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Research Article

FORMULATION, CHARACTERIZATION AND EVALUATION (*INVIVO- INVITRO STUDY*) OF MATRIX TYPE TRANSDERMAL PATCHES OF CARVEDILOL

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ABSTRACT

The present study was carried out to formulate, characterize and evaluate a matrix-type transdermal formulation containing Carvedilol with different ratios of hydrophilic (Eudragit RL100,HPMC) and hydrophobic polymeric (Eudragit RS100,Ethyl Cellulose) combinations plasticizer by the solvent evaporation technique.

The interference of the polymers were ruled out by IR spectroscopy, DSC and XRD and accelerated stability studies as per ICH guidelines. *In-vitro* release study was performed using Keshary-Chein diffusion cell with Himedia dialysis membrane and porcine ear skin as barriers.

The prepared patches were tested for their physicochemical characteristics like thickness, weight and drug content uniformity, water vapour transmission, folding endurance, and tensile strength. *In vitro* release studies of Carvedilol-loaded patches in 30% v/v Methanolic Isotonic Phosphate Buffer (MIPB) of pH 7.4 exhibited drug release in the range of 63.00 to 94.56 % in 24 h. Based on the physicochemical and *in-vitro* skin permeation studies, patches coded as RSL2 (Eudragit RS100: Eudragit RL100, 2:8) and RHE 3 (HPMC: Ethyl Cellulose, 7:3) were chosen for further *in-vivo* studies.

The antihypertensive activity of the patches was studied using methyl prednisolone acetate induced hypertensive rats. It was observed that In Eudragit combinations the RSL 1 formulation and In case of HPMC: EC combinations the RHE 3 formulation was most effective in the reduction of systolic BP. The developed transdermal patches increase the efficacy of Carvedilol for the therapy of hypertension.

Keywords: Carvedilol; Eudragit RS 100, Eudragit RL 100, HPMC, EC; *in vitro* permeation.

1.Introduction

Transdermal drug delivery systems are devices containing drug of defined surface area that delivers a pre-determined amount of drug to the surface of intact skin at a pre-predefined rate.[1] The skin as a route for systemic drug administration has become very

attractive since the introduction of transdermal therapeutic

systems in the form of patches. A transdermal patch is a medicated adhesive patch that is placed on the skin to deliver a time-released dose of medication systemically for treating illnesses. Since early 1980s, this dosage form of transdermal therapeutic system has been available in the pharmaceutical market. The discovery of transdermal drug delivery systems (TDDS) is a breakthrough in the field of controlled drug delivery systems. Preparation of TDDS consists of three basic designs: membrane control or reservoir patches (RPs), matrix or monolithic patches (MPs), and Drug in adhesive patches (DIAPs).[2] Several factors should be considered before choosing an appropriate design for a particular compound: drug solubility, stability and release rate. As a rule of thumb, if a drug permeates or crosses the skin faster than desired, RPs can slow down or control the permeation. Alternatively, if a drug passes through skin at a slower rate than the patch releases it, MPs probably containing a suitable
chemical penetration enhancer may suffice.[2]

1.1 The Skin site for transdermal drug administration:[10]

The skin is one of the most extensive and readily accessible organs of the human body. The skin of an average adult body covers a surface area of approximately $2m^2$ (or 3000 $inch^2$) and receives about one third of the blood circulating through the body.

1.2 Drug transport through human skin:

Human skin is an effective, selective barrier to chemical permeation. Most small water-soluble non-electrolytes diffuse into the systemic circulation a thousand times more rapidly when the horny layer is absent.

Among the various skin layers, stratum corneum (SC) is the rate-limiting barrier to percutaneous drug transport due to its desquamating 'horny' properties comprising about 15–20 rows of flat partially desiccated dead keratinized epidermal cells. Due to the lipid - rich nature of the SC layer (40% lipids, 40% protein and only 20% water) and its low water content transport of hydrophilic or charged molecules across SC is low while transport of lipophilic drugs due to their lipid miscibility with intercellular lipids around the cells in the SC layer.

1.3 Factors affecting transdermal permeability[3]

Physico-chemical properties of the penetrant molecules

a. Partition coefficient: Drugs having both lipid and water solubilities are favorably absorbed through skin. Transdermal permeability

coefficient shows a linear dependency on partition coefficient. A lipid /water partition coefficient of one or greater is generally required.

b. pH conditions

The pH value of high or low can be destructive to the skin. With moderate pH values, the flux of ionisable drugs can be affected by changes in pH that alter the ratio of charged to uncharged species and their transdermal permeability.

c. Penetrant concentration

Increasing concentration of dissolved drug causes a proportional increase in flux. At higher concentration excess solid drug function as reservoir and help to maintain a constant drug concentration for a prolonged period of time.

II. Physico-chemical properties of drug delivery systems

a. Release Characteristic
Solubility of the drug in the vehicle determines the release rate.

b. Enhancement of transdermal permeation

Majority of drugs will not penetrate the skin at rates sufficiently high for therapeutic efficacy. The permeation can be improved by the addition of permeation enhancer into the system.

III. Physiological and pathological condition of skin

a. Reservoir effect of horny layer
The horny layer especially is deeper layer can sometimes act as a depot & modify the transdermal permeation of drugs. This effect is due to irreversible binding of a part of the applied drug with the skin.

b. Lipid film
The lipid film on the skin surface acts as a protective layer to prevent the removal of moisture from the skin and helps in maintaining the barrier function of stratum corneum.

c. Skin hydration
Hydration of stratum corneum can enhance permeability. hydration appears to open up the dense closely packed cells of the skin and increases its porosity.

d. Skin temperature
Raising the skin temperature results in an increase in the rate of skin permeation;

this may be due to availability of thermal energy required for diffusivity.

e. **Regional variation**
Differences in nature and thickness of the barrier layer of skin causes variation in permeability.

f. **Pathological injuries to the skin**
Injuries that disrupt the continuity of the stratum corneum increases permeability due to increased vasodilatation caused by removal of the barrier layer.

g. Cutaneous self metabolism
Catabolic enzymes present in the epidermis may render the drug inactive by metabolism and the topical bioavailability of the drug is greatly reduced.

1.4 Transdermal drug delivery system designs
Transdermal drug delivery can be achieved via active or passive systems depending on whether external energy is used to assist the transport of the drug through the skin. The active systems use heat, electric current (iontophoresis), sound waves (sonophoresis), or transient high-voltage electrical pulses (electroporation) to enhance the delivery of drugs into the systemic circulation.
In passive transdermal drug delivery systems, the drug diffuses through the skin into the systemic circulation by passive means. The concentration gradient of the drug across the skin and the difference in solubility between the adhesive and skin are the driving force for delivery to the surface of the skin. In general, chemical permeation enhancers (pharmaceutical excipients) are required for passive delivery to achieve the required delivery of the drug from a patch of a reasonable size (that is, a surface area of ≤ 40 cm^2).There are four major designs of the conventional passive transdermal drug delivery patches.

2. Materials and methods
Carvedilol was procured as gift sample from Sun Pharmaceutical industries LT.D Bharuch , Gujrat.Euragit RS 100 & Eudragit RL 100 was procured as a gift sample from Evonik Degussa India Pvt Ltd, Mumbai. HPMC (6cps) was procured as a gift sample from Arihant Trading Co. Mumbai. Ethyl cellulose was procured a gift sample from Deepak Cellulose Pvt Ltd, Mumbai.
Triethyl cetrate , dibutyl pthslste , acetone , methanol, mercury were of analytical grade.

2.1 Determination of λ_{max} and preparation of standard calibration curve for Carvedilol[6]
Carvedilol exhibits absorption maxima at 242 nm in 30% v/v Methanolic isotonic phosphate buffer (MIPB) pH 7.4.

2.1.1Preparation of standard solution
100 mg of Carvedilol was accurately weighed into a 100 ml volumetric flask and dissolved in small volume of MIPB pH 7.4 with sonication. The volume was made up to 100 ml with MIPB to get a concentration of 1000 µg/ml (SS-I). From the above solution 10 ml was pipetted in a 100 ml volumetric flask and the volume was made up with MIPB to get a concentration of 100 µg/ml (SS-II). From this, working standard solutions were prepared.

2.1.2 Preparation of working standard solutions
From (SS-II) aliquots of 0.2, 0.4, 0.6, 0.8., 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml were pipetted out into a series of 10 ml volumetric flasks and the volume was made with MIPB to get a concentration ranging from 2-20 µg/ml.
The absorbance of the resulting solutions was then measured at 242 nm using UV spectrophotometer against respective parent solvent as a blank. The standard curve was obtained by plotting absorbance v/s concentration in µg/ml. The standard calibration curve is shown in Graph 1 and the values are tabulated in Table 1.
Beer's range: 2 to 20 µg/ml.

2.2. Pre-formulation studies
2.2.1 Partition coefficient
The oil-water partition coefficient is a measure of lipophilicity of a molecule, which can be used to predict its capability to cross biological membrane. One of the most common ways of measuring partition coefficient is shake flask method.

Procedure
The Carvedilol was added little at once into 5 ml of *n*-octanol until saturated solution was obtained. This solution was filtered to get a clear solution. Three ml of the saturated solution was mixed with 2 ml of fresh *n*-octanol. In total 5 ml of *n*-octanol containing Carvedilol was mixed with 15 ml of water. Then two phases were allowed to equilibrate at 37 ^{o}C for 24 h, on cryostatic constant temperature shaker bath (Research and Test Equipments, Bangalore, India). The concentration of the drug in the aqueous phase and organic phase was determined by UV spectroscopic method after necessary dilution. The apparent partition coefficient *(Kp)* was calculated as the ratio of drug

concentration in each phase by the following equation.

$$K_P = \frac{C_{org}}{C_{aq}}$$

where, C_{org} is concentration of drug in organic phase and C_{aq} is the concentration of drug in aqueous phase.

2.2.2 Melting point determination

Melting point of drug was determined by taking a small amount of drug in a capillary tube closed at one end and was placed in melting point apparatus and temperature at which the drug melts was noted.

2.2.3 Permeability studies through porcine ear skin[6,8,9,17.]

a) Preparation of the skin barrier

From a local abattoir, ears were obtained from freshly slaughtered pigs. The ears were cleaned with water to remove blood stains. The fresh full thickness (0.95 mm) porcine ear skin was used for the study. The epidermis was prepared by soaking the ear in water at 60^0C for 1 min. The intact epidermis from the dorsal side was subsequently teased off from dermis with forceps, rapidly rinsed with isopropyl alcohol to remove the fat adhering to the dermal side, washed with water & used immediately.

b) Determination of drug permeability through porcine ear skin

The permeability study of the drug was carried out across the porcine ear skin using a Keshary-Chien diffusion cell. A 5 mg/ml drug suspension was prepared in phosphate buffer pH 7.4 and sonicated to ensure uniform drug distribution. One ml of the above suspension was taken in the donor compartment. The barrier was mounted between the donor & the receptor compartments in a way that, the dermal side of the skin was facing receptor compartment. The receptor cell contained MIPB of pH 7.4 as the elution medium. The medium was magnetically stirred for uniform drug distribution and was maintained at 37 ± 1^0C. The samples were withdrawn every hour upto 8 hours and estimated spectrophotometrically (UV) at 242 nm after suitable dilutions to determine the amount of drug diffused.

The flux (µg/cm^2/hr) of Carvedilol was calculated from the slope of the plot of cumulative amount of drug permitted per square centimeter of skin at steady state against the time using linear regression analysis.

The steady state permeability coefficient (Kp) of the drug diffused through the porcine skin was calculated using the equation:

$$Kp = \frac{J}{C} \text{---------- (15)}$$

Where, J = Steady state flux

C = Concentration of Carvedilol in donor compartment.

The flux data are tabulated in table 2 and graph 2.

2.2.4 Optimazation of transdermal paches with different plasticizers & in different concentration[7,8,10.]

Drug free patches of Eudragit RL : RS 100 and HPMC 6cps : Ethyl cellulose were prepared by solvent casting on mercury surface (mercury substrate method) along with two different plasticizers i.e. dibutyl phthalate (DBP) and triethyl citrate (TEC).

Polymer solutions were prepared by dissolving in respective solvents by sonication with the aid of a sonicator for 12 mins. For Eudragit and HPMC : Ethyl cellulose patches acetone and a mixture of methylene chloride & methanol were used as solvents respectively. Plasticizers like DBP and TEC at different concentrations based on dry weight of polymer were used to optimize the patches (Table 3 to 5). The prepared patches were then evaluated for physical appearance and folding endurance.

2.2.5 Polymer-Skin compatibility: [4,11,12.]

Compatibility of polymers with skin was determined by performing skin irritation test. The skin irritation test was performed on two healthy albino rabbits weighing between 2.0 to 3.5 kg. Aqueous solution of formalin 0.8% was used as standard irritant. Drug free polymeric patches of 4.5 cm^2 were used as test patches. 0.8% of formalin is applied on the left dorsal surface of each rabbit, where as the test patches were placed on identical site, on the right dorsal surface of the rabbit. The patches were removed after a period of 24 hrs with the help of alcohol swab. The skin was examined for erythema/oedema. The data are tabulated in table 6.

2.2.6 Compatibility studies of drug and polymers

a) FTIR studies[7,12,13,15.,]

The application of infrared spectroscopy lies more in the qualitative identification of

substances either in pure form or in the mixtures and as a tool in establishment of the structure. Since I.R. is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds. In order to establish this point, comparisons were made between the spectrum of the substance and the pure compound. The infrared data is helpful to confirm the identity of the drug and to detect the interaction of the drug with the polymers. Infrared spectra of drug & polymers, alone and in physical mixtures were taken. Then it was investigated for any possible interaction between polymer and drug. I.R spectral data are shown in spectra 2 to 4 & their results are tabulated in table 7.

b) DSC studies

The physicochemical compatibility between drug and polymers to be used in the formulation of transdermal patches was also studied by using differential scanning calorimetry (DSC). The thermograms obtained for drug, polymers and their physical mixtures were compared to ascertain any interactions. The DSC thermograms are shown in spectra 5 to 7 & their results are tabulated in table 8.

2.3 Formulation design

Preparation of Transdermal patches

Transdermal patches containing Carvedilol were prepared by mercury substrate method using varying ratios of different grades of polymers and plasticizers in different concentrations as shown in the table 9 and 10.

a) Procedure for preparation of Eudragit patches[4,7,10.]

The polymers Eudragit RL and RS 100 (total weight = 1000 mg) were weighed in requisite ratios and dissolved in 10 ml of acetone to form a 10% w/v solution. Plasticizers like DBP and TEC were added to the above solution, 25 mg of Carvedilol is then added under mild agitation until drug dissolves. The solution was poured on the mercury placed in a glass Petri dish of 63 cm^2 area and dried at room temperature for 24 hours. The organic solvent evaporates to leave stable Eudragit RL/RS patches (Table 9).

b) Procedure for preparation of HPMC : Ethyl cellulose (EC) patches[8]

Films composed of different ratios of HPMC (6 cps) and EC (total polymer weight = 600 mg) were prepared by mercury substrate method. HPMC and EC were weighed and dissolved in 10 ml of an equal volume of methylene chloride and methanol (5:5 ratio) to form a 6 % w/v solution, which is then plasticized with either TEC or DBP. 25 mg of Carvedilol is added to the above polymer solution under mild agitation until the drug dissolves. The resultant solution was poured on the mercury placed in a glass Petri dish of 63 cm^2 area, dried at room temperature for 24 hours and subsequently oven-dried at 45^0C for 30 min to remove the residual organic solvents

2.4 Evaluation of transdermal formulation:

2.4.I. Physicochemical evaluation:

1. Physical appearance

All the transdermal systems were visually inspected for colour, clarity, flexibility and smoothness.

2. Folding Endurance[4,14]

Folding endurance of the film was determined manually by folding a small strip of the film (4×3 cms) at the same place till it breaks. The maximum number of folding operation done at the same place of the film without breaking, gives the value of folding endurance, where the cracking point of the films were considered as the end point.

3. Thickness of the films[5,8,14]

The thickness of the patches was measured at three different places by using a Digital Screw Gauge micrometer (Mitutoyo, Japan) and mean thickness was calculated.

4. Weight uniformity[4,5]

The dried patches were weighed on electronic balance (Sartorius UK). The average of 3 observations was calculated.

5. Drug content[4,5]

Transdermal systems of specified area (5.088 cm^2) was cut into small pieces and taken into 50 ml volumetric flask, 25ml of MIPB pH 7.4 was added and gently heated to 45^0 C for 15 min and kept for 24 hrs with occasional shaking. Then the volume was made up to 50ml again with MIPB pH 7.4 and further dilutions were made from this solution. Similarly, a blank was carried out using a drug free patch. The solutions were filtered and absorbances were read at 242 nm by UV spectrophotometer. The values for different physicochemical parameters are tabulated in Table 11.

6. Percentage moisture uptake[4,14,15,]

The weighed films were kept in a dessicator at room temperature for 24 hours. They were then taken out and exposed to 84% relative humidity using a saturated solution of

potassium chloride in a dessicator until a constant weight was achieved. Then the films were weighed and percentage moisture uptake was calculated by using the following formula:

Percentage moisture uptake = [Final wt.−Initial wt./Initial wt.]×100 --------

The values are tabulated in table 12 and 13.

7. Percentage moisture content[4,14,15,16]

The prepared films were weighed individually and kept in a desiccator containing fused calcium chloride at room temperature for 24 hours. The films were weighed repeatedly until they showed a constant weight. The percentage moisture content was calculated using the following formula:

Percentage moisture content = [Initial wt.−Final wt./Final wt.]×100 -------- The values are tabulated in table 14 and 15.

8. Tensile Strength & Percentage Elongation[4,5] Tensile strength of the film was determined with Universal Strength Testing Machine (Hounsfield, Slinfold, Horsham, U.K.). The sensitivity of the machine was 1 gram. It consisted of two load cell grips. The lower one was fixed and upper one was movable. The test film of size (4 × 1 cm^2) was fixed between these cell grips and force was gradually applied till the film broke. The tensile strength of the film was taken directly from the dial reading in kg. The values are shown in table 16 & 17. Tensile strength is expressed as follows;

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

9. Water vapour transmission studies (WVT or MVT) [5,11]

MVT is defined as the quantity of moisture transmitted through unit area of film in unit time.

For this study glass vials of equal diameter were used as transmission cells. These cells were washed and dried in an oven. About 1gm of fused calcium chloride was taken in the cells and the polymeric patches (1.30 cm^2 area) were fixed over the brim with the aid of an adhesive. Then the cells were accurately weighed and kept in a closed desiccator containing saturated solution of potassium chloride (200 ml). The humidity inside the desiccator was measured by a digital Hygro thermometer and found to be 84% RH. The cells were taken out and weighed after 1st, 2nd, 3rd, 4th, 5th, 6th & 7th days of storage and weighed accuratly. The amount of water vapour transmitted and rate of WVT were calculated and plotted. The values are shown in table 18 & 19 and graph 3 & 4.

The rate of water vapour transmission (WVT) was calculated using the formula:

$$\text{WVT Rate} = \frac{WL}{S}$$

Where, W = gm of water transmitted

L = Thickness of the film in cm

S = Exposed surface area of the film in cm^2.

10. Skin irritation test[15,39,60]

The skin irritation test was performed on two healthy albino rabbits weighing between 2.0 to 3.5 kg. Aqueous solution of formalin 0.8% was used as standard irritant. Polymeric patches containing drug of 5.088 cm^2 were used as test patches. 0.8% formalin is applied on the left dorsal surface of each rabbit, where as the test patches were placed on identical site, on the right dorsal surface of the rabbit. The patches were removed after a period of 24hours with the help of alcohol swab. The skin was examined for erythema/oedema.

11. Scanning electron microscopy (SEM)[7,14,16]

The surface morphologies of the transdermal patches were analyzed by using a JEOL, JSM-6360A, Japan scanning electron microscope. The samples placed on the stubs were coated finely with gold palladium alloy and examined under the microscope.

12. XRD studies[20]

Samples of Carvedilol its pure crystalline state and the transdermal patches were assessed for crystallinity using Philips analytica X- Ray diffractometer (Model:PW 3710). The voltage and current was 25 kv and 25 mA, respectively. Measurements were carried out in the angular scan range from 10° to 70° (2θ). The XRD spectral data are shown in spectra 10 to 12.

13. Accelerated stability studies of the optimized formulation

Stability of a pharmaceutical preparation can be defined as “the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical,

microbiological, therapeutic and toxicological specifications throughout its shelf life". Optimized formulation of Eudragit RL : RS 100 with TEC as plasticizer (RSL 2) and HPMC : EC with TEC as plasticizer (RHE accelerated stability studies as per ICH guidelines. These two batches were subjected for $40^0C \pm 2^0C$ / 75% RH ± 5% RH for a period of 3 months. These patches were analyzed for physical appearance, folding endurance, weight variation, content uniformity and finally the patches were studied for interaction studies. Results of stability studies are represented in table 21 and spectra 8 and 9.

2.4.II. Adhesive test

1. Thumb tack test[2] One week after the preparation of transdermal patches, the thumb was pressed lightly on a patch for about 5 seconds and then quickly withdrawn. By varying the pressure and time of contact and considering the difficulty of pulling the thumb from the patch, it was possible to set a scoring as to how easily, quickly and strongly the polymer can form a bond with the skin. The entire test was simultaneously performed in blind way on all samples .

Studies are designed to increase the rate of chemical degradation or physical change of an active drug substance or drug product by using exaggerated storage conditions as a part of the formal, definitive, storage program.

ICH specifies the length of study and storage conditions:

- Long term testing: $25^0C \pm 2^0C$ / 60% RH ± 5% RH for 12 months.
- Accelerated testing: $40^0C \pm 2^0C$ / 75% RH ± 5% RH for 6 months.

2.4. III. *In-vitro* membrane/skin permeation study

In-vitro permeation studies were carried out for all the formulations using dialysis membrane as barrier. The optimized patches (patches which showed highest release in 8 hours) were further subjected for *in-vitro* release through porcine ear skin.

1. Keshary-Chien diffusion cell using dialysis membrane[17]

The dialysis membrane soaked in phosphate buffer pH 7.4 for overnight was fixed carefully to the receptor compartment of the diffusion cell so that it just touches the receptor fluid surface. The transdermal system of 5.088 cm^2 area was placed above the dialysis membrane fixed to the donor compartment. The receptor compartment was filled with 48 ml of MIPB of pH 7.4 as diffusion medium. The receptor medium was magnetically stirred using a magnetic bead for uniform drug distribution and was maintained at 37 ± 1^0C. The samples (3 ml) were withdrawn every hour upto 8 hours and estimated spectrophotometrically (UV) at 238 nm to determine the amount of drug released. The volumes withdrawn at each interval were replaced with an equal volume of fresh, pre warmed buffer solution.

The cumulative amount of drug permeated was plotted against time and steady state flux as well as Kp value was determined. The release pattern is shown in graph 5, 6 and 7.

2.4. IV. *In-Vivo* permeation study[4]

Procurement, Identification and Housing of Animals

Thirty six male albino rats (8 weeks old) 230-250 g were supplied by Animal House facility in our college and kept under standard laboratory conditions in 12h light/dark cycle at 25 ± 2 °C. Animals were provided with pellet diet (Lipton, India) and water *ad libitum.* Animals were marked with picric acid solution for easy identification.

All the experimental procedures were carried out accordance with committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. All the experimental procedures were approved by the institutional animal ethical committee (IAEC).

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 non-aggressively 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 (Biopac Systems, Inc Santa Barbara, USA). 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 Rats

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

Post TDDS Treatment BP Assessment of MPA induced Hypertensive Rats

After MPA treatment, groups III, IV, V and VI were subjected to TDDS (formulations RSL-2, RSL-8, RHE-3 and RHE-7, 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 (Johnson and Johnson) 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 upto 12 hours. Results of Systolic BP are represented in table 20.

3. RESULT AND DISCUSSION

3.A) Pre-Formulation studies

Preliminary identification test were either carried out or obtained from literature survey for pure drug on the following parameters:

1. Solubility

The solubility of the drug was determined in distilled water it was insoluble. Further the drug was found to be freely soluble in DMSO, and soluble in methanol, methyl chloride.

2. Partition coefficient

Octanol-water partition coefficient is thought be a good indicator. Partition co-efficient was determined and found to be 0.59 indicating the permeability of Carvedilol may be moderate.

3. Melting point

There is a linear correlation between log flux and reciprocal of melting point, indicating that the lower the melting point, the better the penetration. The melting point of Carvedilol is in the range of 1140C to 1170C.

4. Determination of drug permeability through porcine ear skin

In-vitro permeation of drug through porcine ear skin indicates that Carvedilol has got good skin permeation property. Out of 5 mg/ml drug suspension present in the donor compartment, 4.29 mgs (4290.83µgm) of drug was permeated at the end of 24 hrs. Diffusion rate constant [flux] and permeability coefficient (Kp) were found to be 9.088 µg/cm2/hr and 0.00721 cm/hr respectively (table 2 and graph 2).

5. Compatibility studies of drug and polymers

a)FTIR studies

Physical mixtures of drug and polymers were characterized by Fourier transform infrared spectroscopy. Spectral analysis for physical as well as chemical alteration of IR results showed that there was no interference in the functional groups of the drug. Major peaks of the drug is due to amide-NH and carbonyl C=0. Carvedilol does not contain any major reacting groups hence, no physical interactions were observed between the drug/ polymers/ plasticizers employed in the present investigation. IR spectral data are shown in spectra 2 to 4 and their results are tabulated in table 7.

b) DSC analysis

Compatibility studies were also carried by using Differential Scanning Calorimetry**,** which is a qualitative analytical tool for assessing the interactions. The pure form and in combination forms with polymers are studied after one month storage at room temperature. It was found that the thermal peaks of drug are identical in presence of polymers. This indicates that, there is no interaction in the polymer and drug. DSC spectral data are shown in spectra 5 to 7 and their results are tabulated in table 8.

c) Formulation Design

Fifteen formulations of TDDS containing Carvedilol were prepared using various hydrophilic and hydrophobic polymer combinations viz Eudragit RS : RL 100 and HPMC : Ethyl cellulose in different ratios (Table 9 and 10). For Eudragit and HPMC : EC patches, TEC and DBP were used as plasticizers respectively. The method adopted for casting the film on mercury surface (mercury substrate method) was found to be satisfactory.

7. Evaluation parameters

I. Physico-chemical evaluation

a) Physical appearance

Both polymer combinations used for fabrication of TDDS showed good film forming properties. The fabricated patches were thin, flexible, elastic, smooth and transparent except for HPMC : Ethyl cellulose – TEC patches which were translucent.

b)Folding endurance

The folding endurance for Eudragit formulations ranged between 140 to 241 and for HPMC : EC patches between 95 to 227. For HPMC : EC patches the folding endurance was found to be a function of HPMC content.

c)Thickness of the films

The thickness of each film was measured at 3 different points and S.D values were calculated. In case of Eudragit patches the thickness varied from 0.0833 to 0.1367 mm. For HPMC : Ethyl cellulose patches the thickness were in the range of 0.0910 to 0.1183 mm & the thickness of the film was found to increase with the increase in HPMC content. The orders of thickness for various films were:

- In Eudragit series: RSL 7>RSL 8>RSL 6>RSL 3>RSL 2>RSL 4>RSL 5>RSL 1
- In HPMC : EC series: RHE 6>RHE 5>RHE 4>RHE 2>RHE 3>RHE 1.

d)Weight uniformity

The weight of Eudragit patches varied from 0.762gm to 0.950gm. For HPMC : Ethyl cellulose patches the weight ranged from 0.566gm to 0.637gm.

e) Drug content

Both Eudragit and HPMC patches showed uniform drug content and the values ranged from 88.78 % to 96.36 %. HPMC : EC patches showed higher drug content than Eudragit patches. This may be due to greater solubility of HPMC matrix in the solvent used for drug content estimation. All the values of physico-chemical evaluation parameters are tabulated in table 11.

f)Percentage moisture uptake

Both Eudragit and HPMC patches showed moisture absorbing capacities. HPMC:EC patches showed higher moisture uptake values as compared to Eudragit patches. In case of HPMC:EC patches moisture uptake capacity increased as HPMC concentration increased indicating HPMC has more moisture absorbing capacity as it is an hydrophilic polymer. Similarly for Eudragit patches moisture content increased with increase in RL 100 content of the film.

The order of moisture uptake for various films was found to be:

- In Eudragit series

RSL 4> RSL 2> RSL 5>RSL 6>RSL 3>RSL 8>RSL 7>RSL 1.

- In HPMC series

RHE 5> RHE 4> RHE 6> RHE 7> > RHE 2> RHE 3>RHE1.

Details of moisture uptake are tabulated in Table 12 and 13.

g)Percentage moisture content

The results of moisture content have indicated that, all transdermal systems have specific moisture content in them. Percentage moisture content ranged from 3.22 to 14.28% for Eudragit patches while 5.45 to 12.76% was obtained for HPMC:EC patches. The results indicated a wide difference in moisture contents among the patches of same combinations. However higher content of HPMC in the patches showed more moisture content in them.

The order of moisture content for various films was found to be

- In Eudragit series

RSL 6>RSL 7>RSL 8>RSL 4>RSL 5>RSL 3>RSL 1>RSL 2

- In HPMC series

RHE 7> RHE 2> RHE 1> RHE 6> RHE 3> RHE 4> RHE 5.

The results of moisture content are tabulated in Table 14 and 15.

h)Water vapor transmission (WVT) studies

An increased release rate of drug from transdermal patches may be related to the water vapor permeation of the films. Eudragit patches showed better WVT than HPMC:EC patches. Formulation RLS 7 gave highest WVT value and RHE 1 showed least WVT value indicating that as the ratio of RL 100 in Eudragit patches and HPMC in HPMC : Ethyl cellulose patches increased, the WVT increased.

The WVT rate constant decreased in the following order

- For Eudragit films

RSL 7>RSL 8>RSL 4>RSL 3>RSL 2>RSL 56>RSL 5>RSL1.

- For HPMC EC films

RHE 6>RHE 5>RHE 4>RHE 7>RHE 2>RHE 3>RHE1.

j)Tensile Strength and Elongation of Films

Tensile strength was determined using Hounse Field universal testing machine for drug-loaded films. The results (average of 3

determinations) are given in the Table 16 and 17. The tensile strength of films was increased With increase in Eudragit RS 100 proportion. Similarly in case of HPMC:EC patches With increase in HPMC proportion the tensile strength of films was increased. It reflects that the soluble polymer develops cross linking better than insoluble polymer. This is in agreement with the viscosity determinations. More the solubility of the polymer higher will be the tensile strength. The percent of elongation is inversely proportional to tensile strength of the patches.

The order of Tensile strength for various films was found to be

- For Eudragit films

RSL 7>RSL 6>RSL 5>RSL 4>RSL 3>RSL 2>RSL 8>RSL 1.

- For HPMC : EC films

RHE 1>RHE 2>RHE 7>RHE 3>RHE 4>RHE 5> RHE 6.

k)Skin irritation test

For the transdermal system to be successful there must be compatibility between polymeric patches & skin. The skin irritation test was performed on two healthy albino Rabbits by using drug free as well as drug containing optimized patches (RSL 2 and RHE 3). Formalin solution (0.8%) was used as control. In both cases there were no erythema or oedema while positive control showed clear erythema. This indicates skin compatibility of these polymers for topical application. The results of skin irritancy are tabulated in Table 6.

l)Scanning electron microscopy (SEM)

The surface morphologies of the films were investigated by using a Jeol JSM-6360A analytical scanning electron microscope. The SEM of formulation RSL 2 reveals that the surface of the film was porous, smooth and free from air bubbles. The SEM of formulation RLS 2 taken at different magnifications is shown in Figure 3.

The SEM of formulation RHE 3 reveals that the surface of the film was porous, smooth and free from air bubbles. The SEM of formulation RHE 3 taken at different magnifications is shown in Figure 3.

m) X- Ray Diffraction (XRD) Studies

X-ray diffraction studies were undertaken to confirm the physicochemical characteristics of Carvedilol in the polymeric matrix of transdermal patches. The pure Carvedilol exhibited the diffraction peaks at 2θ value of 13.50°, 15.61°, 18.27°, 24.69°, 25.76°, 27.60°, 29.26°, etc., which indicating the presence of crystalline Carvedilol. But the combination of Carvedilol with Eudragit RL100:RS100 and HPMC:EC polymer matrix does not show the any crystalline peaks. This result implies that Drug molecule was dispersed at the molecular level and the crystallinity of Carvedilol was not shown by X-Ray Diffraction study. This result shows that Carvedilol is present as an amorphous form in the polymer system (Spectra 10 to 12.)

n) Stability studies

Two optimized formulations namely RSL 2 and RHE 3 were selected for accelerated stability studies as per ICH guidelines. The patches were observed for colour, appearance and flexibility for a period of three months. The folding endurance, weight and drug content of the formulations were found to be decreasing. This decrease may be attributed to the harsh environment (40^0C) maintained during the studies. However IR spectra of the patches taken after 3 months revealed no interference in the functional groups of the drug indicating drug polymer compatibility (Table 21; spectra 8 and 9).

II. Adhesive type evaluation

a)Thumb tack test

Eudragit patches had better adhesive property than HPMC : EC patches.

III. *In vitro* membrane/skin permeation studies

a)Keshary-Chien diffusion cell using dialysis membrane

in vitro permeation studies were carried out for Eudragit RL:RS 100 and HPMC:EC formulations using dialysis membrane as barrier. The maximum and minimum drug release obtained for optimized patches were 79.10% (RSL2) & 77.33% (RSL 8) for Eudragit patches and in case of HPMC:EC patches it was 65.63% (RHE 3) & 58. 23% (RHE 7).

The release profiles from various systems were in the following order

- In Eudragit series

RSL 2>RSL 8>RSL 1>RSSL 3>RSL 4>RSL 5>RSL 6>RSL 7 (, graph 5 and6).

- In HPMC : EC series

RHE 3>RHE 7>RHE 6>RHE 5>RHE 2>RHE 4>RHE 1. (graph 7).

In case of Eudragit patches the release rate was found to be dependent on the Eudragit polymer RL 100 and the release rate increased as the RL 100 content in the patches increased. Similarly in case of HPMC:EC patches, the formulations RHE 4 and RHE 5 gave higher drug release. In this

case release rate depends on the concentration of HPMC and the release rate increased as the HPMC content in the patches increased. No significant variation in release rates was observed when DBP or TEC were interchanged as plasticizers.

IV. *In-vivo* Studies

The hypertension was successfully induced in the normotensive rats by MPA administration as highly significant difference was found in the treatment values (Group 2, Table 20). This was authenticated by Dunnet test, which showed significant difference ($P < 0.05$) in BP values of control (1) and toxic control (2) Groups.

On treating experimental hypertensive rats with Carvedilol TDDS, a significant fall in BP ($P < 0.05$) was observed in the treatment groups 3, 4, 5, and 6.

The groups 3 (RSL 2), and 5 (RHE 3) were showed significant fall in BP compared to groups 4(RSL 8) and 6(RHE 7). This was confirmed by Dunnet test, wich revealed that there was significant difference ($P < 0.05$) in the toxic control and treatment groups 3, 4, 5, and 6. The patches produced the peak effect continued for up to 24 hours. This clearly indicates that the transdermal patches release the drug gradually over a period of time. The results are tabulated in the Table no. 20.

4. CONCLUSION

From the above experimental results it can be reasonably concluded that:

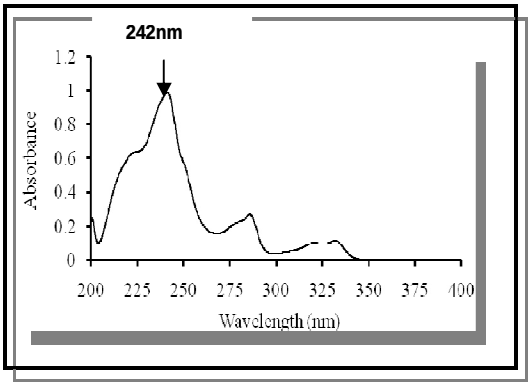
- The formulated TDD patches of both Eudragit and HPMC-Ethyl cellulose series showed good physical properties.
- 18% w/w of TEC and 25% w/w of DBP were suitable plasticizers for Eudragit and HPMC:EC combinations respectively.
- All the optimized patches formulated were stable at room temperature.
- FTIR and DSC studies indicated compatibility between the drug and the excipients employed in the fabrication of TDDS, which was further confirmed by accelerated stability studies as per ICH guidelines.
- SEM micrographs (HPMC:EC, RHE 3) revealed the rough & porous surface nature of the patches.
- Formulated patches did not show any skin irritation reaction as compared to standard.
- In Eudragit series, RLS 2 showed highest release (79.70 %) and in case of HPMC:EC series a maximum of 65.63 % release was obtained for RHE 3 during *in vitro* drug permeation studies through dialysis membrane.
- The release of Carvedilol appears to be dependent on lipophilicity of the matrix. Moderately lipophilic matrices showed best release. The predominant release mechanism of drug through the fabricated matrices was believed to be by diffusion mechanism.
- The *in vitro* release study between dialysis membrane and porcine skin could not be correlated because of difference in release behaviour.
- The *in vivo* release study the groups 3 (RSL 2), and 5 (RHE 3) were showed significant fall in BP compared to groups 4(RSL 8) and 6(RHE 7).

5. ACKNOWLEDGMENT

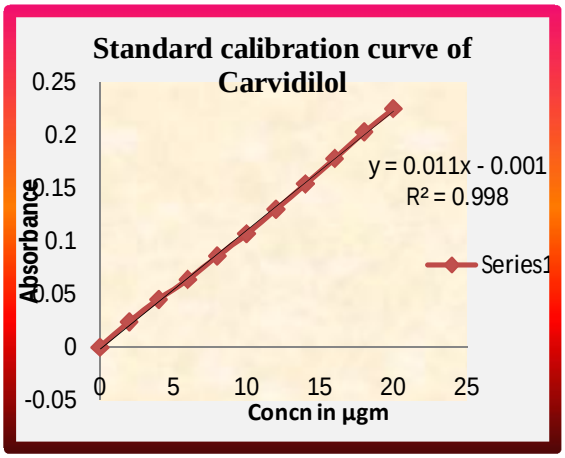
I owe my special debt to Ms. Saumya Shetty of SUN Pharmaceutical Industries Ltd., for providing me a gift sample of drug Carvedilol.

I am really thankful to Evonik Degussa India Pvt. Ltd., and Arihant trading company for providing me a gift sample of polymers

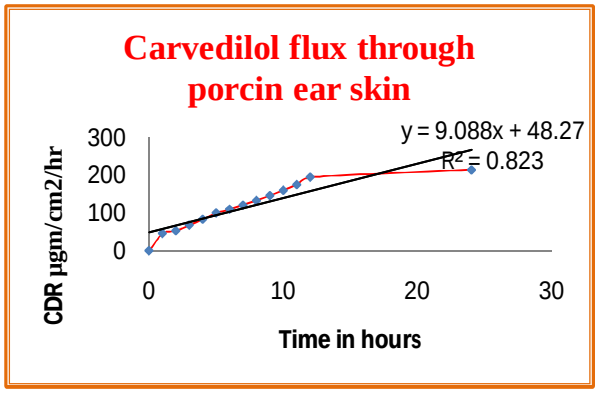
7. Figures, Tables, Spectras and graphs



Spectra 1: Scanning of Carvedilol by UV-spectrophotometer in 30% v/v Methanolic Isotonic Phosphate Buffer (MIPB) of pH 7.4



Graph 1: Standard Calibration curve of carvedilol in 30% v/v Methanolic Isotonic Phosphate Buffer (MIPB) of pH 7.4



Graph 2: Flux of Carvedilol through Porcine ear skin

Table 1: Data for standard Calibration curve of carvedilol in 30% v/v Methanolic Isotonic Phosphate Buffer (MIPB) of pH 7.4

Flask No.	Volume of SS–II (ml)	Volume made up to (ml)	Conc. (µg/ml)	Absorbance at 242 nm				
				Trial 1	Trial 2	Trial 3	Average	S.D.# (±)
1	0.2	10	2	0.024	0.024	0.025	0.024	0.00057
2	0.4	10	4	0.045	0.045	0.045	0.045	0.0000
3	0.6	10	6	0.064	0.065	0.066	0.064	0.0010
4	0.8	10	8	0.088	0.086	0.084	0.086	0.0020
5	1.0	10	10	0.107	0.107	0.108	0.107	0.00057
6	1.2	10	12	0.130	0.130	0.130	0.130	0.0000
7	1.4	10	14	0.154	0.155	0.154	0.154	0.00057
8	1.6	10	16	0.179	0.178	0.178	0.178	0.00057
9	1.8	10	18	0.203	0.203	0.205	0.203	0.00115
10	2.0	10	20	0.227	0.225	0.225	0.225	0.00115

Table 2: Data obtained from *in-vitro* flux study of Carvedilol through Porcine ear skin

S.No	Time in Hours	Absorbance	Conc. in µg/5 ml (with dilution)	Conc. in µg/1 ml (Conc x D.F)	Conc. in µg/48 ml	CDR µgm	CDR µg/cm^2/hr	% CDR
1.	1	0.191	8.63	0	211.59	211.59	45.79	12.46
2.	2	0.212	9.59	8.63	234.97	243.61	52.73	14.35
3.	3	0.265	12	18.22	294	312.22	67.58	18.39
4.	4	0.321	14.54	30.22	356.36	386.59	83.67	22.78
5.	5	0.375	17	44.77	416.5	461.27	99.84	27.18
6.	6	0.401	18.18	61.77	445.45	507.22	109.78	29.88
7.	7	0.431	19.54	79.95	478.86	558.81	120.95	32.92
8.	8	0.463	21	99.5	514.5	614	132.90	36.18
9.	9	0.498	22.59	120.5	553.47	673.97	145.88	39.715
10	10	0.536	24.31	143.0909	595.79	738.88	159.93	43.54
11	11	0.578	26.22	167.40	642.56	809.97	175.31	47.72
12	12	0.634	28.77	193.63	704.93	898.56	194.49	52.95
13	24	0.689	31.27	222.40	766.18	988.59	213.98	58.25

Table 3: Optimization of TDDS patches using Eudragit RL and RS 100 as polymers with DBP as plasticizer

S.No	Ratio of Polymer Eudragit RS 100 : RL 100	% Total Polymer Conc (w/v)	% Conc (w/w) of plasticizer DBP	Observations
1.	1 : 9	10	12	Patches have formed, transparent, non adhesive in nature, initially exhibit good physical properties like flexibility but on storage they were brittle.
2.	2 : 8		15	Patches have formed, transparent, non adhesive in nature, initially exhibit good physical properties like flexibility & elasticity but on storage they were found to be brittle.
3.	3 : 7			
4.	4 : 6		18	Patches have formed, transparent, exhibit good physical properties like flexibility & elasticity, stable on storage. Patches have slight adhesive property.
5.	5 : 5			
6.	6 : 4		21	Patches have formed, transparent; apart from good physical properties like increased flexibility & elasticity they exhibit moderate stickiness, stable on storage. RS 100 patches were found to be more sticky when compared to RL 100 patches.
7.	7 : 3			

Table 4: Optimization of TDDS patches using Eudragit RL and RS 100 as polymers with TEC as plasticizer

S.No	Ratio of Polymer Eudragit RS 100 : RL 100	% Total Polymer Conc (w/v)	% Conc (w/w) of plasticizer TEC	Observations
1.	1 : 9	10	10	Patches have formed, transparent, brittle, non adhesive in nature.
2.	2 : 8		15	Patches have formed, transparent, exhibit mild elasticity & flexibility, non adhesive in nature and stable on storage.
3.	3 : 7			
4.	4 : 6		18	Patches have formed, transparent, exhibit more elasticity & flexibility and stable on storage. Patches have slight adhesive property.
5.	5 : 5			
6.	6 : 4		20	Patches have formed, transparent; apart from good physical properties like increased flexibility & elasticity they exhibit moderate stickiness and stable on storage. RS 100 patches are more sticker when compared to RL 100 patches.
7.	7 : 3			

Table 5: Optimization of TDDS patches using HPMC and EC as polymers with DBP & TEC as plasticizers

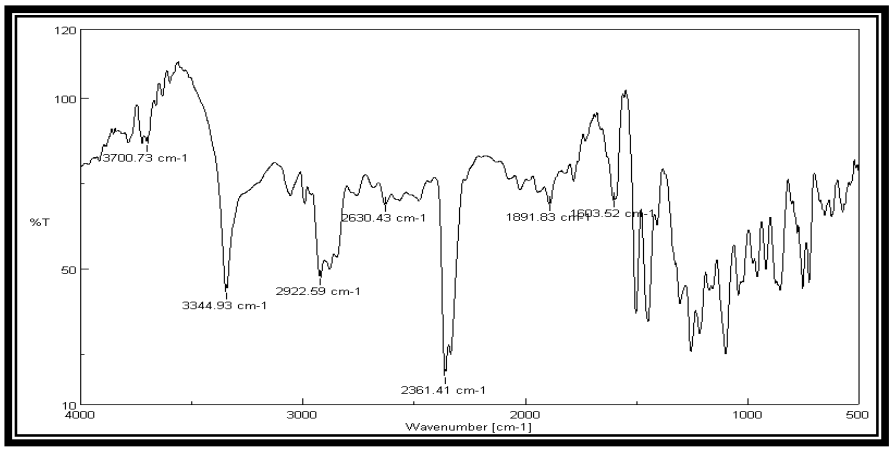
S. No	Ratio of Polymer HPMC : EC	% Total Polymer Conc (w/v)	% Conc (w/w) of plasticizer DBP	Observations
1.	10 : 0	6	25% of DBP	Patches have formed, translucent, non adhesive in nature. As the ratio of ethyl cellulose increases the softness & flexibility of the patches increase. HPMC patches (10:0, 9:1 and 8:2) were hard, non brittle and crackle to some extent when folded where as other patches (7:3, 6:4 and 5:5) were soft, flexible in nature and do not crackle when folded.
2.	9 : 1			
3.	8 : 2			
4.	7 : 3		25% of TEC	Patches have formed, transparent, soft, flexible, non adhesive in nature and stable on storage. Patches do not crackle when folded.
5.	6 : 4			
6.	5 : 5			

Table 6: Data obtained from skin irritation test for drug free and optimized polymeric patches

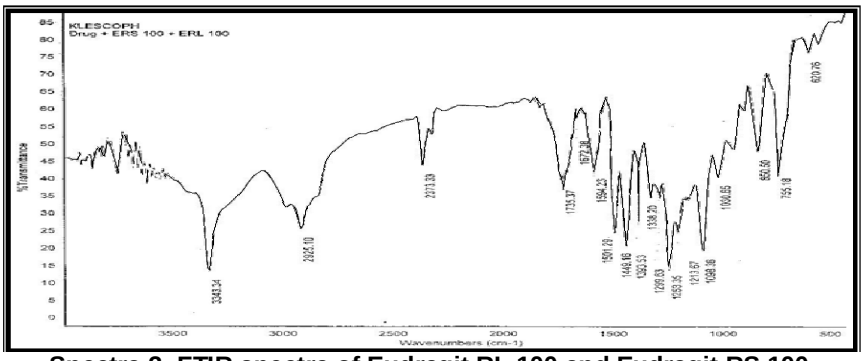
S .No	Formulation	Control	Test 1	Test 2
1	Patch without drug Eudragit RS : RL100 (1:1)	++	---	---
2	Patch without drug HPMC : EC (5:5)	++	---	---
3	Optimized patch with Eudragit RS:RL 100 & drug (RLS 2)	+++	---	---
4	Optimized patch with HPMC:EC & drug (RHE 3)	++	---	+

--- = No Erythema
\+ = Very slight erythema
\+ + = Well defined erythema
\+ + + = Moderate to severe erythema

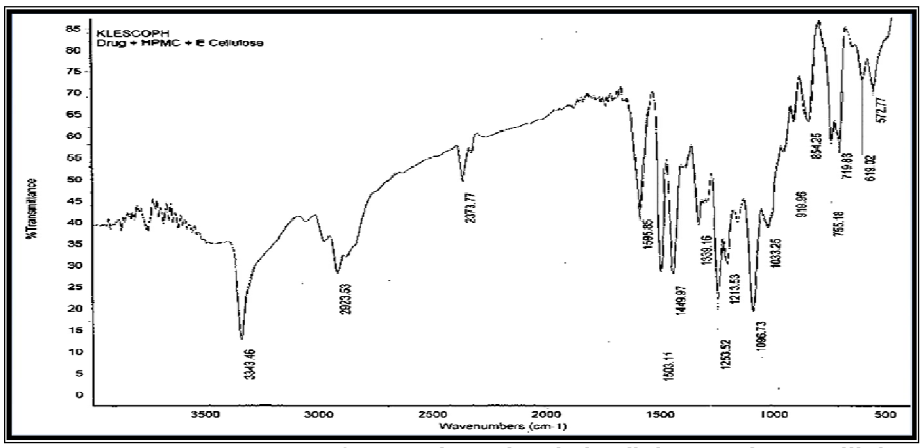
Compatibility studies of drug and polymers by FTIR spectroscopy



Spectra 2: FTIR spectra of Carvedilol



Spectra 3: FTIR spectra of Eudragit RL 100 and Eudragit RS 100



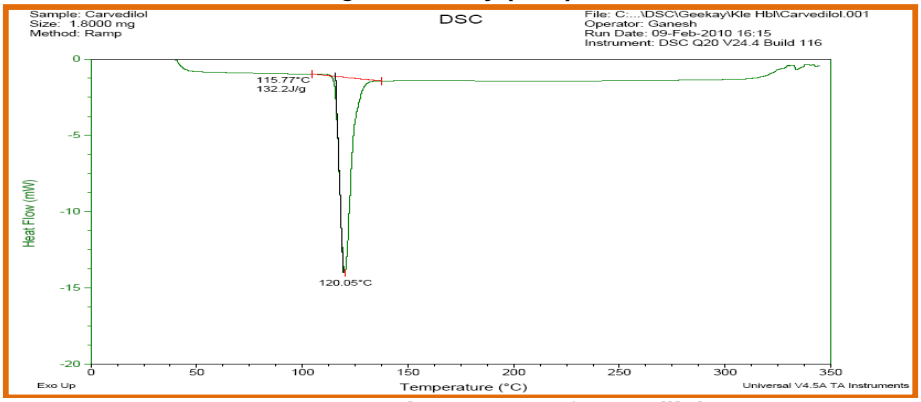
Spectra 4: FTIR spectra of HPMC (6 cps), Ethyl cellulose and Carvedilol

Table 7: Data obtained from compatibility studies of drug and polymers by FTIR spectroscopy

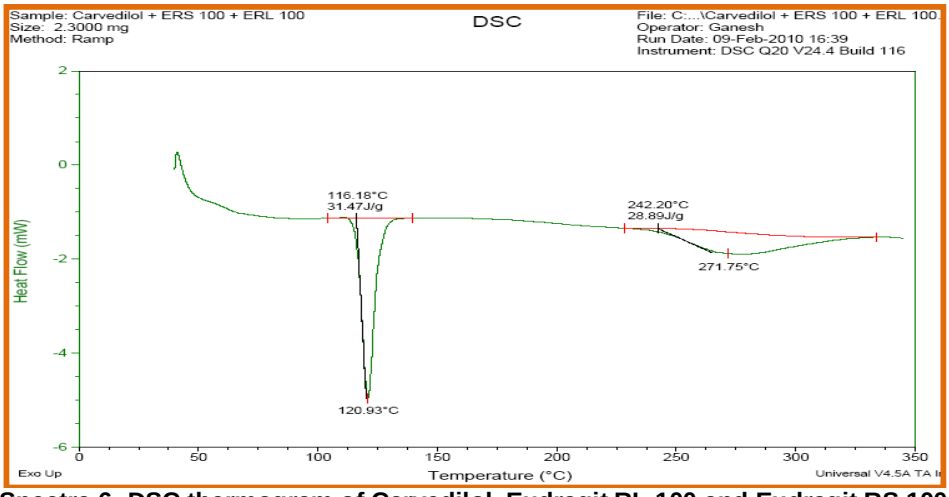
Drug/Polymer	Important IR spectral peaks of different groups expressed in wave number (cm^{-1})							
	N-H/-N-2^0amide/quaternary ammonium group	C-O-C stretch (ether)	C-H stretch (aliphatic)	OH stretch (alcohol/ acid)	C=O stretch (acid)	C=O stretch (amide/ester)	Aromatic C=C stretch	C-O-H bend
Carvedilol	3344.93	1214.70 and 1034.36	2922.56	Broad peak (3200 — 2583.03)	1603.53	1636.78	1562.20 and 1493.74	---
Carvedilol + Eudragit RL+RS 100	3343.34	1213.57 and 1099.66	2925.10	Broad peak (3200 — 2500)	1594.23	1637.40 (D) 1730 (P) (W)	1564.44 and 1492.35	---
Carvedilol + HPMC (6cps) + EC	3343.46(W)	1213.63(D)(W) 1087.81 (P) 1096.73 (P)(W)	2923.53	3478.81 (B)	1595.85	1639.77	1568.30 and 1451.48	1380.31

(D) — drug peak (Carvedilol) (W) — a weak peak EC — Ethyl cellulose (P) — polymer peak (B) — broad peak due to hydrogen bonding.

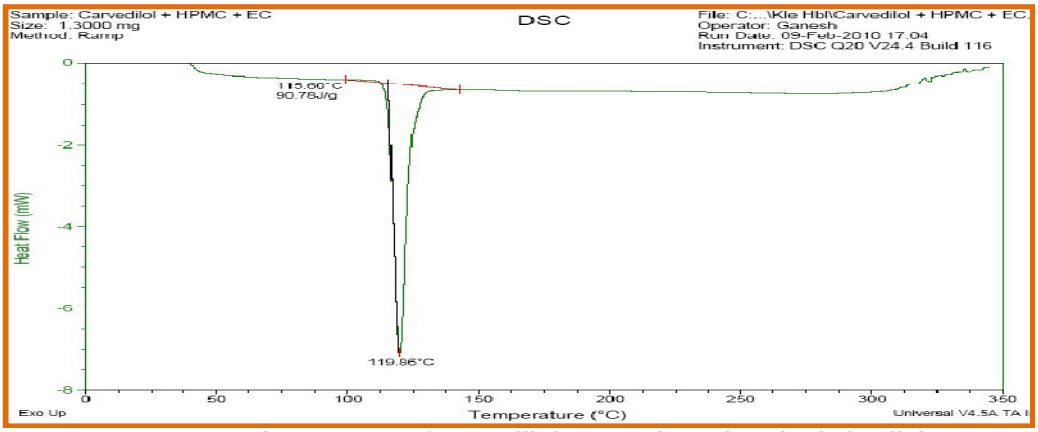
Compatibility studies of drug and polymers by differential scanning calorimetry (DSC) studies



Spectra 5: DSC thermogram of Carvedilol



Spectra 6: DSC thermogram of Carvedilol, Eudragit RL 100 and Eudragit RS 100



Spectra 7: DSC thermogram of Carvedilol, HPMC (6 cps) and Ethyl cellulose

Table 8: Data obtained from compatibility studies of drug and polymer by DSC thermograms.

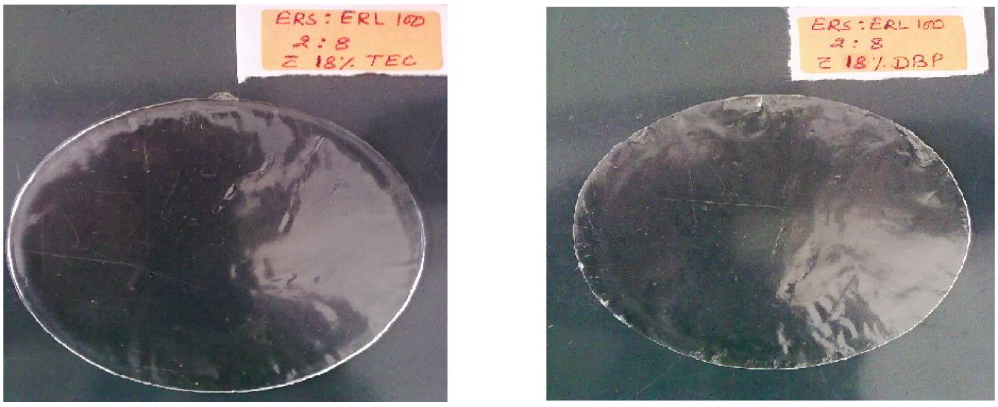
Drug/Drug-Polymer combination	Observed Peaks			
Carvedilol (drug)	120.05^{0}C	---	---	---
Carvedilol + Eudragit RL 100+RS 100	120.93^{0}C (drug)	---	---	---
Carvedilol + HPMC (6 cps) + EC	119.86^{0}C (drug)	---	---	250.39^{0}C (EC)

Table 9: Formulation design for Eudragit combination patches

S.No	Ratio of Eudragit RS 100 : RL 100	% Total polymer Conc (w/v)	% Conc (w/w) of plasticizer		Drug (mg)	Formulation Code
			DBP	TEC		
1	1 : 1	10	---	18	25	RSL 1
2	2 : 8	10	---	18	25	RSL 2
3	3 : 7	10	---	18	25	RSL 3
4	4 : 6	10	---	18	25	RSL 4
5	5 :5	10	---	18	25	RSL 5
6	6 : 4	10	---	18	25	RSL 6
7	7 : 3	10	---	18	25	RSL 7
8	2 : 8	10	18	---	25	RSL 8

Table 10: Formulation design for HPMC (6cps) : Ethyl Cellulose (EC) patches

S. No.	Ratio of HPMC : EC	% Total polymer Conc(w/v)	% Conc (w/w) of plasticizer		Drug (mg)	Formulation Code
			DBP	TEC		
1.	5 : 5	6	---	25	25	RHE 1
2.	6 : 4	6	---	25	25	RHE 2
3.	7 : 3	6	---	25	25	RHE 3
4.	8 : 2	6	---	25	25	RHE 4
5.	9 : 1	6	---	25	25	RHE 5
6.	10 : 0	6	---	25	25	RHE 6
7.	7 : 3	6	25	---	25	RHE 7



Formulation RSL2 **Formulation RSL8**

Fig 1: Photographs of Eudragit RS: RL 100 patches

Formulation RHE 3 **Formulation RHE 7**

Fig 2: Photographs of HPMC: Ethyl cellulose patches

Table 11: Summary of data showing physical parameters and drug content of TDDS

SL. No.	Formulation Code	*Folding Endurance ± S.D (No. of foldings)	Weight (gm)	*Thickness (mm) ± S.D	*Drug content (%) ± S.D
1.	RSL 1	217 ± 7.506	0.863	0.0833 ± 0.0115	89.48 ± 0.6351
2.	RSL 2	241 ± 7.024	0.859	0.1103 ± 0.0351	96.36 ± 0.7045
3.	RSL 3	206 ± 12.000	0.950	0.1033 ± 0.0208	92.23 ± 0.9022
4.	RSL 4	140 ± 11.140	0.762	0.1100 ± 0.0100	92.16 ± 0.3119
5.	RSL 5	159 ± 5.850	0.887	0.0933 ± 0.0251	91.81 ± 0.4038
6.	RSL 6	178 ± 6.506	0.902	0.1133 ± 0.0152	90.94 ± 0.2237
7.	RSL 7	156 ± 17.217	0.813	0.1367 ± 0.0503	88.78 ± 0.6602
8.	RSL 8	239 ± 12.290	0.837	0.1166 ± 0.0472	93.84 ± 0.6842
9.	RHE 1	95 ± 2.517	0.566	0.0910 ± 0.0168	92.74 ± 0.5703
10.	RHE 2	120 ± 4.726	0.592	0.0973 ± 0.0283	93.72 ± 1.270
11.	RHE 3	179 ± 8.082	0.597	0.0923 ± 0.0155	96.34 ± 1.007
12.	RHE 4	227 ± 6.391	0.637	0.1000 ± 0.0174	92.27 ± 0.4022
13.	RHE 5	170 ± 15.020	0.612	0.1037 ± 0.0232	91.45 ± 0.5950
14.	RHE 6	216 ± 13.111	0.603	0.1183 ± 0.0293	90.77 ± 0.5972
15.	RHE 7	103 ± 3.512	0.589	0.0993 ± 0.0070	95.84 ± 0.2831

* = Average of 3 observations.

Table 12: Data obtained from percentage moisture uptake for Eudragit RS : RL 100 patches

S. No.	Formulation Code	Moisture uptake (gm) for films containing drug		% Moisture uptake
		Initial wt in gm	Final wt in gm	
1.	RSL 1	0.227	0.229	0.88
2.	RSL 2	0.235	0.238	1.27
3.	RSL 3	0.215	0.217	0.93
4.	RSL 4	0.214	0.217	1.41
5.	RSL 5	0.195	0.197	1.02
6.	RSL 6	0.199	0.201	1.00
7.	RSL 7	0.221	0.223	0.90
8.	RSL 8	0.218	0.220	0.91

Table 13: Data obtained from percentage moisture uptake for HPMC : Ethyl cellulose patches

S. No.	Formulation Code	Moisture uptake (gm) for films containing drug		% Moisture uptake
		Initial wt in gm	Final wt in gm	
1.	RHE 1	0.172	0.174	1.12
2.	RHE 2	0.163	0.165	1.23
3.	RHE 3	0.167	0.169	1.19
4.	RHE 4	0.175	0.178	1.71
5.	RHE 5	0.129	0.132	2.32
6.	RHE 6	0.137	0.139	1.42
7.	RHE 7	0.147	0.149	1.36

Table 14: Data obtained from percentage moisture content for Eudragit RS : RL 100 patches

S. No.	Formulation Code	Moisture content (gm) for films containing drug		% Moisture content
		Initial wt in gm	Final wt in gm	
1.	RSL 1	0.060	0.058	3.44
2.	RSL 2	0.064	0.062	3.22
3.	RSL 3	0.045	0.043	4.65
4.	RSL 4	0.051	0.046	10.86
5.	RSL 5	0.054	0.051	5.88
6.	RSL 6	0.024	0.021	14.28
7.	RSL 7	0.041	0.036	13.88
8.	RSL 8	0.061	0.055	10.90

Table 15: Data obtained from percentage moisture content for HPMC : Ethyl cellulose patches

S. No.	Formulation Code	Moisture content for films containing drug		% Moisture content
		Initial wt in gm	Final wt in gm	
1.	RHE 1	0.053	0.048	10.41
2.	RHE 2	0.060	0.054	11.11
3.	RHE 3	0.061	0.057	7.01
4.	RHE 4	0.057	0.054	5.55
5.	RHE 5	0.058	0.055	5.45
6.	RHE 6	0.054	0.050	8.00
7.	RHE 7	0.053	0.047	12.76

Table 16: Data obtained from Tensile strength and Elongation of Eudragit RL: RS 100 patches

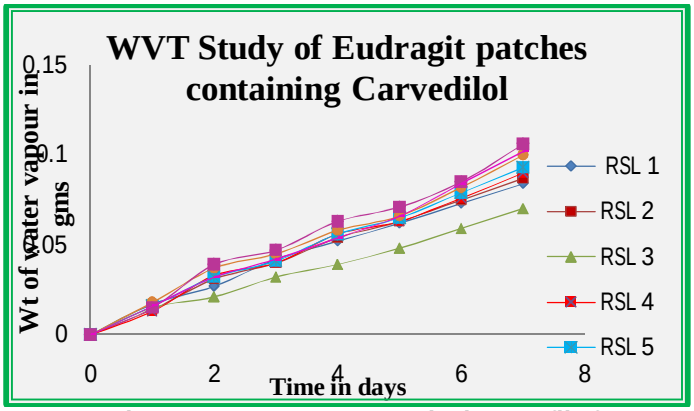
S. No	Formulation Code	Tensile strength (Kg ± SD)	Elongation (mm ±SD)
1.	RSL 1	0.4245 ± 0.0122	15.48 ± 1.1511
2.	RSL 2	0.3874 ± 0.0118	18.61 ± 0.7703
3.	RSL 3	0.3604 ± 0.0164	22.46 ± 1.1452
4.	RSL 4	0.2877 ± 0.0101	27.17 ± 1.3499
5.	RSL 5	0.2736 ± 0.0119	31.76 ± 0.8637
6.	RSL 6	0.2381 ± 0.0102	35.92 ± 1.2550
7.	RSL 7	0.1999 ± 0.0107	41.88 ± 1.6258
8.	RSL 8	0.3747 ± 0.0068	18.56 ± 0.8748

Table 17: Data obtained from Tensile strength and Elongation of HPMC: EC patches

S. No	Formulation Code	Tensile strength (Kg ± SD)	Elongation (mm ± SD)
1.	RHE 1	0.2333 ± 0.0125	35.29 ± 0.8045
2.	RHE 2	0.3246 ± 0.0126	32.45 ± 0.8558
3.	RHE 3	0.3842 ± 0.0130	26.51 ± 2.1601
4.	RHE 4	0.4758 ± 0.0093	24.56 ± 0.7006
5.	RHE 5	0.5759 ± 0.0134	23.35 ± 1.7043
6.	RHE 6	0.385 ± 0.0100	20.49 ± 1.0864
7.	RHE 7	0.3784 ± 0.0130	27.51 ± 1.7601

Table 18: Data obtained from water vapor transmission studies for Eudragit RS : RL 100 patches

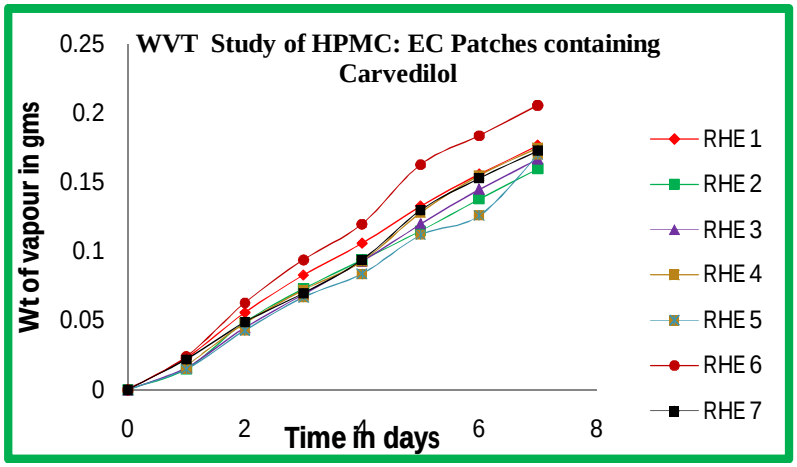
S. No	Formulation Code	Amount of water vapor transmission for drug containing patches in gm							WVT rate constant (gm.cm/cm^2.24 hrs)
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
1.	RSL 1	0.017	0.027	0.042	0.052	0.062	0.073	0.084	5.381×10^{-3}
2.	RSL 2	0.014	0.031	0.040	0.054	0.063	0.075	0.087	6.652×10^{-3}
3.	RSL 3	0.015	0.021	0.032	0.039	0.048	0.059	0.070	6.674×10^{-3}
4.	RSL 4	0.013	0.033	0.040	0.056	0.063	0.076	0.090	7.124×10^{-3}
5.	RSL 5	0.016	0.032	0.041	0.056	0.065	0.079	0.093	6.021×10^{-3}
6.	RSL 6	0.018	0.037	0.045	0.058	0.066	0.082	0.100	6.647×10^{-3}
7.	RSL 7	0.015	0.039	0.047	0.063	0.071	0.085	0.106	8.814×10^{-3}
8.	RSL 8	0.016	0.032	0.042	0.054	0.066	0.084	0.102	7.531×10^{-3}



Graph 3: Water vapour transmission profile for Eudragit RL: RS 100 patches

Table 19: Data obtained from water vapour transmission studies for HPMC : Ethyl cellulose patches

Sl. No	Formulation Code	Amount of water vapour transmission for drug containing patches in gm							WVT rate constant (gm.cm/cm^2 .24 hrs)
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
1.	RHE 1	0.023	0.056	0.083	0.106	0.133	0.156	0.177	**5..880 × 10^{-3}**
2.	RHE 2	0.015	0.049	0.073	0.094	0.115	0.138	0.160	**6.284 × 10^{-3}**
3.	RHE 3	0.016	0.045	0.069	0.093	0.120	0.145	0.167	**5.963× 10^{-3}**
4.	RHE 4	0.017	0.048	0.072	0.093	0.128	0.155	0.175	**6.472 × 10^{-3}**
5.	RHE 5	0.015	0.043	0.067	0.084	0.112	0.126	0.170	**6.700 × 10^{-3}**
6.	RHE 6	0.024	0.063	0.094	0.120	0.163	0.184	0.206	**7.644. × 10^{-3}**
7.	**RHE 7**	**0.022**	**0.049**	**0.070**	**0.094**	**0.130**	**0.153**	**0.173**	**6.416 × 10^{-3}**



Graph 4: Water vapour transmission profile for HPMC: Ethyl cellulose patches

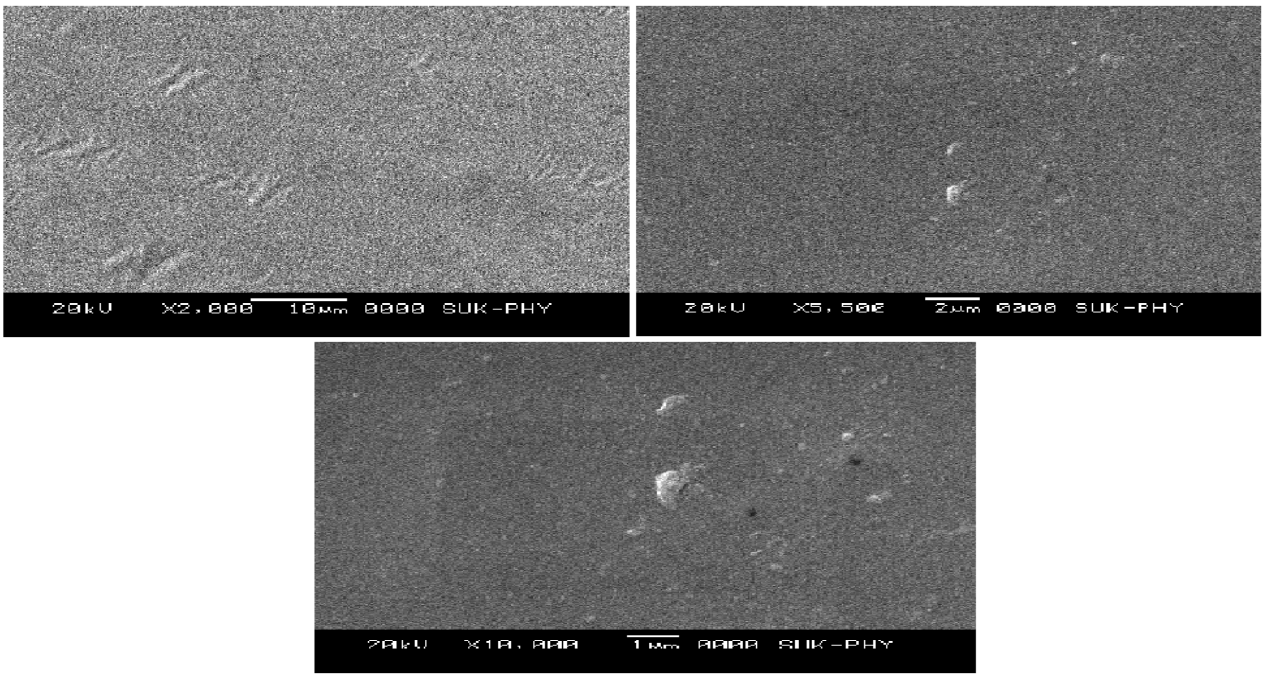
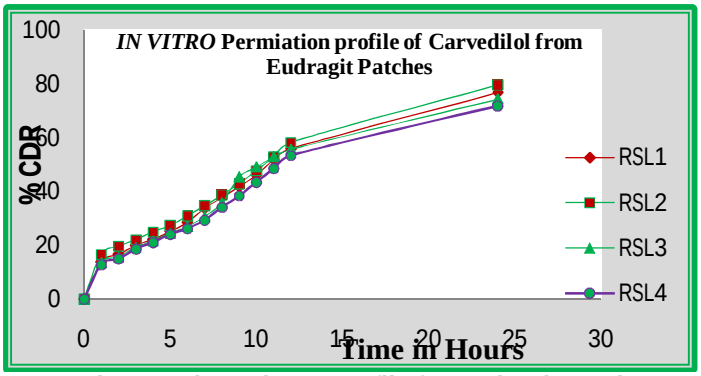
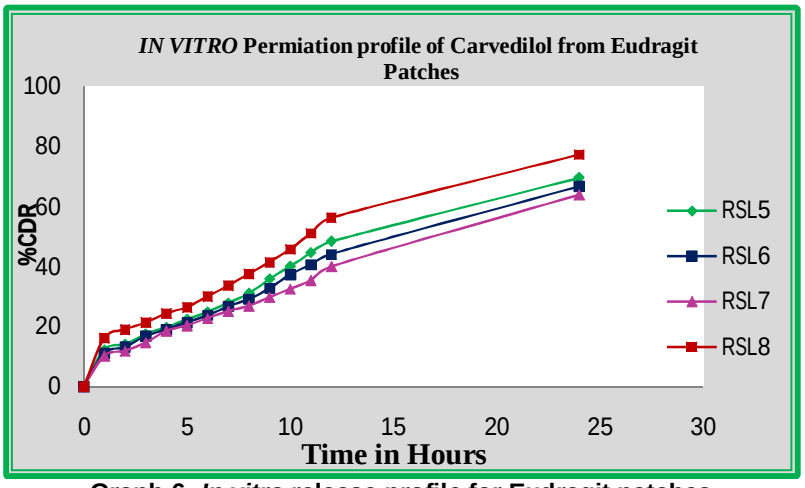


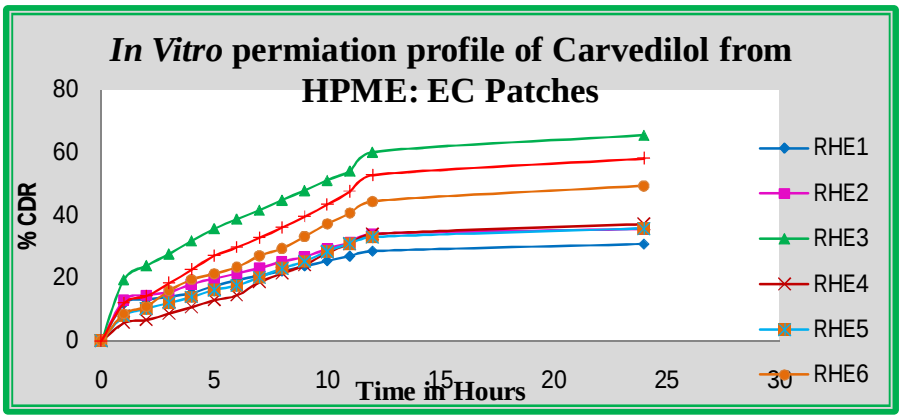
Fig 3: Scanning Electron Microscopy of formulation RSL 2



Graph 5: *In vitro* release profile for Eudragit patches containing 25 mgs of Carvedilol through dialysis membrane



Graph 6: *In vitro* release profile for Eudragit patches containing 25 mgs of Carvedilol through dialysis membrane



Graph 7: *In vitro* release profile for HPMC : Ethyl cellulose patches containing 25 mgs of Carvedilol through dialysis membrane

Table 20: Effect of Transdermal drug delivery systems of Carvidilol on mean blood pressure in control and methyl prednisolone acetate (MPA) induced hypertensive rats

Group	Treatment	Initial	1 hour	2 hour	4hour	6hour	10hour	12hour
1	Control [a]	113.8 ±1.639	113.9 ±1.122	112.2 ± 0.9058	114.2 ± 1.664	113.0 ± 1.123	112.7 ± 1.332	113.4 ± 1.392
2	MPA Control [b]	156.6± 1.367	157.2 ± 1.621	155.6 ±1.679	157.3 ±1.558	156.9 ± 1.2785	157.9 ± 4.3697	155.3± 1.3845
3	ERL:ERS(4:1) [c]	158.6± 1.3706	154.8 ± 0.3867**	148.6± 1.370***	142.6 ±1.6133***	138.6 ± 1.1829***	132.9 ± 1.3603***	126.8 ± 1.7648***
4	ERL:ERS(1:4) [c]	157.6± 0.3552	155.1± 0.3965*	152.6 ± 1.311***	148.9 ± 1.611***	144.9± 1.139***	139.9 ± 1.613***	135.1 ± 1.395***
5	HPMC:EC(8:2)[c]	158.6± 1.237	153.2 ± 0.3481***	149.3 ± 1.264***	142.3 ± 1.7252***	139.1 ± 1.3451***	134.8 ± 0.1187***	129.2 ± 1.2740***
6	HPMC:EC(5:5)[c]	156.5± 1.4561	154.6± 0.6423**	152.9± 1.378***	146.1 ± 0.3408***	141.8 ± 1.369***	135.6 ± 1.838***	132.9 ± 1.3829***

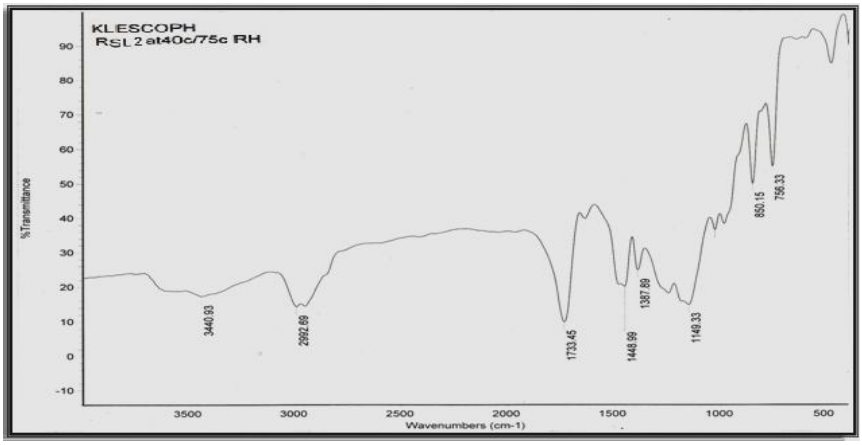
***Mean BP (mm Hg) ± SEM**

[a]**Control Group**: Received no treatment.
[b]**Toxic Control Group:** Received Methyl prednisolone acetate s.c. 20mg/Kg/week for two weeks.
[c]**Treatment Groups:** Received Methylprednisolone acetate s.c. 20mg/Kg/week for two weeks followed by Carvidilol(Drug) loaded Transdermal Patches. *Significant compared with MPA control (P < 0.05).

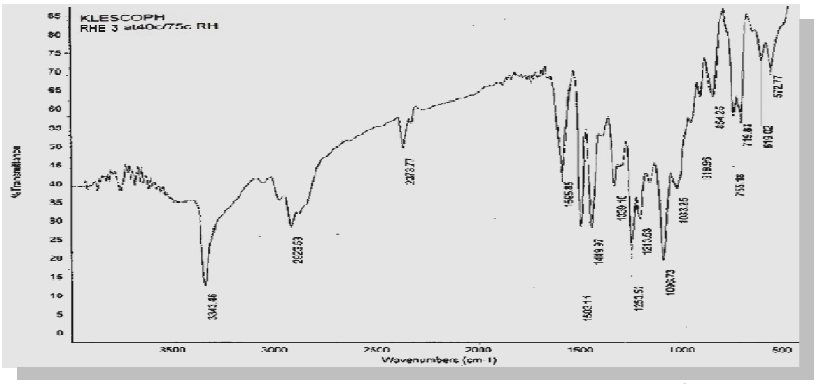
Table 21: Data obtained from stability studies for physico-chemical parameters of optimized patches

S.N o.	Time Interval	Physical appearance		Folding Endurance		Decrease in weight (gm)		Drug content	
		RSL 2	RHE 3	RSL 2	RHE 3	RSL 2	RHE 3	RSL 2	RHE 3
1.	0 days	+ +	+ +	157	117	0.751	0.638	95.21	98.07
2.	15 days	+ +	+ +	158	125	0.747	0.630	93.67	98.69
3.	30 days	+ +	+ +	133	105	0.742	0.621	94.37	97.15
4.	60 days	+ +	+	176	98	0.738	0.616	93.55	97.42
5.	90 days	+ +	+	182	90	0.735	0.613	93.70	96.34

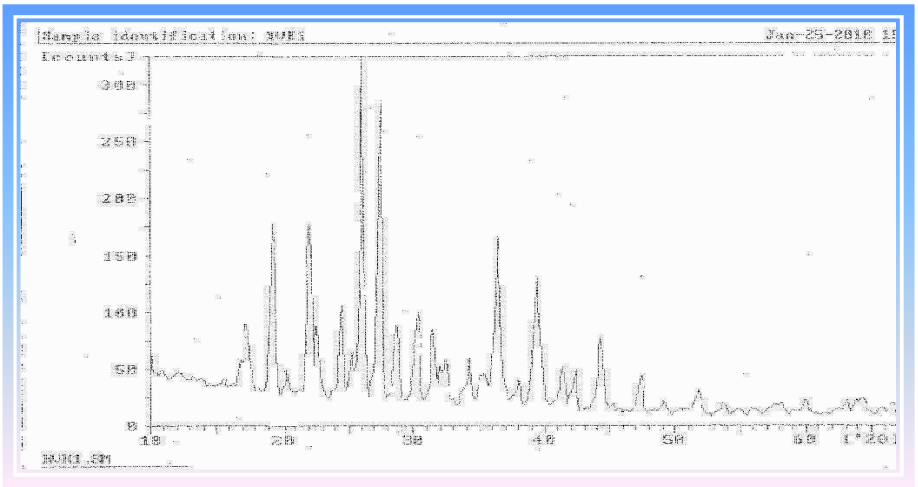
+ + — No change in physical appearance
+ — Slight change in physical appearance.



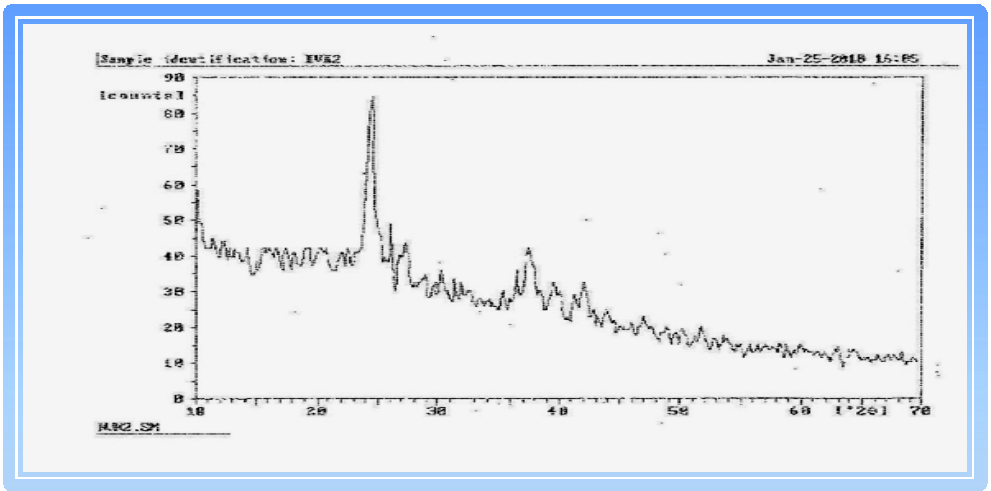
STABILITY STUDIES » Spectra 8: IR spectra of formulation RSL 2 at 400C ± 20C / 75% RH ± 5% RH



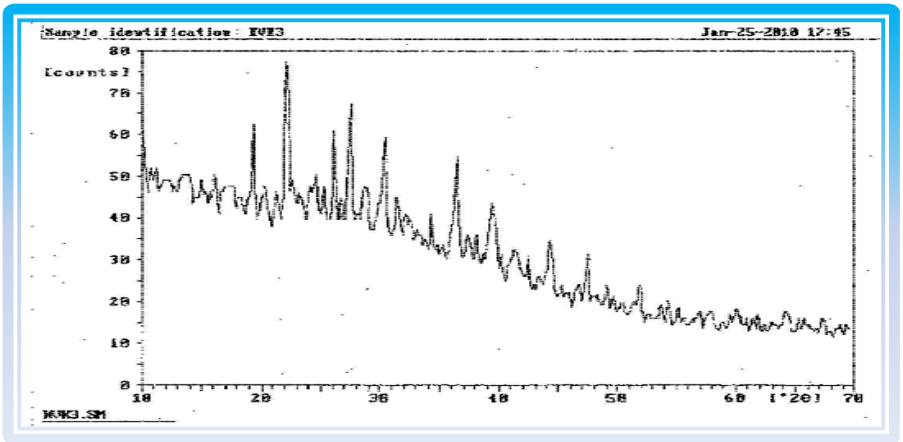
STABILITY STUDIES » Spectra 9: IR spectra of formulation RHE 3 at 40^0C ± 2^0C / 75% RH ± 5% RH



Spectra 10: XRD spectra of Carvedilol



Spectra 11: XRD spectra of Carvedilol+Eudragit RS 100 +RL 100



Spectra 12: XRD spectra of Carvedilol+HPMC+EC

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