameter were prepared by cutting and packed in an aluminium foil and stored in a dessicator.

Table 1: - Composition of various formulation of carvedilol patches.

Formulation	F4	F5
Drug (%w/w)	13	13
HPMCK ₁₅ M (parts)	300	-
PVPK ₃₀ (parts)	200	400
EC (parts)	-	100
PEG-400(%w/w)*	40	-
Dibutyl phthalate(%w/w)*	-	40
Tween 80(%w/w) *	30	-
DMSO (%w/w)*	-	25
DCM/Methanol(ml)	12	-
Chloroform(ml)	-	5

*Based on polymer weight

Evaluations of Transdermal Patches^[5, 6,7,8,9]

Weight variation

Weight variation was studied by taking individual weight of ten randomly selected patches for each formulation prepared in different batches. The weights were taken in electronic digital balance.

Folding Endurance

A strip of specific area (2 cm*2 cm) was cut evenly and repeatedly folded at the same place till it broke. The

number of times the film was folded at the same place without breaking gave the value of the folding endurance.

Thickness Uniformity

The thickness of each film was measured by using screw gauze. The thickness was measured at three different places on each film and the average thickness of the film was taken as the thickness of the patches.

Percentage Moisture content

The prepared films were weighed individually and to be kept in a desiccators containing fused calcium chloride at room temperature for 24 hrs. After 24hrs the films are to be reweighed and determine the percentage moisture content from the below mentioned formula.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

Percentage Moisture uptake

The weighed films were kept in desiccators at room temperature for 24hrs containing saturated solution of potassium chloride in order to maintain 84% RH. After 24hrs the films are to be reweighed and determine the percentage moisture uptake from the below mentioned formula.

$$\text{Percentage moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Tensile strength

Mechanical properties of the polymeric patches were conveniently determined by measuring their tensile strength. The tensile strength of the patches was determined by using a tensile strength instrument (Fig. 1). Average reading of three patches was taken as the tensile strength. The transdermal patch was fixed to the assembly, the weights required to break the patch was noted and simultaneously elongation was measured with the help of a pointer mounted on the assembly and calculated the tensile strength of the patch using the following formula.

$$\text{Tensile Strength} = \frac{\text{break force}}{a.b} (1 + \frac{\Delta L}{L})$$

Where a, b and L are width, thickness and length of the patch respectively.

ΔL is the elongation of patch at break point.

Break force = Weight required to break the patch (Kg).

Drug content uniformity

The patch (1 cm) was transferred into a graduated glass stopper flask containing 100 ml of phosphate buffer saline 7.4 P^H. The flask was shaken for 4hr in a mechanical shaker. Then the solution was filtered and 1 ml diluted to 10 ml with of phosphate buffer and the absorbance was measured at 243 nm using the placebo patch solution as blank and the drug content was calculated.

In-vitro permeation studies

A Franz diffusion cell was used in this experiment. The Franz diffusion cell was fabricated from borosilicate glass and consisted of two compartments i.e. receptor and donor. The cell had lower jacketed halve through which water maintained at $37 \pm 5^\circ\text{C}$ was circulated and this lower halve had an effective receptor volume of 25ml and skin surface area of 3.14 cm^2 . The two halves of the cell were secured in place with the help of strong metallic clips. The receptor solution was stirred by a Teflon bead of 12 mm length on a magnetic stirrer.

Selection of animal

The experiment was conducted according to the protocol approved by the institutional animal ethics committee (IAEC NO: NIPS/05/29/2016). For in-vitro evaluation of TDDS, albino rat was selected because of its easy availability and suitability. Animal was sacrificed and skin was excised from the abdomen. The rat abdominal skin was immersed in 0.32N ammonium hydroxide solution to facilitate removal of hairs. The dermal side of the skin was thoroughly cleaned of any adhering tissues or blood vessels and washed with distilled water before running the experiment. The skin was trimmed into a circular section of about 2cm diameter.

Procedure for In-vitro permeation studies

The in-vitro permeation study of transdermal patches of carvedilol was carried out by using excised rat abdominal skin and Franz diffusion cell. The skin was sandwiched between donor and receptor compartments of the diffusion cell, so that the epidermis faces the donor compartment. A 2.0cm diameter patch was placed in intimate contact with the stratum corneum side of the skin; the top side was covered with Aluminium foil as a backing membrane. Teflon bead was placed in the receptor compartment filled with 25 ml of phosphate buffer saline pH 7.4. The cell contents were stirred with a magnetic stirrer and a temperature of $37 \pm 5^\circ\text{C}$ was maintained throughout the experiment. Samples of 1ml were withdrawn through the sampling port at different time intervals for a period of 24 hours. The samples were analyzed spectrophotometrically.

Characterization of API and Polymer

IR spectroscopy study was carried out to determine the physical or chemical interaction between drug and polymers.

Drug release kinetics^[10]

In order to investigate the mechanism of release of drug from the films, the release data was analyzed with the following mathematical release models:

Model		Equation
Zero order kinetics	:	$Q = Q_0 - K_0 t$
First order kinetics	:	$Q = Q_0 (1 - e^{-K_1 t})$
Higuchi square root model	:	$Q_t = K_H t^{1/2}$
Hixson-Crowell's cube root model	:	$\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = K_{HC} t$
Korsmeyer-peppas model	:	$\frac{Q_t}{Q_\infty} = K_k t^n$

Skin irritancy study^[11]

The primary skin irritation test (IAEC NO: NIPS/05/29/2016) was performed on healthy rats weighing between 200-250 g. the hairs on the dorsal side of Albino rats was removed by clipping 1 day before this portion of the experiment. The rats were divided into 4 groups (n=2). Group I served as control, group II received transdermal patch F4, group III received a blank transdermal patch and group IV received a 0.8% (v/v) aqueous solution of formalin as a standard irritant. Area on the back of rats was clean shaved 24 hours prior to testing. Optimize patch was applied to the clean area and kept in its place by adhesive tape. After 24 hour, the patch was removed with the help of alcohol swab and observed visually for signs of edema or erythema.

Antihypertensive study^[12,13]

The experimental procedure and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by the ethical approval was obtained from the institutional animal ethics and care committee (IAEC NO: NIPS/05/29/2016) at the department of pharmacology, NRI Institute of pharmaceutical sciences Bhopal.

Procurement, Identification and Housing of Animals

Twenty four male albino rats (8 weeks old) 230-250 g were supplied by Animal House facility in NIPS college and kept under standard laboratory conditions in 12h light/dark cycle at $25 \pm 2^\circ\text{C}$. Animals were provided with pellet diet and water ad libitum. Animals were marked with picric acid solution for easy identification.

Conditioning/Training of Animals

For conducting the BP measurement studies, the animals were required to be kept in a restrainer (rat holder). It had only one side open for entry/exit of the animal with proper ventilation at all other sides. As the rats were unaccustomed to remain in the restrainer in a calm and non-aggressive manner, animals were trained for their stay in the restrainer as a slight movement in and aggression by the animal would have led to variation BP reading. For this, a rat was inserted in the restrainer headlong until the whole body got conveniently accommodated inside. The restrainer was locked by screwing the open side of the apparatus leaving the tail

outside. The exercise was repeated several times until the animals learnt to stay in restrainer nonaggressive and familiarized with the conditions.

Measurement of Initial Systolic BP of Rats

The initial BP of all the rats was recorded using Non-invasive blood pressure apparatus the restrainer carrying the rat was placed in the rat holder with tail protruding out. Systolic blood pressure was measured indirectly in conscious and slightly restrained, pre-warmed rat by the tail cuff method. An average of ten consecutive readings was noted.

Induction of Hypertension in Normotensive

The animals were divided into four groups' six animals each. Group I was taken as control. Hypertension was induced in the remaining four groups (Groups II to IV) by subcutaneous injection of methyl prednisolone acetate (20mg/Kg/week). Two weeks later, rats with a minimum mean BP of 150 mmHg were selected.

MPA induced Hypertensive Rats

After MPA treatment, groups III, IV, were subjected to TDDS (formulations F₄, F₅, respectively). Group II served as toxic control and received no further treatment. The TDDS was applied to the previously shaven abdominal area of rat skin with the entire release surface in intimate contact with the stratum corneum. The patch was applied over the stratum corneum, over the patch an aluminum foil was placed for avoid the backward movement of drug through the adhesive tape. A microporous adhesive tape was then rolled over to keep the patch secured at the site of application. The rat was then placed in the restrainer and the Systolic BP was recorded up to 24 hours.

Stability studies^[14]

Stability studies were carried out at 40°C and 75% RH for 90 days on optimized formulation batches (F₄ & F₅). The parameters studied for stability studies are thickness, drug content, assay, water vapor transmission rate, moisture absorption and in vitro drug release.

RESULT AND DISCUSSION

Table 2: Physico-Chemical Evaluation of Transdermal patches.

Formulation Code	Thickness (mm)	Weight variation (mg)	% Drug Content	Folding endurance	Tensile strength Kg/mm ²
F ₄	0.17±0.008	0.160±0.011	99.65±2.42	60±24.33	3.85±1.80
F ₅	0.35±0.09	0.149±0.017	98.36±2.02	58±22.03	3.92 ±1.84

Formulation Code	% Elongation	% Moisture Content	% Moisture uptake	Swelling index
F ₄	26.25±4.12	3.2±1.82	4.7±0.85	23.41±0.74
F ₅	28.04±4.71	2.7±0.98	5.7±1.45	22.82±1.25

Table 3: Kinetic Release of Dissolution Data.

Sr. No	Zero Order (r ²)	First Order (r ²)	Higuchi (r ²)	Korsmeyer-Peppas (r ²)	(n)	Best Fit Model
F ₄	0.994	0.852	0.995	0.926	0.12	Higuchi matrix
F ₅	0.916	0.882	0.988	0.906	0.28	Higuchi matrix

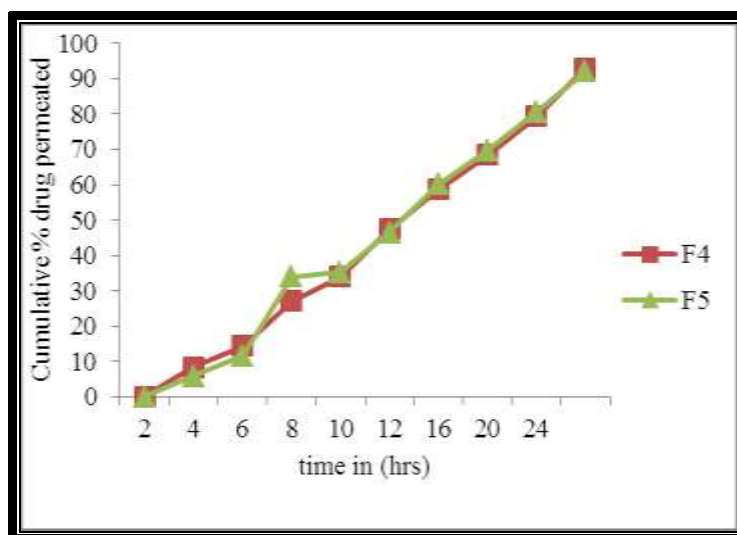


Fig. No. 1: Zero Order Release Kinetic Profile of Carvedilol TDDS.

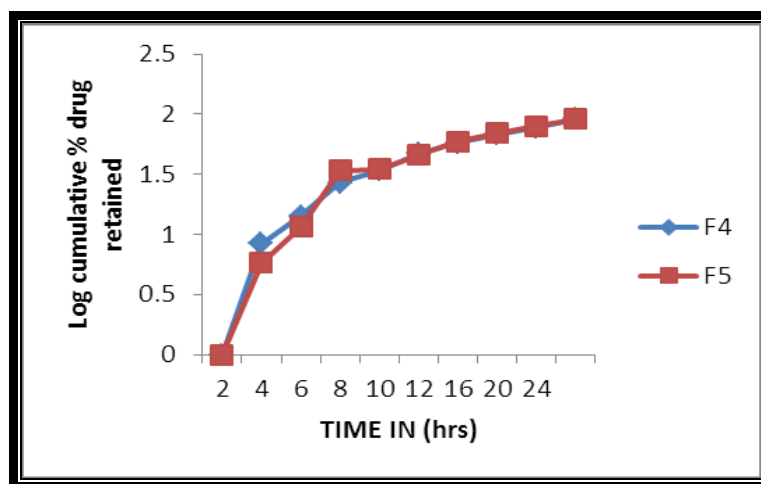


Fig. No. 2: First Order Release Kinetic Profile of Carvedilol TDDS.

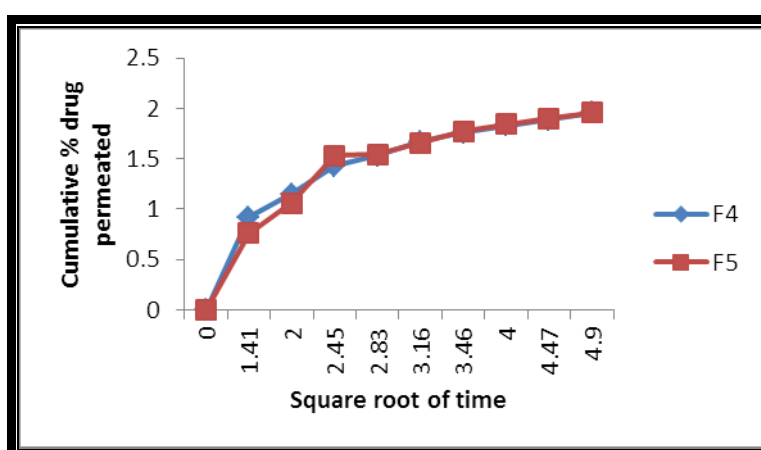


Fig. No. 3: Higuchi Release Kinetic Profile of Carvedilol TDDS.

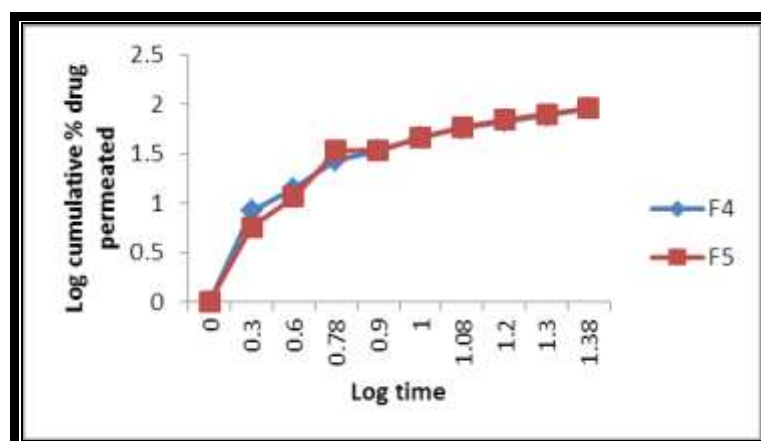


Fig. No. 4: Korsmeyer-peppas Release Kinetic Profile of Carvedilol TDDS.

Skin Irritation Test

Data from the primary skin irritation test for prepared formulations.

Normal	F4:Drug	Blank film	Formalin
-	-	-	+++
-	-	-	+++

Erythema

- Nil
 + Mild
 ++ Severe
 +++ Very severe

Edema

- Nil
 * Mild
 ** Severe
 *** Very severe

IN-VIVO ANTIHYPERTENSIVE STUDY WITH NON INVASIVE BLOOD PRESSURE MEASUREMENT APPARATUS (NIBP).

Effect of Transdermal Drug Delivery Systems (F4, F5,) of Carvedilol on Mean Blood Pressure, in Control and Methyl Prednisolone Acetate Induced Hypertensive Rats.

Table no.-4

Group	Treatment	Initial	2 hrs	6 hrs	12 hrs	16 hrs	24 hrs
1	Control	114.6±1.52	113.3±0.80	115.2±1.5	113.3±1.4	118.4±1.36	117.3±1.24
2	MPA Control	157.8±1.28	156.7±1.57	158.4±1.4	158.6±1.36	155.4±1.37	153.5±1.34
3	F4	156.5±1.24	147.6±1.23	141.2±1.53	131.3±1.35	128.3±1.67	124.2±1.55
4	F5	158.7±0.38	149.4±1.34	144.8±1.56	134.2±1.65	126.2±1.37	121.3±1.48

MEAN B.P. (mm Hg) ± SEM

Reduction in B.P.= $156.5-124.2/156.5 \times 100=20.63$

= $158.7-121.3/158.7 \times 100=23.56$

Control group-received no treatment

Toxic control group- Received methyl prednisolone acetate S.C. – 20mg/kg/week for two week.

Treatment group-Received methyl prednisolone acetate S.C. – 20mg/kg/week for two week. Followed by carvedilol transdermal patch.

Stability Study of batch F4

Sr. no	Evaluation Parameter	At o day	After 90 days
1	Thickness (mm)	0.35±0.09	0.34 ± 0.06
2	Weight variation	0.225±0.017	0.224± 0.011
3	% Drug Content	96.36±2.02	95.68 ± 1.25
4	Folding endurance	58±22.03	57 ± 12.14
5	Tensile Strength Kg/mm ²	2.41±0.86	2.40 ± 1.64
6	% Elongation	28.04±4.71	26.41 ± 4.3
7	% Moisture content	3.23±2.78	2.5 ± 0.94
8	% Moisture uptake	5.7±1.45	4.89 ± 3.03
9	Swelling index	22.82±1.25	21.46 ± 0.97
10	% Drug permeated	92.5	91.38

The selected optimized formulation F4 had a residual drug content of more than 90% after weekly checking up to 3 month when stored at room temperature 0^oc, 25^oc at 60% RH & 40^oc at 75% RH. These results indicate that the selected formulation exhibited good stability during storage & they were stable.

CONCLUSION

Transdermal drug delivery systems are ideally suited for drugs that undergo hepatic first pass metabolism. Hypertension being a very common disorders leading to preventable death & been treated with different classes of drugs in form of several dosages. On the basis of good mechanical properties like tensile strength, thickness elongation at break, better compatibility and stability. The matrix type transdermal drug delivery system of Carvedilol was successfully designed & developed by trial and error method. The transdermal patches were prepared using combination of HPMCK₁₅M, PVPK30 and ethyl cellulose in various ratios. From the present study, the following conclusions were observed.

- The formulated transdermal patches of carvedilol showed good thickness, drug content uniformity, folding endurance and tensile strength.

- The used polymers (HPMCK₁₅M, PVPK30 and ethyl cellulose in various ratios) can be used to develop transdermal patches in different proportion.
- Form the formulated patches, formulation F4 showed highest permeation through rat skin.
- The permeation of carvedilol form formulated transdermal patch was found to follow diffusion mechanism.
- From the result it was conclude that the highest release i.e.F4 and F5 gave 93.00%, 92.05% with suitable polymer ratio and penetration enhancer.
- As the concentration of PVP and ethyl cellulose increase, moisture content of patches was also increase. The folding endurance was measured manually all the formulation shows good folding endurance.
- The ex-vivo permeation study in rat's skin indicated that the flux and permeability co-efficient of optimized F-1 patch was 47.5±0.054 µg/cm²/h and 0.0073 µg/cm²/h, respectively,
- The description of dissolution profiles by a model function has been attempted using different kinetics (zero-order, first-order, Pappas, matrix, Higuchi kinetics and Hixson–Crowell model). Higuchi

kinetics model seemed to be the most appropriate model describing release kinetics from all patches.

- Skin irritation test on rat showed no sign of skin reaction and erythema.
- Stability study performed on optimized formulation. No significant changes were found in the parameters during study period, thus it could be concluded that formulation was stable.
- *In-vivo* study the groups F4, F5 were showed significant falls in B.P (SBP). By using cuff tail method.
- Administration of carvedilol patch resulted in gradual fall in BP decrease in BP started from 1 hr effect continue for 24 hr this clearly indicate that the transdermal patch released drug gradually over a period of time.

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REFERENCES

1. Wikipedia-the free encyclopedia, Carvedilol.
2. R. Panner Selvam, Anoop Kumar Singh, T. Sivakumar., Transdermal drug delivery systems for antihypertensive drugs - A review, *Int J Pharm Biomed Res*, 2010; 1(1): 1-8.
3. Biana Godin, Elka Touitou., Transdermal skin delivery: Predictions for humans from in vivo, ex vivo and animal models *Advanced Drug Delivery Reviews*, 59 (2007).
4. Dey B.K, Nath L.K., Mohanti B, Bhowmik B.B, Development and Evaluation of Propranolol Hydrochloride Transdermal Patches by using Hydrophilic and Hydrophobic Polymer. *Indian J Pharm. Educ. Res.*, 2007; 41(4): 388-392.
5. Jain, P. S., Talele G.S., Talele S. G., Surana S. J., Spectrophotometric determination of carvedilol from bulk and formulations, *Indian Journal of Pharmaceutical Sciences*, May 2005; 358-359.
6. Patel D.M., Kavitha K., Formulation & Evaluation aspects of transdermal drug delivery system, 2011; 6(2): 83-90.
7. Rajesh N., Siddaramaiah, Gowda D.V., Somashekar C.N., Formulation and evaluation of biopolymer based transdermal drug delivery, *Int J Pharmacy Pharm Sci*, 2010; 2(2): 142-146.
8. Animesh Gairola, Urmi Chaurasia, Anupama Singh and Vikas Anand Saharan., Development and evaluation of transdermal patches of aceclofenac, *The Thai Journal of Pharmaceutical Sciences*, 2013; 90-97.
9. Gupta JRD, Tripathi P, Irchhiaya R, Garud N, Dubey P, Patel JR, Formulation and Evaluation of matrix type transdermal drug patches of Glibenclamide, *Int. J. Pharm. Sci. Drug. Res.*, 2009; 1: 46-50.
10. Costa P. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.*, 2001; 13: 123-133.
11. Praveen M, Someswara Rao B, Kulkarni S.V., Basavaraj C.S., Formulation and Evaluation of Tizanidine Hydrochloride Transdermal Patches, *Int. J. Drug. Pharma. Res.*, 2011; 2(2): 298-31.
12. Vilegave K., Dantul B., Chandankar P., Kharjul A, Kharjul M., Analytical methods, Preformulation study and physicochemical evaluation techniques for transdermal patches of antihypertensive drug, *Int. J. For Pharma. Res. Scholar*, 2003; 2(1): 71-82.
13. Mohd. Aqil, Asgar Ali, Yasmin Sultana, Kiran Dubey, Abul K. Najmi and Kollapa K. Pillai, *In Vivo Characterization of Monolithic Matrix Type Transdermal Drug Delivery Systems of Pinacidil Monohydrate: A Technical Note AAPS Pharm Sci Tech*, 2006; 7(1): Article 6.
14. Jadhav Jaydatt, Sreenivas S.A. Formulation & *In-vitro* Evaluation of Drug Reservoir Transdermal Patches of Piroxicam Using Polymers HPMC E15, PVP K30 & Eudragit L-100. *Int. J. Pharm*, 2013; 3(5): 67-80.