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DEVELOPMENT OF CLOPIDOGREL BISULFATE TRANSDERMAL PATCHES AND ESTIMATION OF SIGNIFICANT EFFECT OF VARIABLES BY USING FACTORIAL DESIGN.

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Abstract:

The transdermal drug delivery system now becomes a promising and efficient route of drug delivery system. It has a possible benefit of avoiding first pass metabolism, improved patient compliance, decrease side effect and improved bioavailability.The purpose of this research was to develop a matrix type transdermal system containing drug Clopidogrel bisulfate with polymer ratio of HPMC E5 and ERS-100 by solvent evaporation technique by using polyethylene glycol 400 as a plasticizer and varying concentration of penetration enhancers (Tea tree oil, Sweet basil oil, Eucalyptus oil, Dimethyl sulphoxide and clove oil). The prepared formulations were characterized by FT-IR and DSC to estimate the incompatibility. The physical appearance, thickness, percent moisture absorption, percent moisture loss, water vapour transmission rate, folding endurance, and medication content of the prepared transdermal patches were all assessed. The *in-vitro* diffusion studies of formulations were completed by using Franz diffusion cell, from the above study it was found that batch SN6 (A1) and SP5 (A2) showed highest cumulative percent drug release. The microscopic research done out by using scanning electron microscope.

Keywords: TDDS, Clopidogrel bisulfate, Polyethylene glycol, *in-vitro* diffusion study.

I. INTRODUCTION

A transdermal patch, referred to as a skin patch, is a medicated adhesive patch that is applied to the skin and is used to deliver a particular dose of medication into the bloodstream. TDDS are adhesive drug-delivery devices with a specific surface area that deliver a predetermined amount of drug to the intact skin's surface at a predetermined rate, and maintain the rate for extended period of time thus eliminating numerous problems associated with oral dosing including product stability, decrease the drug load as compare to oral drug delivery, bioavailability and the peaks and troughs of pulse dosing, enhance patient compliance. For a variety of reasons, the development of technology for controlled drug release into systemic circulation via the skin has become popular. It includes innovative drug delivery systems and can be used to achieve an efficient systemic effect by bypassing hepatic first pass metabolism and increasing the fraction absorbed.

The screening and testing of polymers for use in transdermal drug delivery needs the knowledge of placebo patches. Plasticizers, which are added to polymeric systems to change their physical properties and improve their film forming qualities, can drastically alter polymers' viscoelastic behaviour. Transdermal administration of lipophilic medicines in systemic circulation has been proved to be a viable option after extensive research, e.g. Nitro-glycerine, Ephedrine, Ketoprofen, Propranolol and Estradiol([1,2,3]).

Among the various types of systems, drug-in-adhesive products, which contain the medicine in the adhesive layer that comes into contact with the skin, are highly popular since they are thin and comfortable. More and more efficient systems are introduced into the market, with the advantage of reducing the size of the patch to the size of a stamp.[(4)] The development of

transdermal drug delivery systems is a multidisciplinary activity that includes everything from selecting a drug molecule to demonstrating sufficient drug flux in an in-vitro and in-vivo model to fabricating a drug delivery system that meets all of the stringent requirements that are unique to the drug molecule (Physicochemical and stability factors), the patient (Comfort and cosmetic appeal), and the manufacturer.[5,6,7]

II. MECHANISM OF ACTION OF TRANSDERMAL PATCH

Various methods are used to apply the transdermal patch and to transport the active drugingredient from the patch to the circulatory system via the skin.[8,9,10]

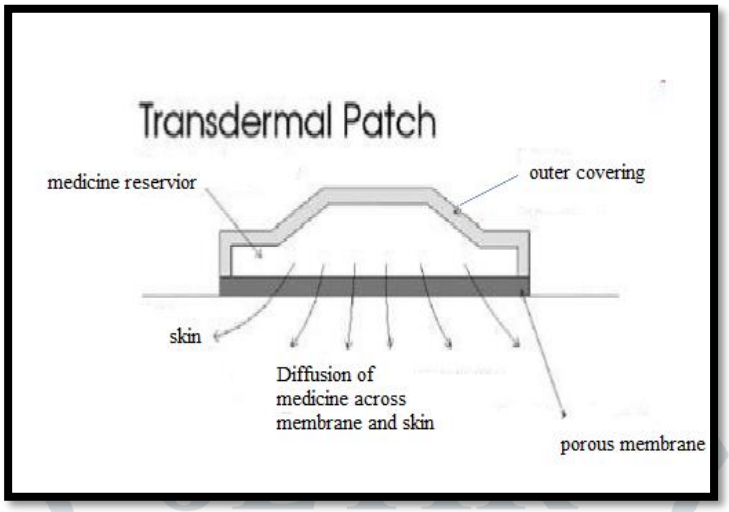


Fig. No. 1 Representing mechanism of drug release from transdermal patch

III. FACTORS AFFECTING TRANSDERMAL PERMEABILITY

Various elements, including drug or skin physiology, are involved in controlling and rendering drug permeability via the skin. There is no single factor that affects drug permeation; rather, a variety of factors are involved, all of which are mutually dependent on one another and are characterized as follows:

3.1 Physiochemical properties of drug and formulation characteristics.

3.2 Skin physiology and pathology.

3.1 Physicochemical properties of drug and formulation characteristics:

The following are some of the physicochemical features of drugs that can affect their absorption and diffusion through the skin:

3.1.1 Size of drug molecules and molecular weight:

The size of drug molecules is inversely proportional to their ability to penetrate the skin. Percutaneous transfer of drugs with molecular weights more than 500 Dalton is problematic. The lesser the molecular weight, the lower the absorption.

3.1.2 Partition coefficient and solubility:

Because drugs are either lipophilic or hydrophilic, their solubility or diffusion in lipids and aqueous environments is determined by their partition coefficient. The skin is made up of a lipid bilayer, drugs that have both lipid and water solubility is ideal for percutaneous absorption. Drugs should have some lipid solubility for absorption but also some hydrophobicity to diffuse inside the skin in an aqueous environment. As a result, a medication candidate should have the best partition co-efficient possible. A drug's partition co-efficient can be changed by changing the solvent system or making chemical changes to the drug candidate's structure without impacting the medication's pharmacological activity.

3.1.3 Drug concentration:

Passive diffusion is responsible for drug absorption through the skin. The drug follows a concentration gradient, moving from a high to a low concentration. As a result, the rate of diffusion over the skin is determined by the concentration of drug in the formulation applied to the skin; the higher the concentration, the greater the permeation.

3.1.4 pH conditions:

Most drugs are acidic or basic in nature and their ionization at the skin surface results in better absorption than ions or ionic species. As a result, pH plays a major role in regulating the extent of drug penetration, and ionisable species movement in aqueous environments is pH dependent.

3.1.5 Formulation characteristics:

The following are some of the formulation characteristics that can affect drug molecule penetration through the skin:

a. Release rate of the drug:

The affinity of the carrier for the drug in the formulation, the solubility of the drug in the solvent, and the interfacial partitioning of the drug from formulation to skin all influence the medication's release rate.

b. Ingredients of formulation:

By modifying the physicochemical properties of the drug or skin physiology, various excipients and polymers contained in the formulation can affect either drug release or drug penetration through the skin.

c. Presence of permeation enhancers:

Permeation enhancers of various types are used to promote drug permeation through the skin by temporarily altering the skin's integrity (physicochemical and physiological change) and opening the skin pores for absorption. Permeation enhancer may be chemical substance acts by chemically or physically.

3.2 Physiological and pathological condition of the skin:

The physiological and pathological conditions of the skin alter and affect the permeation of drug by changing the properties of the skin.

3.2.1 Hydration of skin:

Hydration of the skin causes the swelling of stratum corneum of the skin and provides some fluidity to the skin, hence increases the per minute solubility and partitioning from vehicle to the membrane, hence the permeation of drug molecules occurs easily through the hydrated skin.

3.2.2 Skin temperature:

Percutaneous absorption of the medicine increases as the skin temperature rises due to fluidization of lipids and dilatation of blood vessels. Increased blood flow to the skin enhances absorption via the skin.

3.2.3 Skin age:

It is assumed that skin of young and elderly are more permeable than middle aged persons, because in premature infants stratum corneum is absent and children are more susceptible to toxic effects of drugs through the skin.

3.2.4 Blood flow:

Changes in peripheral circulation do not affect transdermal absorption but an increase in blood flow increase the concentration gradient across the skin and reduces the total time of residence of the drug molecules in the dermis by continuously removing it.

3.2.5 Pathology of the skin:

Disease of the skin and any injury to the skin causes the rupturing of the lipid layers of the stratum corneum which alters the skin penetration of drugs. Pathogens disrupt skin layers by digesting them and can form pores in the skin, causing changes in the skin's integrity in both pathological and injury situations.

3.2.6 Regional site of skin:

Anatomical aspects of the skin, such as the thickness of the stratum corneum, the number of hair follicles, and the number of sweat glands per unit surface area, vary from location to site, person to person, and species to species. As a result, percutaneous absorption varies from one example to the next.

3.2.7 Skin flora and enzymes:

Various metabolizing enzymes and microbes are present in the skin which metabolizes the drugs passing through the skin, a few drug candidates reaches in active form in the circulation. e.g., 95% of the Testosterone absorbed gets metabolized in the skin.[11,12,13]

IV. TYPES OF TRANSDERMAL DRUG DELIVERY SYSTEM

4.1 Single-layer Drug-in-Adhesive:

The drug is also contained in the adhesive layer system, which is surrounded by a temporary liner and a backing. The adhesive layer not only helps to glue the various layers together, as well as the entire system to the skin, but it is also responsible for the drug's release. [14] As Shown in figure no.2.

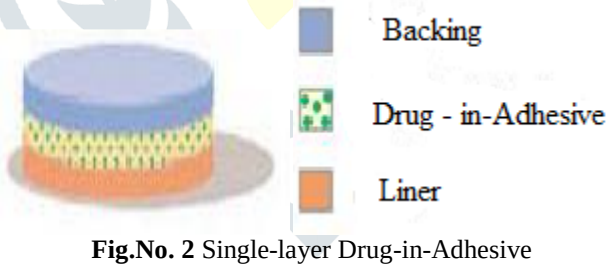


Fig.No. 2 Single-layer Drug-in-Adhesive

4.2 Multi-layer Drug-in-Adhesive:

The multi-layer and single-layer drug-in adhesive patches are similar to the single-layer approach in that the drug is released via both adhesive layers. The multi-layer system is distinct in that it includes an additional layer of drug-in-adhesive, usually separated by a membrane, as well as a temporary liner layer and a permanent backing. [15] As Shown in figure no.3.

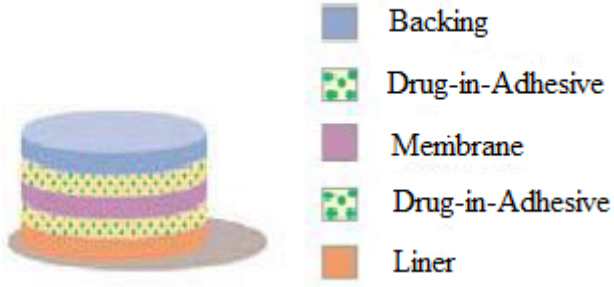


Fig.No.3 Multi-layer Drug-in-Adhesive

4.3 Reservoir:

The reservoir transdermal system has a separate drug layer, which is a liquid compartment containing a drug solution or suspension separated by the adhesive layer, this patch is backed by the backing layer, and the rate of release is zero order, unlike single-layer and multi-layer Drug-in-Adhesive systems. [16] As Shown in figure no.4.

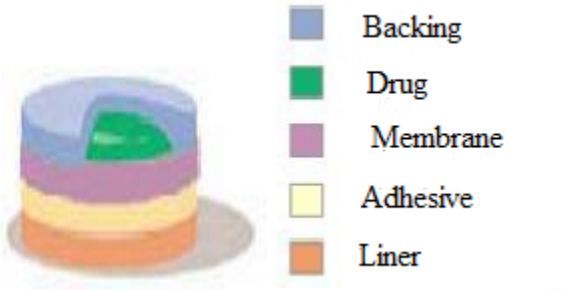


Fig.No.4 Reservoir transdermal system

4.4 Matrix:

A drug layer of a semisolid matrix holding a drug solution or suspension is present in the Matrix system, and the adhesive layer in this patch partially overlays the drug layer. As Shown in figure no.5.

Fig.No.5 Matrix system

4.5 Vapour Patch:

The adhesive layers in a vapour patch not only serves to adhere the various layers together, but also to release vapour, release essential oils for up to 6 hours, and are primarily used in cases of decongestion. Other vapour patches include controller vapour patches that improve sleep quality and reduce the number of cigarettes smoked per month.[17]

V. VARIOUS METHODS FOR PREPARATION TDDS

5.1 Asymmetric TPX membrane method:

For this, a heat sealable polyester film (Type 1009, 3m) with a 1cm diameter concave will be utilised as the backing membrane, the drug sample will be dispensed into the concave membrane, covered with a TPX {poly (4-methyl-1-pentene)} asymmetric membrane, and sealed with an adhesive.

Asymmetric TPX membrane preparation:

The dry/wet inversion procedure is used to make these; TPX is dissolved in a solvent (cyclohexane) plus non-solvent additives at 60°C to generate a polymer solution. The polymer solution is maintained at 40° C for 24 hours before being cast on a glass plate with a gardening knife to a pre-determined thickness. After that, the casting film is evaporated for 30 seconds at 50°C, and the glass plate is immediately immersed in the coagulation bath [temperature kept at 25° C]. After 10 minutes of soaking, the membrane can be removed and dried in a circulation oven at 50° C for 12 hours.

5.2 Circular Teflon mould method:

Solutions containing polymers in various ratios are used in an organic solvent, a calculated amount of medication is dissolved in half of the same organic solvent, and enhancers in various concentrations are dissolved in the other half of the organic solvent and added. As a plasticizer, di-N-butyl phthalate is added to the drug polymer solution, which is then agitated for 12 hours before being poured into a circular Teflon mould. In a laminar flow hood model with an air speed of 0.5 m/s, the moulds should be positioned on a leveled surface and covered with an inverted funnel to manage solvent vaporization. To prevent ageing, the solvent is allowed to evaporate for 24 hours before the dried films are stored in a desiccator containing silica gel for another 24 hours at 25 0.5°C. Within one week of their preparation, the type films must be reviewed.

5.3 Mercury substrate method:

In this procedure, the drug is dissolved in a polymer solution with the plasticizer, agitated for 10 to 15 minutes to generate a homogeneous dispersion, and then poured onto a leveled mercury surface, covered with an inverted funnel to prevent solvent evaporation.

5.4 By using "IPM membranes" method:

This approach involves dispersing the drug in a mixture of water and propylene glycol containing carbomer 940 polymers and stirring it for 12 hours in a magnetic stirrer. The dispersion is then neutralized and made viscous by adding triethanolamine. If the drug solubility in aqueous solution is low, a buffer pH 7.4 can be employed to make a solution gel, which will subsequently be integrated into the IPM membrane.

5.5 By using "EVAC membranes" method:

1 percent carbopol reservoir gel, polyethelene (PE), ethylene vinyl acetate copolymer (EVAC) membranes can be employed as rate control membranes to construct the target transdermal treatment system; if the drug is not soluble in water, propylene glycol is utilised to prepare the gel. The drug is dissolved in propylene glycol, then carbopol resin is added to the solution and neutralised with a 5% w/w sodium hydroxide solution. Finally, the drug (in gel form) is deposited on a backing layer sheet that covers the necessary area. To create a leak-proof device, a rate-controlling membrane will be placed over the gel and the borders will be sealed with heat.

5.6 Aluminium backed adhesive film method:

If the loading dose is larger than 10 mg, a transdermal drug delivery system may yield unstable matrices; in that case, an aluminum-backed adhesive film approach is appropriate; for preparation, chloroform is the solvent of choice because most drugs and adhesives are soluble in chloroform. The drug will be dissolved in chloroform, and the adhesive substance will be added and dissolved in the drug solution. Aluminum foil is used to line a custom-made aluminium former, and the ends are blanked off with securely fitting cork blocks.

5.7 Preparation of TDDS by using proliposomes:

The proliposomes are made utilising a carrier technology and a film deposition technique; an optimal ratio of 0.1:2.0 for drug and lecithin can be used. The proliposomes are made by placing 5 mg of mannitol powder in a 100 mL round bottom flask that is held at 60-70° C and rotated at 80-90 rpm for 30 minutes while vacuum drying the mannitol. After drying, the temperature of the water bath is adjusted to 20-30 °C. After dissolving the drug and lecithin in a suitable organic solvent mixture, a 0.5 mL aliquot of the organic solution is injected into the round bottomed flask at 37 °C, followed by the addition of second 0.5 mL aliquots of the solution after full drying. After the final loading, the flask holding proliposomes is linked in a lyophilized, and the drug-loaded mannitol powders (proliposomes) are desiccated overnight before sieving through 100 mesh. The gathered powder is placed in a glass bottle and kept frozen until it is time to characterize it. [18]

5.8 By using free film method:

Casting on a mercury surface produces a free cellulose acetate film. Chloroform will be used to make a 2 percent w/w polymer solution. Plasticizers should be used at a concentration of 40% by weight of the polymer. In a glass petri dish, five mL of polymer solution was put into a glass ring that was positioned over the mercury surface. By placing an inverted funnel above the petri dish, the rate of solvent evaporation can be adjusted. After the solvent has completely evaporated, observe the mercury surface for the formation of a film. The dry film will be separated and stored in a desiccator between wax paper sheets until needed. By varying the volume of the polymer solution, free films of various thicknesses can be created.[19,20]

VI. METHODS AND MATERIALS

6.1 Methods:

Aluminium backed adhesive film method:

If the loading dose is greater than 10 mg, the transdermal drug delivery system may produce unstable matrices; therefore, this method is appropriate. Chloroform is the solvent of choice because most drugs and adhesives are soluble in chloroform; therefore, the drug is dissolved in chloroform, and the adhesive material is added to the drug solution and dissolved. Aluminum foil is used to line a custom-made aluminium former, and the ends are blanked off with securely fitting cork blocks.

6.2 Materials:

Clopidogrel bisulfate was received as a gift sample from Ajanta Pharma.Ltd, Paithan. Eudragit RS-100 were received as a gift sample from EvonikPharma, Mumbai, Hydroxy Propyl Methyl Cellulose, Dimethyl sulfoxide and Propylene glycol were purchased from LobaChemie, Pvt.Ltd, Mumbai and Tea tree and sweet basil oil were purchased from Rajesh Chemicals, Mumbai.

VII. EXPERIMENTAL WORK

7.1 Preformulation studies:

Preformulation is a phase of drug development in which the physical, chemical, and mechanical properties of drug components are assessed separately and in combination with excipients in order to generate a stable, safe, and effective dosage form. Physical qualities may eventually provide a reason for formulation design, support the necessity for molecular modification, or simply indicate that there are no significant barriers to formulation development. As a result, colour, taste, solubility analysis, melting point determination, and compatibility investigations are all conducted on the medication sample obtained.

7.2 Physical Characteristic:

In physical characteristic we checked drug physical parameter such as colour, odour and surface nature. In this, colour of drug checked by visual observation, and odour check by taking a smell of drug, and surface texture checked by visual observation.Experimental data is given in Table No. 5.

7.3 Melting point:

Melting point of Clopidogrel bisulfate was determined by using Thiel's tube, in which sample was inserted into a capillary tube having one end sealed, then this filled capillary tube was inserted in the Thiel's tube which was filled with paraffin oil up to a certain level. Then this tube was heated into controlled manner with the help of the burner. The temperature at which drug sample starts to melt was noted as melting point temperature.Experimental data is given in Table no 6.

7.4 Thin layer chromatography (TLC):

TLC was performed for determination of impurity in the drug, using stationary phase, mobile phase and visual detector as follows:

7.4.1Preparation of Mobile Phase:

A mixture of Carbon tetrachloride: chloroform: acetone (6:4:0.15 v/v/v) was used as a mobile phase. This mixture was poured into a TLC tank, covered with a lined lid and presaturated with the solvent vapour system for at least 30 min at room temperature before use.

7.4.2 Procedure:

Firstly, paste of silica gel was prepared and spread it over glass slide, to develop thin layer on glass slide activated by keeping it in oven. Sample solutions was prepared by dissolving Clopidogrel bisulfate in ethanol, were applied to the marked start edge of the TLC plate (1- cm height) using the prepared capillary. The sample volumes for the assay experiments were 10 µL, and the volumes spotted for the purity. The plate was then allowed to air-dry for 10 min and then inserted into the TLC tank for development. Then plate was removed from TLC tank and air dry for 10 min, and exposed to iodine vapours. Spot were developed, its distance was measured from the starting point and Rf values were determined. Experimental data is depicted in Fig. no.7

7.5 Compatibility studies:

7.5.1 FT-IR:

Clopidogrel bisulfate was confirmed by FT-IR spectroscopy using Shimadzu 8300 spectrometer and Hyper FT-IR software from Shimadzu, Japan. The drug sample was dispersed in the KBr (200-400 mg) using a mortar, triturated the material into fine powder, and compressing the powder bed into the holder using a compression gauge with 140 mps pressure. The pellet was placed in the light path and the spectrum was recorded. The unique peaks of the functional groups were interpreted and compared with IR spectrum as specified in pharmacopoeial requirements.Spectral analysis data are given in Table no. 7 to 9 and Fig. no.8 to 10.

7.5.2 Differential scanning calorimetry:

7.5.2.1 Procedure:

Difference scanning calorimetry was performed to obtain suitable thermo grams. The accurately weighed sample was placed in an aluminium pan and an empty aluminium pan was used as reference. The experiment was performed under nitrogen flow, at a scanning rate 30 ^{0}C/min. in range of 50-300 ^{0}C.

7.6 Analytical methodology:

7.6.1 Determination of λ_{max} and calibration curve of Clopidogrel bisulfate in different solvent for authentication of the drug solubility, *in-vitro* and *in-vivo* studies:

7.6.2 U.V spectrophotometric analysis:

7.6.3 Determination of λ_{max} and calibration curve ofClopidogrelbisulphate in ethanol or methanol, or and distilled water pH 5.5.

7.6.4 Determination of λ_{max} of Clopidogrelbisulphate in ethanol or methanol, or and distilled water pH 5.5.:

Clopidogrel bisulfate (100 mg) was accurately weighed and transferred to 100 mL volumetric flask and diluted up to the mark with ethanol or methanol, or and distilled water pH 5.5 to obtain final concentration of 1000 µg/mL and used as a stock solution. From the stock solution working standard solutions from 10 µg/mL was prepared by appropriate dilution with ethanol. They were scanned in the UV region of 400-200 nm. The spectrum was obtained and the maximum absorbance was found out for detection λ_{max} of Clopidogrel bisulfate in ethanol, or methanol, or and distilled water pH 5.5.Experimental data are given in Table no. 10 to 15 and Fig.no. 11 to 16 respectively.

7.6.5 Construction of calibration curve of Clopidogrel bisulfate in ethanol or methanol, or and distilled water pH 5.5

Clopidogrel bisulfate (100 mg) was accurately weighed and transferred to the 100 mL volumetric flask and diluted up to the mark with ethanol or methanol, or and distilled water pH 5.5 to obtain final concentration of 1000 µg/mL and by using the above stock solution with appropriate dilutions of the 2, 4, 6, 8, and 10 µg/mL concentrations were prepared and absorbance of these solutions were estimated using UV spectrophotometer. Calibration graph was plotted concentrations solution verse absorbance.Experimental data are given in Table No.10 to 15 and Fig. No. 11 to 16 respectively.

7.7 Selection of polymer concentration for preparation of transdermal patches:

For selection of polymer concentration for preparation of transdermal patches, the varying concentration of (HPMC E5 and ERS 100) polymers were taken along with plasticizer PEG 400-103mg and Clopidogrel bisulfate 66 mg. The patches were prepared by solvent evaporation technique. The prepared patches were evaluated for physical appearance, thickness, % moisture content, % moisture absorption, drug content, and *in –vitro* diffusion studies.

Table No. 1 Composition of polymer concentration.

Sr.no.	Polymers	
	HPMC E5	ERS 100
1	100	100
2	200	100
3	300	100
4	100	200
5	100	300

7.8 Calculation of dose for preparation of transdermal patches:

The dose of Clopidogrel bisulfate has been calculated on the basis of various parameters such as pharmacokinetic properties as well as pharmacodynamics properties. The various factors of drug are taken into consideration such as saturation solubility of the drug, elimination half-life of drug, therapeutic concentration of drug, clearance rate and protein binding etc. From these values the dose has been calculated.

Table No. 2 Standard parameters for dose calculation of Clopidogrel bisulfate

Sr.no.	Parameters	Values
1	Saturated solubility of drug	50.78mg/L
2	Half life	8 h.
3	Total body clearance	30
4	Therapeutic concentration	2.2 ng/mL
5	Area	$10cm^2$
6	Bioavailability factor	F=1

$T_{1/2}= 0.693/K_E$ ---------- (1)

$K_E = 0.693/8 = 0.0866$

From the above mentioned values we can calculate the volume of distribution of drug

$Cl_T=K_E \ XV_d$---------- (2)

Thus, $V_d=Cl_T/K_E$

$V_D= 30/0.0866$

V_d=346 mL/kg/min

Plasma conc of drug (C_{SS}) =2.2 ng/mL

Output rate=$Cl_T\times$ 70Kg (Normal body weight of human) ------- (3)

= 30×70= 210mL/min=12600ml/h.

Jss i.e. Total Flux can be calculated by using following formula

$J_{SS}=Cl_T X\ CP_{SS}/A$ ---------- (4)

$J_{SS}= 12600 \times 0.0022/10$

$J_{SS}= 6.6mg/cm^2/h.$

For $10cm^2$, J_{ss}= 66mg

Mass of drug (M) delivered across skin= $P_{estimate}X\ C_S$ ---------- (5)

= 2.5×0.05078

= $0.1269\mu g/cm^2/h.$

Table No. 3 Formulation composition

Formulation Composition:

Sr. No.	Ingredients	Uses
1	Clopidogrel bisulphate	API
2	Polyethylene Glycol 400	Plastisizer
3	Dimethyl Sulfoxide	Penetration Enhancer
4	Euvalyptus Oil	Penetration Enhancer
5	Clove Oil	Penetration Enhancer
6	Tea Tree	Penetration Enhancer
7	Sweet Basil Oil	Penetration Enhancer

Table No. 4 Formulation table

Formulation Table:

Batch no.	Penetration enhancers			Batch no.	Penetration enhancers		
	DMSO	Oil			Oil		
		Eucalyptus	Clove		TT*	Eucalyptus	SB**
SN1	0	5	10	SP1	5	5	10
SN2	0	10	5	SP2	5	10	5
SN3	5	10	0	SP3	10	5	5
SN4	10	5	0	SP4	0	10	10
SN5	10	0	5	SP5	10	10	0
SN6	5	0	10	SP6	10	0	10

SB** Sweet Basil Oil TT* Tea Tree Oil

7.9 *IN- VIVO* DRUG CONTENT STUDIES BY USING RP-HPLC

Four male rats of 10-12 weeks old weighing 200 gm were selected. They were kept with husk beeding and were fed with standard rodent pellet diet and water. Light and dark cycle with 12 h light and 12 h dark was maintained. The temperature and relative humidity conditions were 28 °C±2 °C and 60 ±15% respectively. Before the experiment, the animal hair at the patch application site was cut at night. Patch samples were put to the shaved spot in the dorsal surface after the skin was gently cleaned with warm water, followed by an alcohol swab and patted dry. The films were carefully removed at precise intervals of time and evaluated for drug content deducted from the original content in the film using RP-HPLC at 215 nm. The mobile phase consisted of 0.1% trifluroacetic acid in water: 0.1% trifluroacetic acid in acetonitrile. The mobile phase was set at a flow rate of 1 mL/min. The run time of sample was kept 10 min. The ultraviolet detection was performed at 215 nm. The % drug content was calculated according to area obtained. Results are given in Table no. 26 to 27 and Fig. no. 31 to 36.

7.10. FACTORIAL DESIGN

The goal of employing a full 3^3 factorial experimental design was to perform a thorough investigation of the effect of process parameters and their interactions with the use of appropriate statistical tools. (Design –Expert DX10 software).

The amount of DMSO (X_1), eucalyptus oil (X_2), and clove oil (X_3) were chosen as independent variables for A1 batch and tea tree oil (X1) eucalyptus oil (X_2) and sweet basil oil (X_3) were taken as independent variables for A2 batch, three factors were evaluated, each at 5 levels. Experimental trials were performed at all 6 possible combinations. Multiple linear regression analysis, ANOVA, and graphical representation of the influence of factor by contour and 3D surface plots were performed. The factorial design batches were evaluated for % moisture content, thickness, diffusion at 1, 4 and 6 h (5 levels) respectively were taken as dependent variables. Results are depicted in Table no. 28 to 35 and Fig.no. 37 to 46.

VIII. RESULT AND DISCUSSION

8.1 Evaluation of transdermal patches formulations:

8.1.1 Physical Appearance: All the prepared patches were visually inspected for colour, clarity, flexibility and smoothness.

8.1.2 Thickness of the patch:

Using a digital micrometer, the thickness of the drug-loaded patch was measured at several spots and the average thickness and standard deviation were calculated to ensure the thickness of the created patch.Results are given in Table no. 18.

8.1.3 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 h. After 24 h the films are to be reweighed and determine the percentage moisture content from the below mentioned formula.Results of are given in Table no. 19.

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

8.1.4 Percentage moisture uptake

To maintain a RH of 84 %, the weighted films were maintained in desiccators at room temperature for 24 hours in a saturated potassium chloride solution. After 24 hours, reweigh the films and use the calculation below to calculate the percentage moisture uptake.[99]Results are given in Table no. 20.

Percentage Moisture Uptake=Final Weight − Initial Weight/Initial Weight ×100

8.1.5 Folding endurance:

A strip of a certain area was cut uniformly and folded over and over until it snapped. The value of the folding endurance was determined by the number of times the film could be folded in the same location without breaking.[100] Results are given in Table no. 21.

8.1.6 Water vapour permeability (WVTR):

Glass vials with a capacity of 5 mL were properly cleaned and dried in an oven to a consistent weight. The vials were filled with about 1 gramme of fused calcium chloride, and the polymer films were adhered to the brim with adhesive tape. The vials were then weighed and stored for 24 hours in a humidity chamber with a RH of 85 percent. To record the weight gain, the vials were withdrawn and weighed at various time intervals such as 3, 6, 12, 18, and 24 hours.Results are given in Table no. 22.

$$\text{Transmission rate} = \frac{\text{Final weight- Initial Weight}}{\text{Time} \times \text{Area}}$$

8.1.7 Drug content:

A 1 cm^2 film was cut into little pieces, placed in ethanol, and shook constantly for 24 hours. After that, the entire mixture was ultrasonicated for 15 minutes. After filtration, the drug was spectrophotometrically assessed to ascertain its content.Results of drug content determination are given in Table no. 23.

8.1.8 *In-vitro* drug diffusion studies:

8.1.8.1 Preparation of skin:

The Long-Evans rat's abdomen skin was utilized. After being sacrificed by protracted chloroform inhalation, hairs on the abdomen area were shaved. The subcutaneous tissue was surgically removed and the abdominal skin was excised. To eliminate any remaining fat, the dermis side was cleaned with isopropyl alcohol. After that, the skin was cleansed with distilled water. The entire thickness skin was treated for 6 hours with a 2M sodium bromide solution in water. Using a cotton swab wet with distilled water, the epidermis was detached. After that, the epidermis sheet was cleansed with distilled water. The skin was then wrapped in Aluminium foil and stored in the freezer at -20°C until needed.

The permeation experiments were carried out using a thermostatically controlled Franz diffusion cell setup. The receptor compartment was covered with excised rat epidermis, with the dermis facing the donor compartment. Samples were withdrawn from the sampling port at predetermined intervals of 30 min, 1 h, 2h, 3h, 4h, 5h and 6h, and the same quantity of fresh buffer was replaced at the same time to maintain sink conditions. Distilled water of pH 5.5 was filled in receptor compartment. The temperature was maintained at 32±2 °C and stirring rate was 100 rpm. The diffusion studies were carried out for 6 h. Samples were analyzed spectrophotometrically at absorption maxima of 219.5 nm. Results are depicted in Table no. 24 and 25.

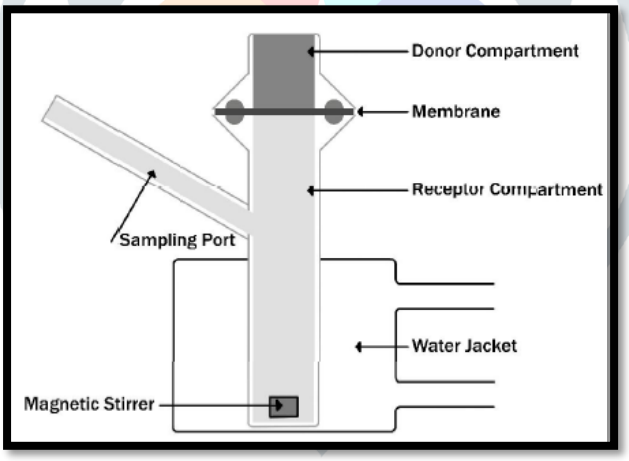


Fig. No. 6 Franz diffusion cell

8.1.9 Microscopic studies by using scanning electron microscopy:

SEM was used to verify the uniformity of particle shape and size. The sample was spread on a brass stub with a small piece of adhesive carbon tape. The sample, then subjected to gold coating using sputtering unit (model: JFC1600) for 10 sec at 10 mA of current. The gold coated sample placed in chamber of SEM (Jeol, JSM 6390 LA) and secondary electron/Back Scattered electron images are recorded. Analysis is given in Fig.no. 27 and 28.

8.2 Preformulation study:

8.2.1 Physical characteristic of Clopidogrel bisulfate:

8.2.1.1 Identification of pure drug:

Table No. 5 Physical characteristic of Clopidogrel bisulfate

Sr.no.	Test	Observation	Inference
1	Colour	White	Complies with I.P.

2	Odour	Odorless	Complieswith I.P
3	Surface nature	Crystalline powder	Complieswith I.P

8.2.1.2 Melting point:

Table No. 6 Melting point of Clopidogrel bisulfate

Sr. no.	Standard	Practically found	Inference
1	182-184 ^{0}C	184 ^{0}C	Complies with I.P

Melting point of Clopidogrel bisulfate was found to be 184 ^{0}C while as per standard literature; it is reported to be 182-184 ^{0}C. So it can be concluded that Clopidogrel bisulfate was in a pure state.

8.2.1.3 Thin layer chromatography:

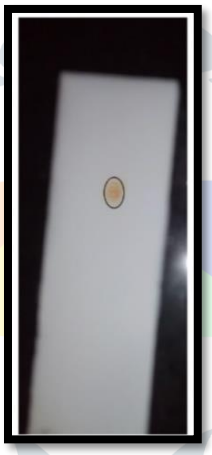


Fig. No. 7 TLC of Clopidogrel bisulfate

Retention factor of Clopidogrel bisulfate was calculated by using following formula

$$\text{Rf value} = \frac{\text{Distance travelled by solute from base line}}{\text{Distance travelled by solvent from base line}}$$

From this formula the R_f value of Clopidogrel bisulfate was found to be 0.36. From this it could be concluded that R_f value of Clopidogrel bisulfate matches to the std. Clopidogrel bisulfate. In the Fig.no.7 showed only one spot of Clopidogrel bisulfate from this it may be concluded that there was no presence of any impurities.

8.2.1.4 Identification and characterization of drug and excipientsby FT-IR absorption spectroscopy:

The FT-IR spectroscopy is one of the most powerful analytical techniques which offer the possibility of chemical identification and authentication. The characteristic FT-IR absorption regions of important bands necessary in the elucidation of drug and excipients presented below.

a) FT-IR spectra of Clopidogrel bisulfate:

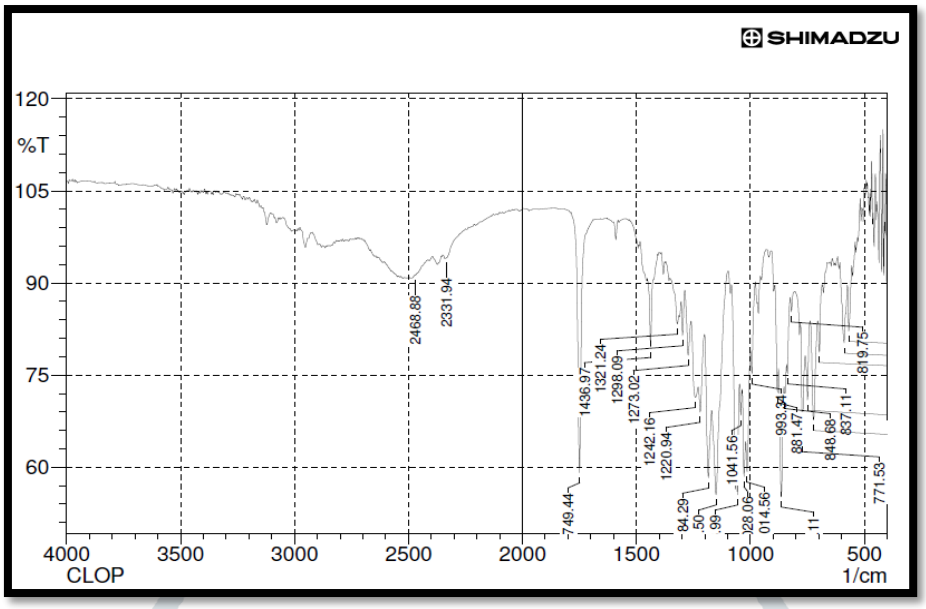


Fig. No.8 FT-IR spectra of Clopidogrel bisulfate

FT-IR interpretation data of Clopidogrel bisulfate

Table No. 7FT-IR interpretation of Clopidogrel bisulfate

Sr.no.	Compound	Frequency (cm^{-1})	Functional group
1	**Clopidogrel bisulfate**	2468	C-H str. (Alkanes)
2		2331	C-H str.
3		1730	C=Ostr. Ketones
4		1436	CH_3 Ben.
5		1321	S=O Sulfates
6		1220	R-COOstr. Esters
7		1041	C-N Amines
8		993	C-Hstr Aromatics
9		771	C-X str Chlorides

The principle absorption peak of drug showed C-H str. at 2468 cm^{-1}, C=O str. at 1730 cm^{-1},CH_3 str. at 1436 cm^{-1}, S=O str. at 1321 cm^{-1},C-O str. at 1220 cm^{-1}, C-N str. at 1041 cm^{-1}, and C-X Structureat771 cm^{-1}. From this we could identified and authenticate that the given drug was Clopidogrel bisulfate.

b) FT-IR Spectra of Eudragit RS-100 (ERS-100):

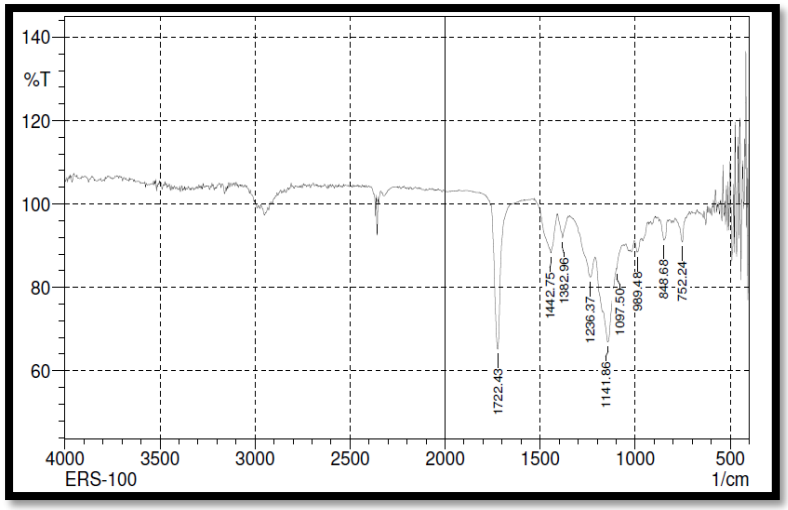


Fig. No. 9 FT-IR spectra of ERS-100

Table no. 8FT-IR interpretation of ERS-100

Sr.no.	Compound	Frequency (cm^{-1})	Functional group
1	**Eudragit RS-100**	1722	C=O str.Ketone
		1442	CH_2 ben
		1382	C-N str. Amines
		1236	C-Ostr.Ether
		1097	CH_3 ben
		752	C-Cl str.

The principle absorption peak of excipient showed RC=O str. at 1722.94 cm^{-1}, CH_2 ben. at 1442 cm^{-1}, C-N str. at 1382 cm^{-1}, C-O str. at 1236 cm^{-1}, C-X str. at 752 cm^{-1}, From this we could identified and authenticate that the given excipient was ERS-100.

c) FT-IR Spectra of HPMCE5:

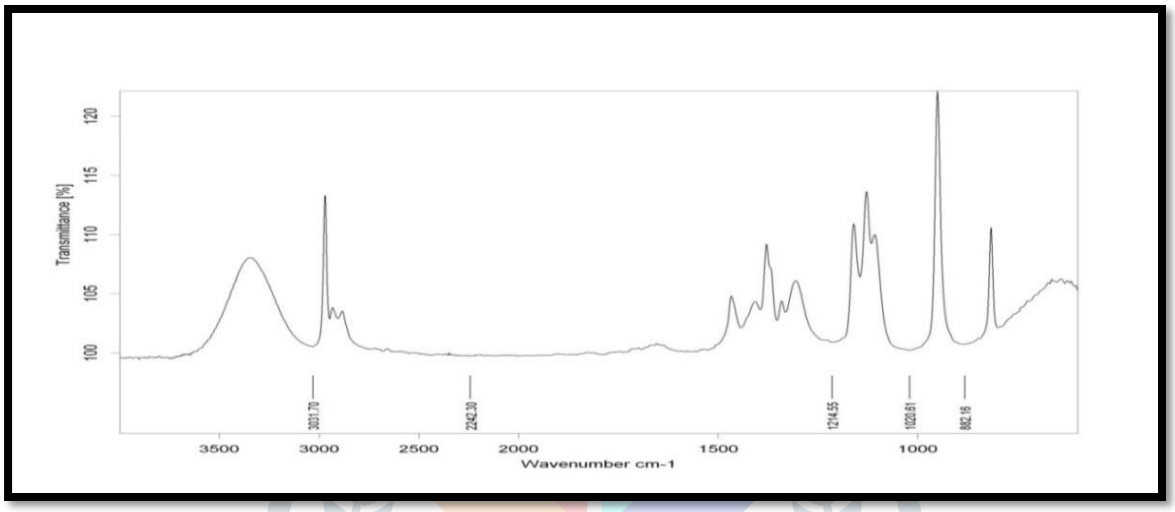


Fig. no. 10FT-IR Spectra of HPMCE5

Table no. 9FT-IR Interpretation of HPMC E5

Sr. no.	Compound	Frequency (cm^{-1})	Functional group
1	**HPMC E5**	3031	C-H str.
		1665	C=C str.
		1330	OH str.

XI. ANALYTICAL METHODOLOGY

9.1 UV spectrophotometric analysis

9.1.1 Determination of λ_{max} and calibration curve of Clopidogrel bisulfate in ethanol

Determination of λ_{max} of Clopidogrel bisulfate in ethanol at 213.5 nm

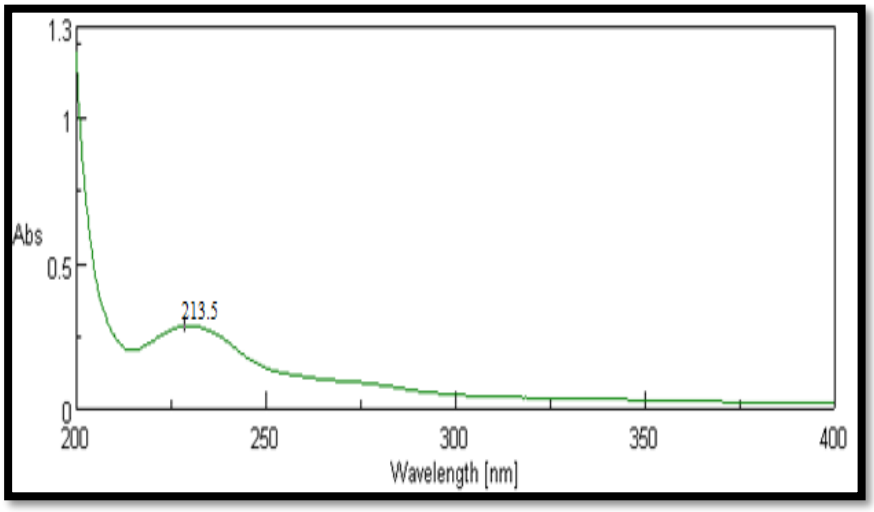


Fig. No.11 λ_{max} of Clopidogrelbisulphate in ethanol

Absorption spectra in the range (200-400 nm) were obtained forClopidogrel bisulfate in ethanol. The drug exhibited an absorption maximum at 213.5 nm. A linear relationship between the λ_{max} (213.5) and the concentration ofClopidogrel bisulfate was established over the examined concentration range (2-10 µg mL^{-1}).

Construction of calibration curve of Clopidogrel bisulfate:

Absorbance and conc. data of Clopidogrel bisulfate in ethanol at 213.5 nm:

Table no.10 Absorbance and conc. data of Clopidogrel bisulfate in ethanol

Sr.no.	Conc. µg/mL	Absorbance
1	2	0.2199
2	4	0.4086
3	6	0.6812
4	8	0.9214
5	10	1.2612

Calibration curve of Clopidogrel bisulfate in ethanol:

Determination of calibration curve of Clopidogrel bisulfate in ethanol:

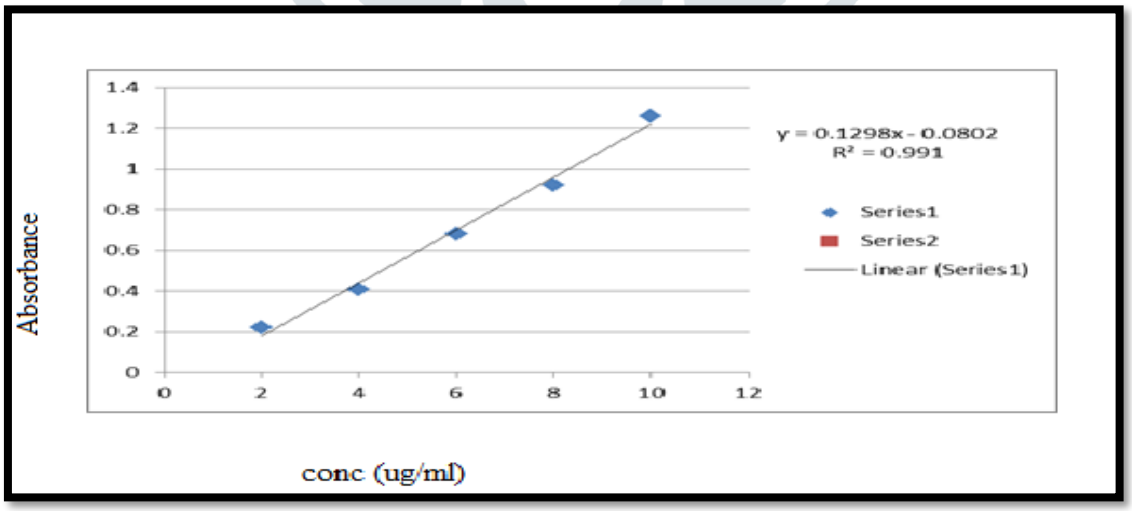


Fig. No.12 Calibration curve of Clopidogrel bisulfate in ethanol

Calibration data of Clopidogrelbisulphate in ethanol:

Table no. 11 Calibration data of Clopidogrel bisulfate in ethanol

Sr. no.	λ max (nm)	Solvent used	Conc. range (µg/mL)	Regression equation	Regression coefficient (R^2)
1	213.5 nm	Ethanol	2-10	y = 0.1298x	0.991

The calibration curve of Clopidogrel bisulfate in ethanol was found to be linear in the range of 2-10 µg/mL and coefficient of correlation was found to be 0.991.

9.1.2 Determination of λ max and calibration curve of Clopidogrel bisulfate in methanol:

Determination of λ max of Clopidogrel bisulfate in methanol at 228.4nm:

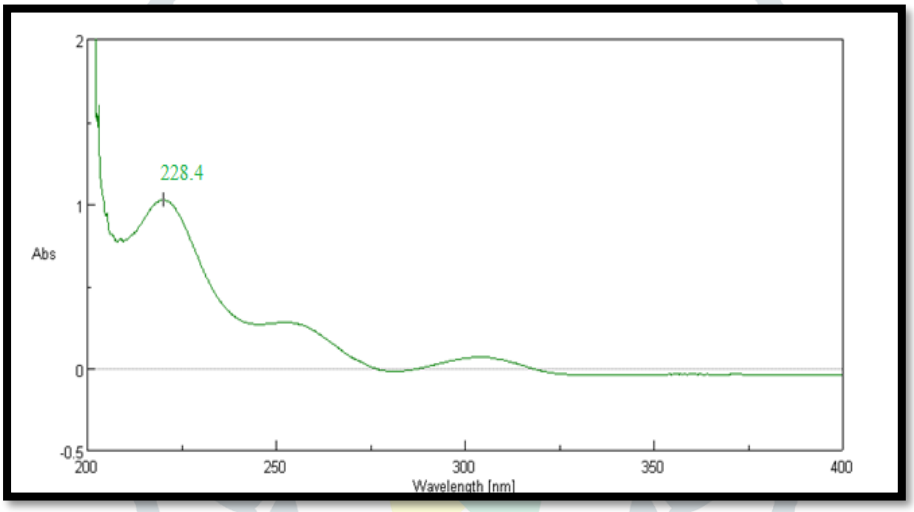


Fig. No. 13λ max of Clopidogrel bisulfate in methanol

Absorption spectra in the range (200-400 nm) were obtained for Clopidogrel bisulfate in methanol. The drug exhibited an absorption maximum Clopidogrel bisulfate at 228.4 nm. A linear relationship between the λ max (228.4nm) and the concentration of was established over the examined concentration range (2-10 µg mL^{-1}).

Construction of calibration curve of Clopidogrel bisulfate:

Absorbance and conc. data of Clopidogrel bisulfate in methanol at 228.4 nm:

Table no. 12 Absorbance and conc. data of Clopidogrel bisulfate in methanol

Sr.no.	Conc. (µg/mL)	Absorbance
1	2	0.2240
2	4	0.4367
3	6	0.6417
4	8	0.8714
5	10	0.9987

Calibration curve of Clopidogrelbisulphate in methanol:
Determination of calibration curve of Clopidogrel bisulfate in methanol:

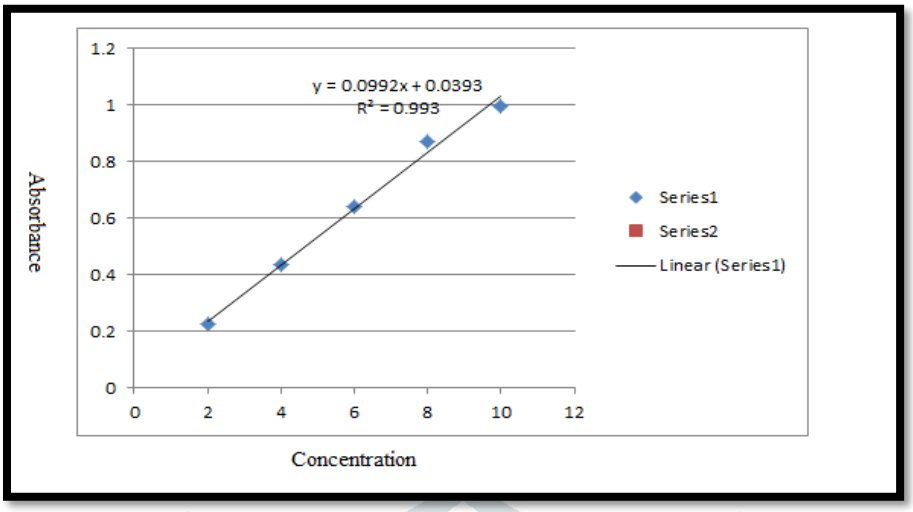


Fig. No.14 Calibration curve of Clopidogrel bisulfate

Calibration data of Clopidogrel bisulfate in methanol

Table no. 13 Calibration data of Clopidogrel bisulfate in methanol

Sr. no.	λ max (nm)	Solvent used	Conc. range (µg/mL)	Regression equation	Regression coefficient (r^2)
1	228.4 nm	Methanol	2-10	y = 0.0992x	0.993

9.1.3 Determination of λ max and calibration curve of Clopidogrel bisulfate in distilled water pH 5.5
Determination of λ max of Clopidogrel bisulfate in distilled water pH 5.5 at 219.9 nm

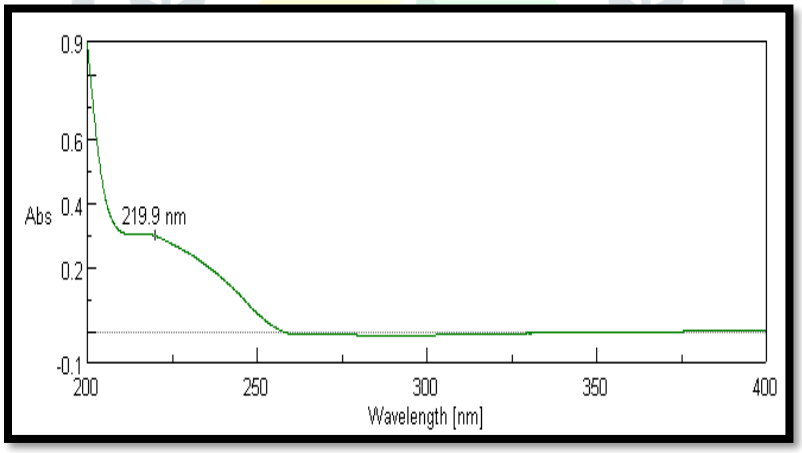


Fig.No.15λ_{max} of Clopidogrel bisulfate in distilled water pH 5.5

Absorption spectra in the range (200-400 nm) were obtained for Clopidogrel bisulfate in distilled water pH 5.5. The drug exhibited an absorption maximum at 219.9 nm. A linear relationship between the λ_{max} (219.9 nm) and the concentration ofClopidogrel bisulfate was established over the examined concentration range (2-10 µg mL^{-1})

Construction of calibration curve of Clopidogrel bisulfate in distilled water pH 5.5: Absorbance and conc. data of Clopidogrel bisulfate in distilled water at pH 5.5:

Table No. 14 Absorbance and conc. data of Clopidogrel bisulfate in distilled water at pH 5.5

Sr.no.	Conc. (µg/mL)	Absorbance
1	2	0.1229
2	4	0.2572
3	6	0.4321
4	8	0.6423
5	10	0.8251

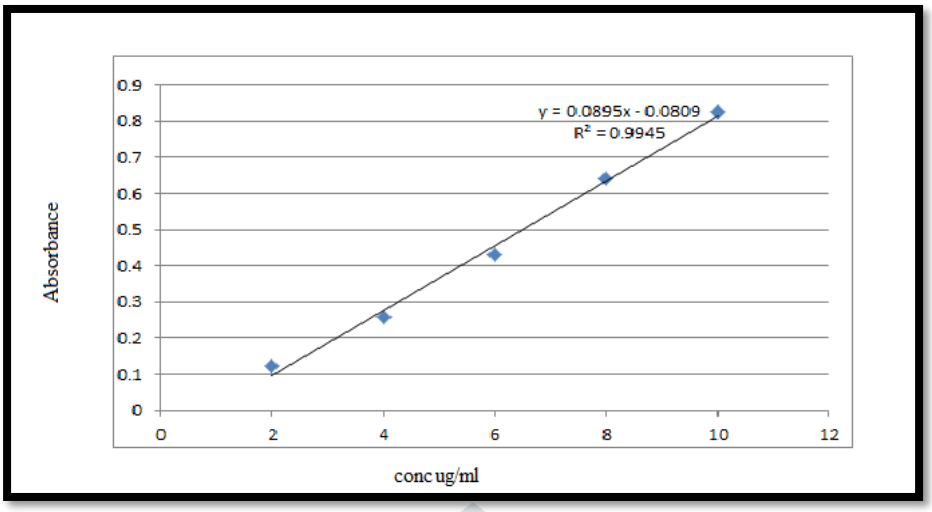


Fig. No.16 Calibration curve of Clopidogrel bisulfate in distilled water pH 5.5

Calibration data of Clopidogrel bisulfate in distilled water pH 5.5 :

Table No. 15 Calibration data of Clopidogrel bisulfate in distilled water pH 5.5

Sr. no.	**λ_{max}** (nm)	**Solvent**	**Conc. Range (µg/mL)**	**Regression equation**	**Regression coefficient** (R^2)
1	219.9	Distilled water pH 5.5	2-10	y = 0.0895x	0.9945

The calibration curve of Clopidogrel bisulfate in distilled water pH 5.5 was found to be linear in the range of 2-10 µg/mLand coefficients of correlation was found 0.9945

9.2 Drug excipients interaction study:

(a) Drug-excipient interaction study by DSC thermogram:

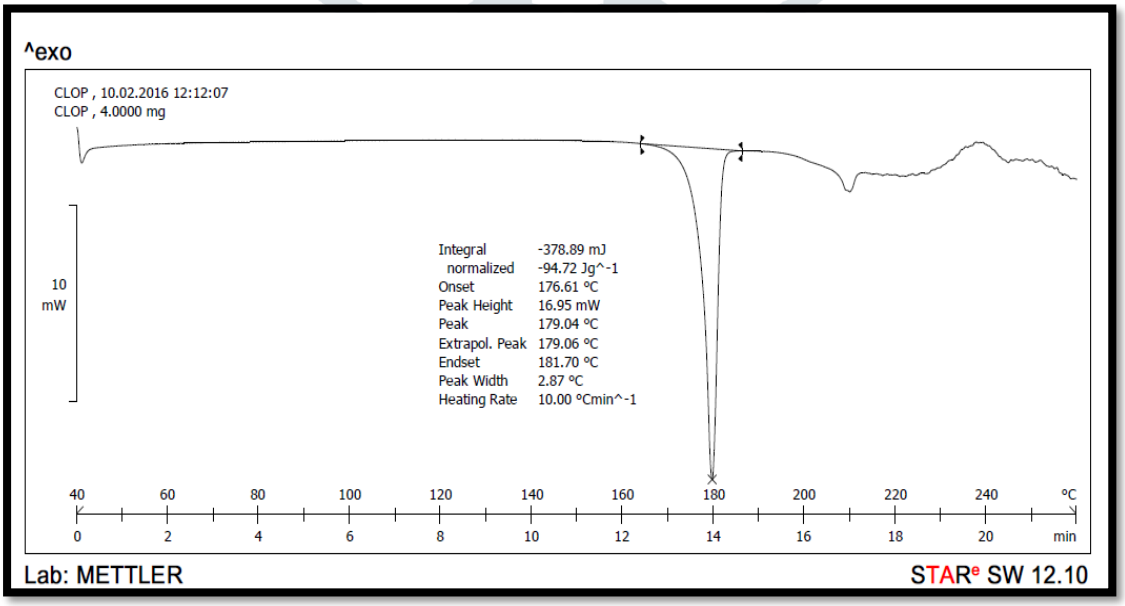


Fig. No. 17 DSC thermogram of Clopidogrel bisulfate

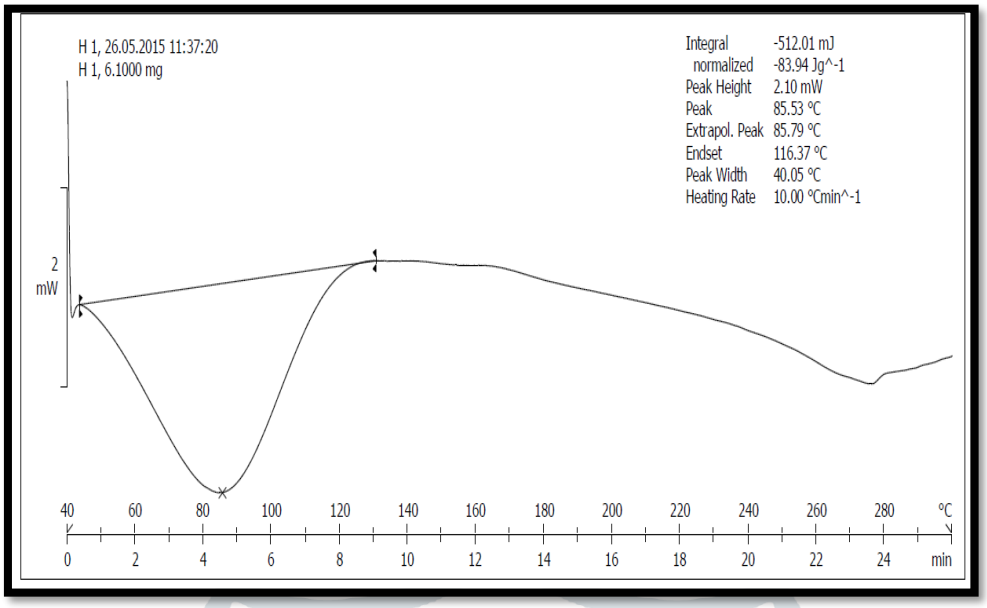


Fig. No. 18 DSC thermogram of HPMC E5

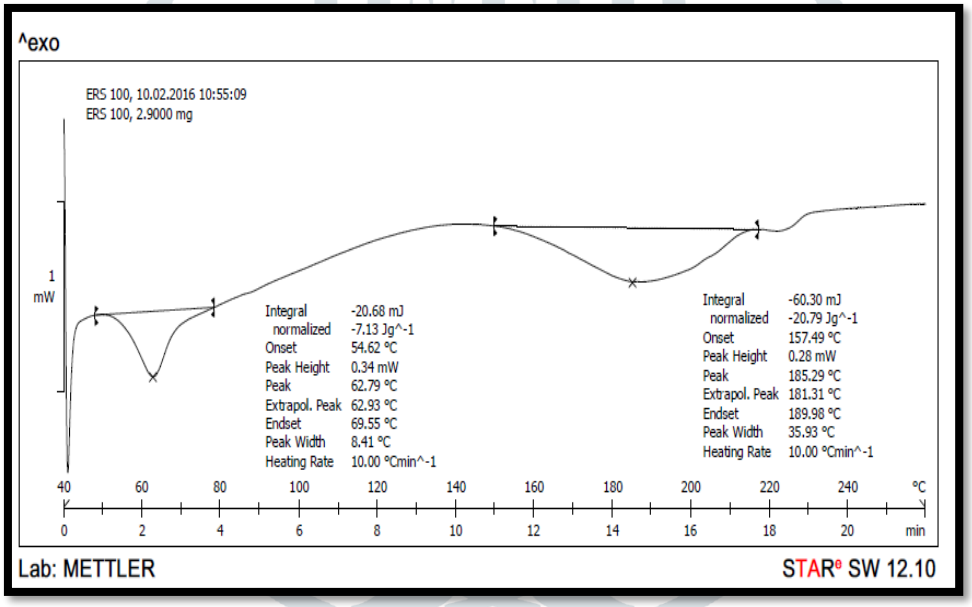


Fig. No.19 DSC thermogram of ERS -100

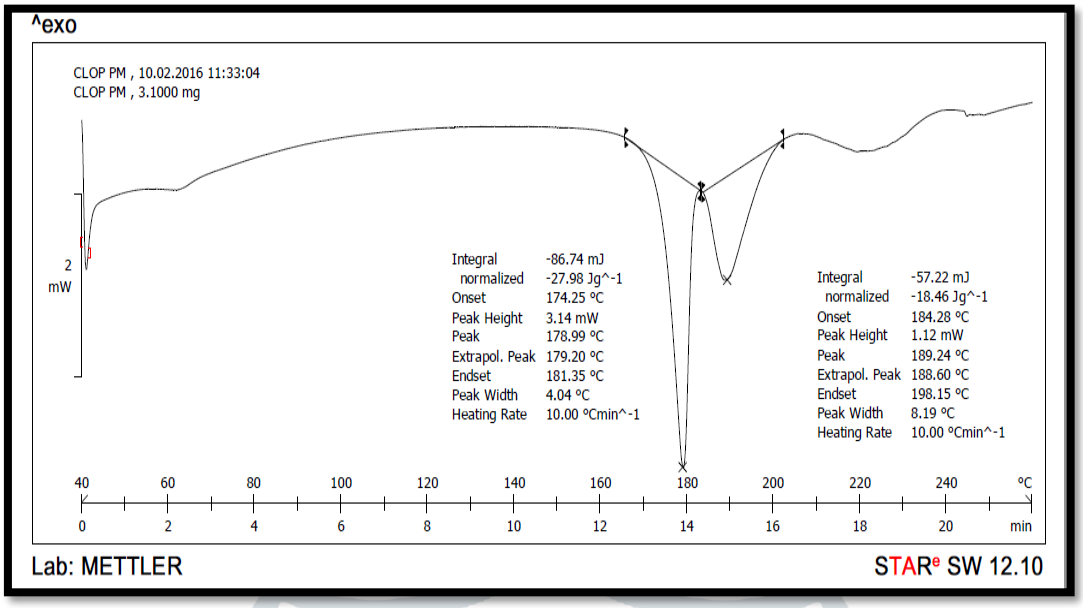


Fig. No.20 DSC thermogram of physical mixture of drug Clopidogrel bisulfate, HPMC E5 and RS -100

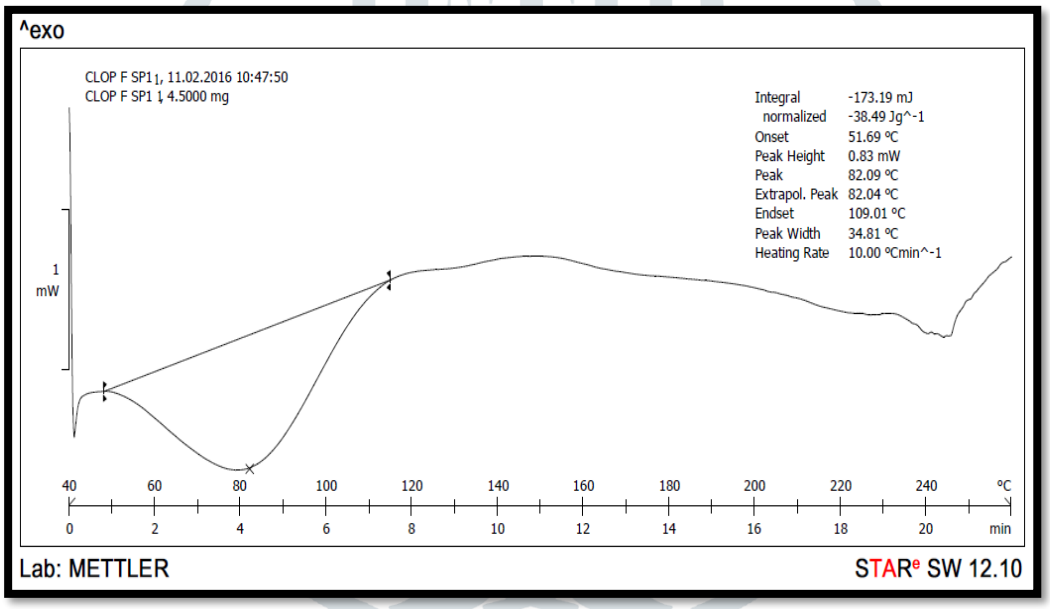


Fig. No.21 DSC thermogram of formulation SP5

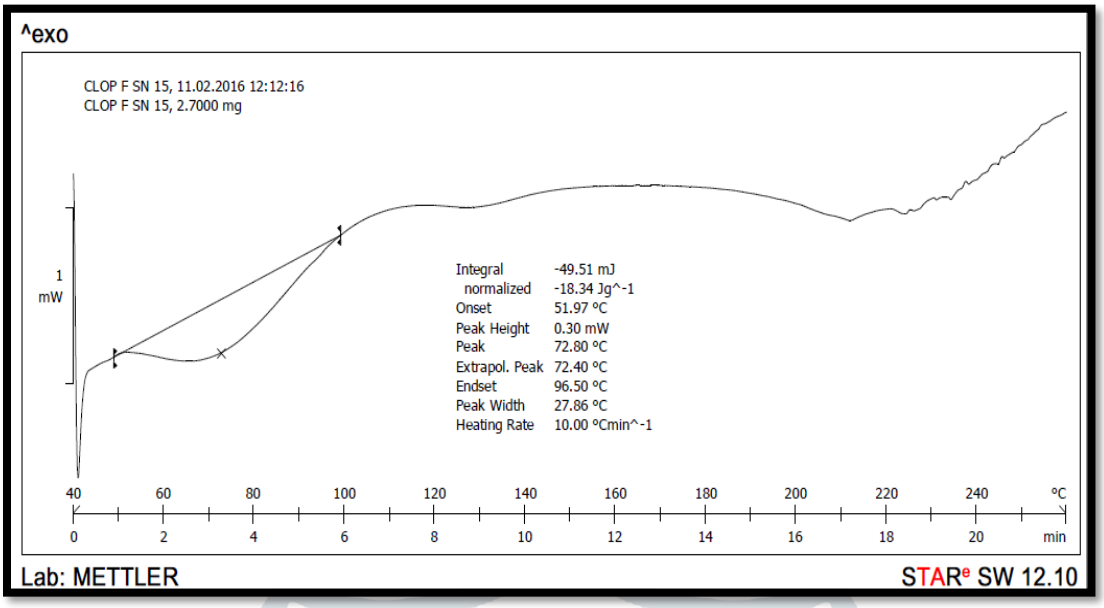


Fig. No.22 DSC thermogram of formulation SN6

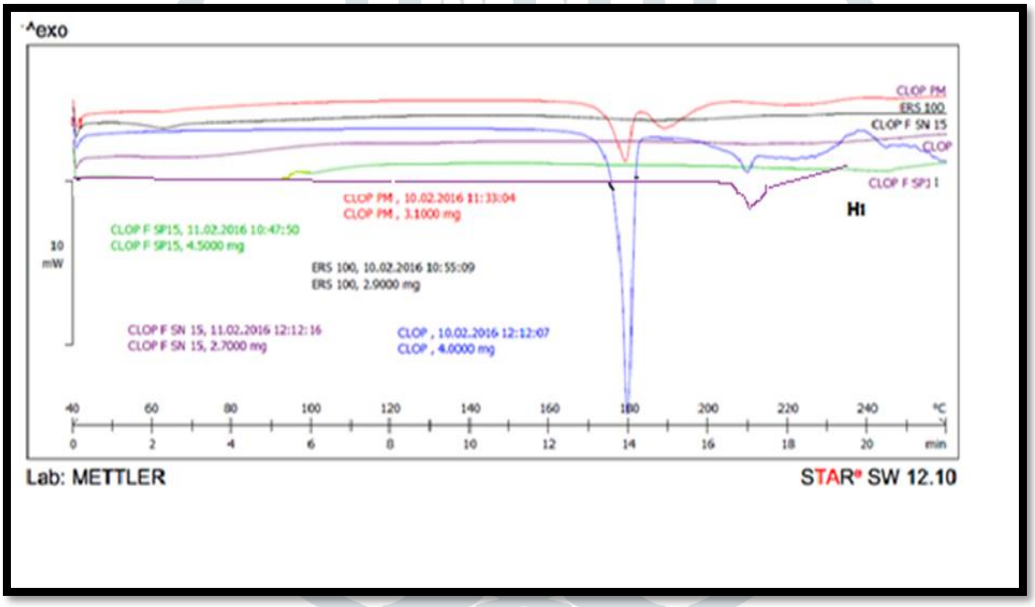


Fig. No.23 DSC overlay thermogram of ERS-100, HPMC E5, Clopidogrel bisulfate, SP5, and SN6

The DSC results provided both qualitative and quantitative information about thephysicochemical state of the drug present in formulation. The DSC thermograph of theClopidogrel bisulphate, polymers and mixture of Clopidogrel bisulfate, HPMCE5 and Eudragit RS 100 were obtained. The thermograph of pure drug showed a melting endothermic peak at 180.70 °C. In the thermograph of the mixture peak was observed at 180 °C. The DSC thermograms of the mixture showed sharp distinct endothermic peaks for Clopidogrel bisulfate. This corresponds to the individual drug and polymer peaks without any modification, indicating that the drug did not interact with the transdermal patch excipients. This confirmed that the presence of other excipients did not affect the drug stability.

(b) Drug- excipient compatibility study by FT-IR:

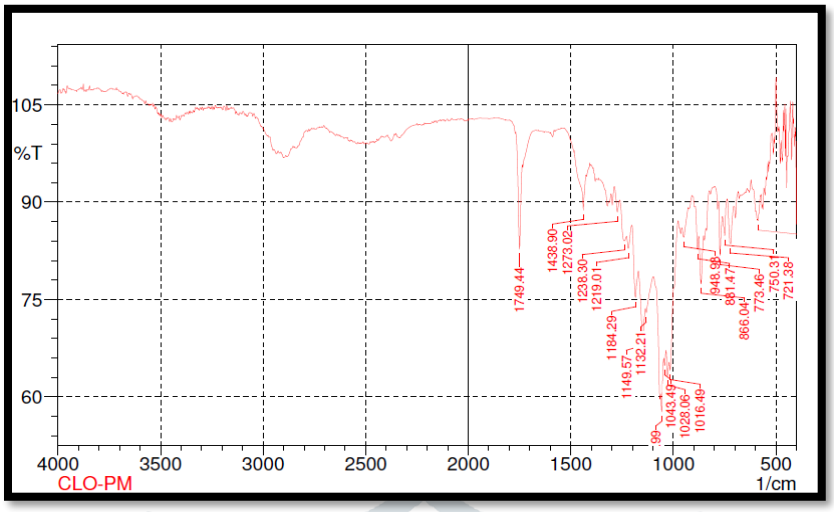


Fig. no. 24 FT-IR spectra of physical mixture of Clopidogrel bisulfate, ERS-100, and HPMC E5

Table No.16FT-IR interpretation data of physical mixture of physical mixture of Clopidogrel bisulfate, HPMCE5, and ERS-100

Sr.no.	Functional group	Drug	HPMCE5	ERS 100	Drug+ HPMCE5 +ERS 100
1	C-H str.(alkanes)	2468	2242	-	2621
2	C-H str.	2331	-		-
3	C=O str.Ketones.	1730	-	1722	-
4	CH_3 ben	1436	-	1442	
5	C-OHstr. Alcohols	1330	1330	-	-
6	S=Ostr. Sulfates	1321	-	-	1043
7	C-O str. Esters	1220	1330	1236	1238
8	C-N str.Amines	1041	-		1438
9	C-H str.Aromatics	993	-		948
10	C-Clstr.	771	-	752	773

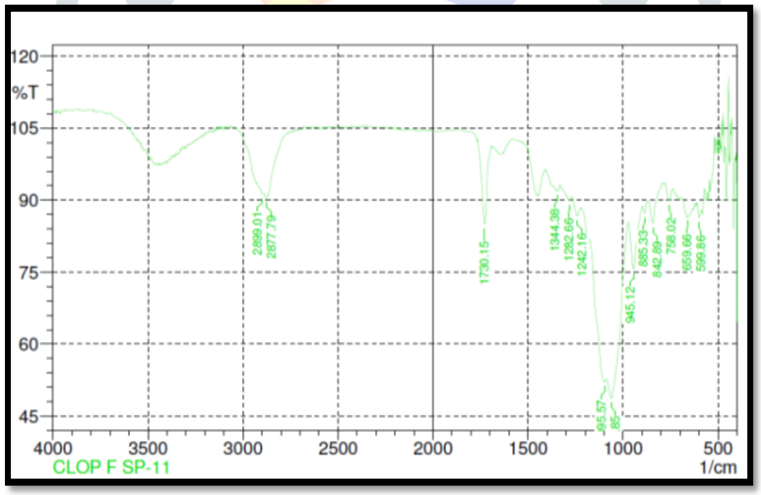


Fig.No.25 FT-IR of formulation SP5

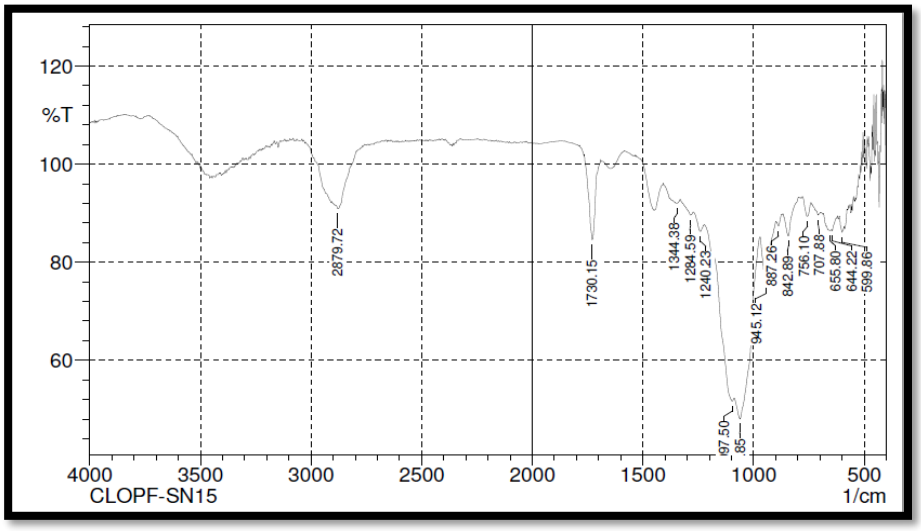


Fig.No. 26 FT-IR of formulation SN6

Table No.17FT-IR interpretation data of formulations

Sr.no.	Formulation	Frequency(cm^{-1})	Functional group
1	SN6	2899	C-H str.
2		2877	C-H str
3		1730	C=O str.Ketone
4		1314	C-N str.Amines
5		1282	C-O str.Esters
6		758	C-X str. Chlorines
7	SP5	2879	C-H str
8		1730	C=O str. ketone
9		1344	S=O str.Sulfates
10		1284	C-O str.Esters
11		756	C-Cl str.

X. EVALUATION OF CLOPIDOGREL BISULPHATE TRANSDERMAL PATCHES:

10.1 Physical appearance:

The formulated patches were thin flexible smooth uniform. The polymer combinations used generate a transparent, smooth, uniform, substantial, flexible, and desirable thickness film for Clopidogrel bisulfate transdermal medication delivery systems.

10.2 Thickness:

Table No. 18 Thickness of batch A1 and A2

Batch A1	Thickness	Batch A2	Thickness
SN1	0.12±0.0084	SP1	0.136±0.012
SN2	0.128±0.0084	SP2	0.148±0.016
SN3	0.17±0.0062	SP3	0.183±0.011
SN4	0.163±0.0084	SP4	0.117±0.0080
SN5	0.17±0.0108	SP5	0.132±0.0057
SN6	0.173±0.0023	SP6	0.108±0.0083

A1** DMSO, Eucalyptus oil and Clove oil.A2** Tea tree oil, Eucalyptus oil and Sweet basil oil.

The thickness of the given batches was found in the rage of0.12±0.0084 to 0.173±0.0023 and 0.108±0.0083 to 0.183±0.011 and it is found in the linear range due to same concentration of the polymers. The minimum standard deviation values assumed that the process used for preparing the drug delivery system is capable of giving reproducible result.

10.3 Percent moisture content:

Table No. 19 Moisture content of batch A1 and A2

Batch A1	% Moisture content	Batch A2	% Moisture content
SN1	9.3±0.012	SP1	4.28±0.93
SN2	3.14±0.89	SP2	4.95±0.04
SN3	3.46±0.12	SP3	7.14±0.98
SN4	4.34±0.77	SP4	19.62±0.007
SN5	5.47±0.44	SP5	7.62±0.32
SN6	3.28±0.65	SP6	2.89±0.145

A1** DMSO, Eucalyptus oil and Clove oil.A2** Tea tree oil, Eucalyptus oil and Sweet basil oil.

The percent moisture content of the prepared transdermal patches was found to be between 3.14±0.89 to 9.3±0.012 and 2.89±0.145 to 19.62±0.007. The formulation SN1 (A1) Showed highest moisture content of 9.3±0.012 reveals its higher hydrophilicity and formulation SN2 absorb least amount of moisture content of 3.14±0.89.The formulation SP4 (A2) showed highest moisture content of 19.62±0.007 and formulation SP6 absorb least amount of moisture content of 2.89±0.145.

10.4 % Moisture uptake:

Table No. 22 Moisture uptake of batch A1 and A2

Batch A1	% Moisture uptake	Batch A2	%Moisture uptake
SN1	7.88	SP1	6.60
SN2	7.61	SP2	4.80
SN3	6.42	SP3	4.24
SN4	4.3	SP4	3.12
SN5	4.22	SP5	3.95
SN6	2.9	SP6	11.02

A1** DMSO, Eucalyptus oil, clove oil, A2** Tea tree oil, Eucalyptus oil, sweet basil oil.

The % moisture uptake was found in between range of 2.9 to 7.88 and 3.12 to 11.02.The highest moistureuptake was found in the formulation SN1 and lowest amount was found in formulation SN6 of A1. For batch A2, the highest amount of moistureuptakewas found in the formulation SP6 and lowest amount was found in formulation SP4.

10.5 Folding endurance:

Table No. 21 Folding endurance of batch A1 and A2

Batch A1	Folding endurance	Batch A2	Folding endurance
SN1	96	SP1	99
SN2	98	SP2	88
SN3	92	SP3	98
SN4	102	SP4	79
SN5	101	SP5	93
SN6	99	SP6	85

A1** DMSO, Eucalyptus oil. , clove oil, A2** Tea tree oil, Eucalyptus oil, Sweet basil oil

The folding endurance was measured manually; films were folded 102 times maximum in Formulation SN4 (A1) and 103times maximum in Formulation SP1 (A2) if the film showed any cracks it was taken as end point.

10.6 Water vapour transmission rate (WVTR):

Table No. 22WVTR rate of batch A1 and A2

Batch A1	WVTR	Batch A2	WVT R
SN1	0.075	SP1	0.025
SN2	0.08	SP2	0.037
SN3	0.08	SP3	0.0416
SN4	0.104	SP4	0.054
SN5	0.12	SP5	0.03
SN6	0.039	SP6	0.045

A1** DMSO, Eucalyptus oil, and clove oil, A2** Tea tree oil, Eucalyptus oil and sweet basil oil

The water vapour transmission rate was found between range of 0.08 to 0.104 and 0.025 to 0.054.Water vapour transmission study determines the permeability characteristics of the patches. The result of water vapour transmission study revealed that all the formulations are permeable to water vapour.

10.7 Drug content:

Table No. 23 Drug content of batch A1 and A2

Batch A1	Drug content	Batch A2	Drug content
SN1	53.24±0.041	SP1	62.06±0.0124
SN2	85.46±0.423	SP2	74.09±0.0094
SN3	23.7±0.084	SP3	36.41±0.0169
SN4	52.42±0.02	SP4	77.48±0.0124
SN5	28.52±0.091	SP5	95.57±0.0081
SN6	92.81±0.040	SP6	44.56±0.0081

A1** DMSO, Eucalyptus oil, clove oil and A2** Tea tree oil, Eucalyptus oil, Sweet basil oil as penetration enhancers.

The developed formulation's drug content uniformity demonstrated that the procedure employed to prepare the transdermal film in this investigation was capable of producing film with uniform drug content. The result of drug content indicates that drug is uniformly dispersed in formulation. The formulation SN6 (A1) shows maximum drug release of 92.81±0.040 with maximum uniform dispersion of drug and the formulation SP5 (A2) shows highest drug release of 95.57±0.0081.

10.8 *In- vitro* drug diffusion study:

Table No. 24% Cumulative drug release of batch SN1-SN6

Time (hrs)	% Cumulative drug release					
	SN1	SN2	SN3	SN4	SN5	SN6
0.5	8.17 ±0.01	3.28 ±0.53	5.93 ±0.56	9.65 ±0.6	13.21±0.9	**6.72 ±0.21**
1	15.75 ±0.1	9.02±0.51	13.57±0.53	15.35 ±0.5	20.05±0.6	**25.11 ±0.45**
2	31.40± 0.1	20.44 ±0.33	21.06±0.23	21.25 ±0.3	28.22±0.2	**40.81 ±0.056**
3	45.12± 0.5	28.15 ±0.07	28.73±0.21	36.61 ±0.8	33.93±0.6	**51.12 ±0.012**
4	57.59± 0.3	33.38 ±0.44	33.18±0.24	47.24 ±0.3	57.78±0.5	**68.39 ±0.07**
5	62.9 ±0.07	40.16 ±0.63	42.11±0.24	56.21 ±0.0	66.23±0.2	**88.43 ±0.8**
6	67.72± 0.4	42.18 ±0.62	45.46±0.37	68.52 ±0.0	67.29±0.7	**92.47 ±0.9**

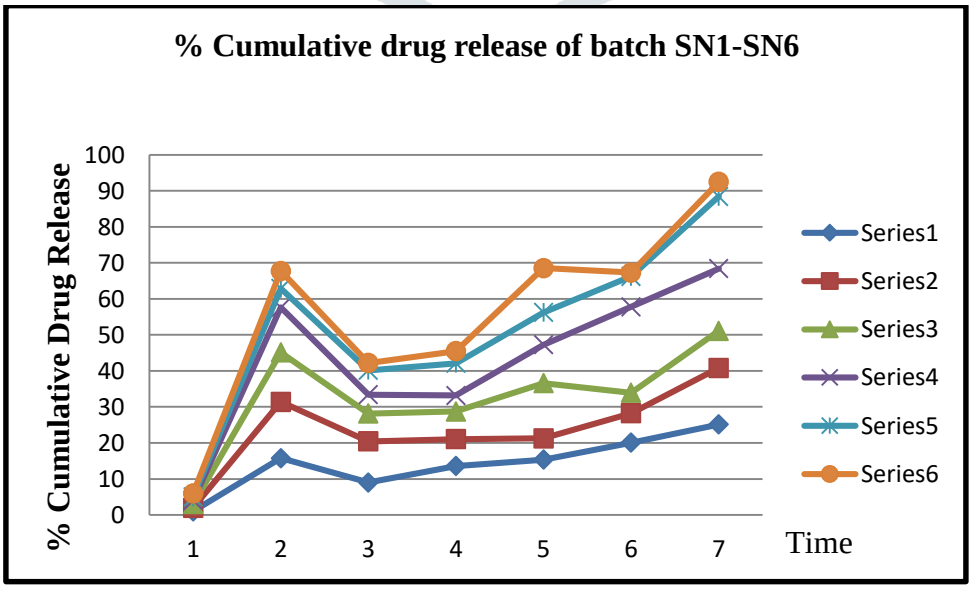


Fig.No.27 % Cumulative drug release of batch SN1-SN6

Table No. 25% Cumulative drug release of batch SP1-SP6

Time (hrs)	% Cumulative drug release					
	SP1	**SP2**	**SP3**	**SP4**	**SP5**	**SP6**
0.5	8.18 ±0.01	6.42 ± 0.34	6.64 ± 0.12	13.51 ±0.12	**6.74 ± 0.21**	5.009 ±0.02
1	15.60 ±0.23	15.41 ±0.14	23.62 ±0.23	28.02 ±0.01	**26.54 ±0.32**	7.85 ±0.07
2	21.67 ±0.34	30.20 ±0.45	31.81 ±0.31	33.92 ±0.71	**42.42 ±0.51**	15.35 ±0.11
3	29.37 ±0.07	46.29 ± 0.46	45.11 ±0.32	57.58 ±0.23	**54.51 ±0.02**	21.89 ±0.66
4	57.16 ±0.11	59.11 ±0.17	70.42 ±0.31	68.61 ±0.76	**66.06 ±0.23**	27.52 ±0.78
5	64.09 ±0.34	80.20 ± 0.18	71.06 ±0.16	71.05 ±0.34	**96.66 ± 0.11**	34.09 ±0.34
6	68.62 ±0.56	86.21 ±0.45	76.11 ±0.76	74.73 ±0.15	**96.67 ±0.42**	33.98 ±0.42

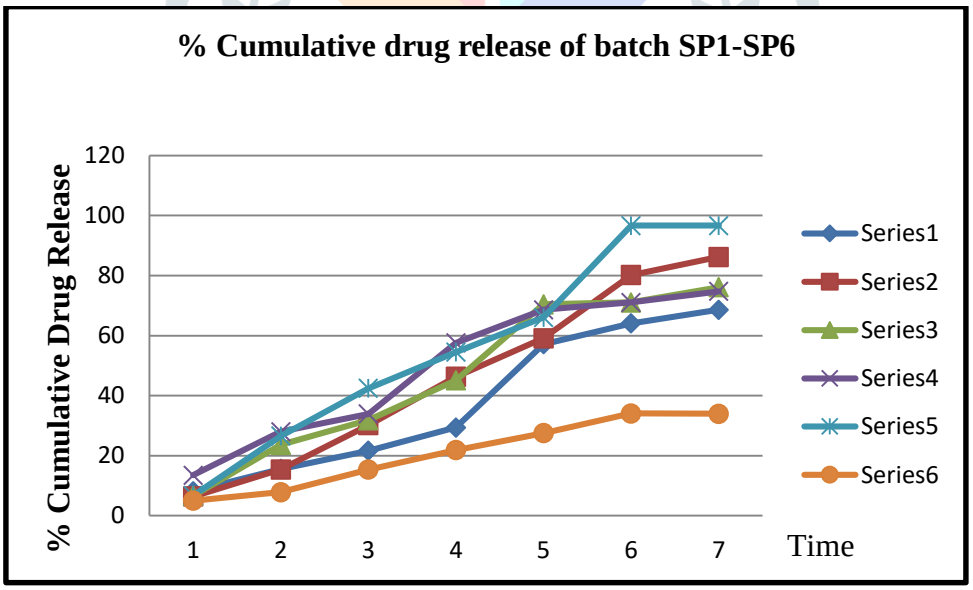


Fig.No.28 % Cumulative drug release of batch SP1-SP6

In-vitro drug release studies were carried out for the different formulations using Franz diffusion cell. The Medicated films showed drug Release study in % Cumulative release, by varying amount of penetration enhancer in polymer film, percent release can be varied, also the comparative effect of various penetration enhancers on % cumulative drug release were studied. Drug polymer affinity can be major factor that control release of drug from formulation for given time interval. Maximum % of drug release were observed in formulation SN6 (A1) i.e. 92.47 ±0.9% and for SP5 (A2) it was found to be 96.67 ±0.42. The addition of hydrophilic polymers such as HPMC E5 plays an important role in enhancement of drug release constant.

From the above study, the selection of batches were carried out on the basis of first five highest % cumulative drug release in given batches, from this data the linear graph of % cumulative drug release were plotted.

10.9 Microscopic evaluations:
10.9.1 Scanning electron microscopy:

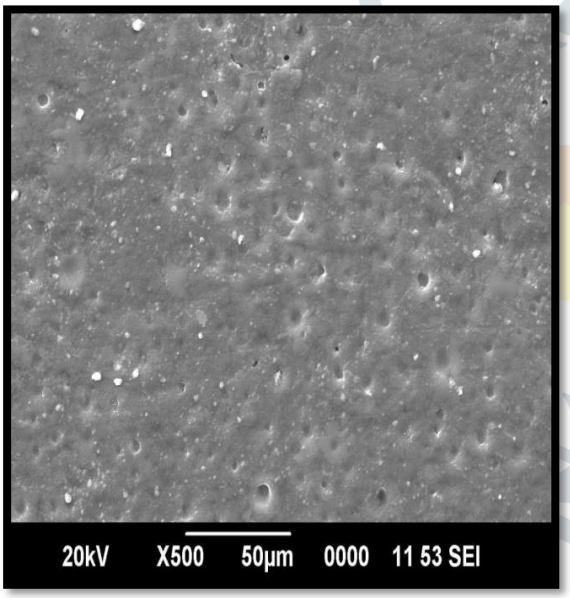


Fig. No.29SEM of formulation SN6

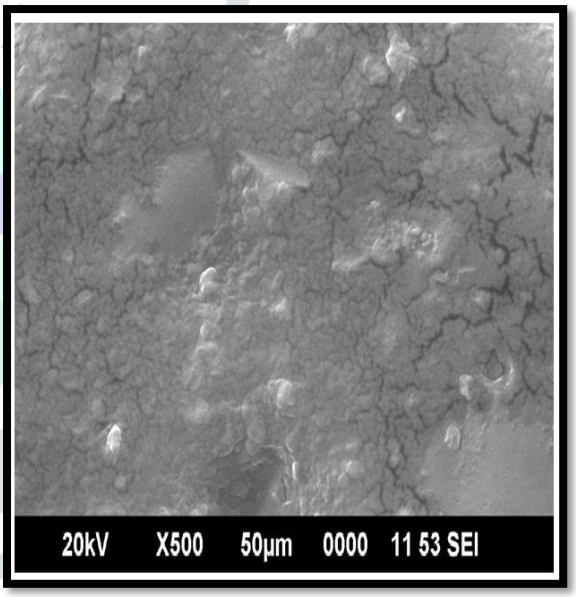


Fig.No.30SEM of formulation SP5

Scanning electron microscopy showed that the surface of formulation SN6 and SP5 seems to be rough but with structures like crystals, which seems to be more uniform. On the basis of SEM we could concluded that irregular the surface or rough the surface of transdermal patches, more and easy release of drug, due to lack of contact angle. Hence it was concluded that formulation SN6 and SP5 showed higher drug release than other batches.

10.10 *In-vivo* drug release by using RP-HPLC

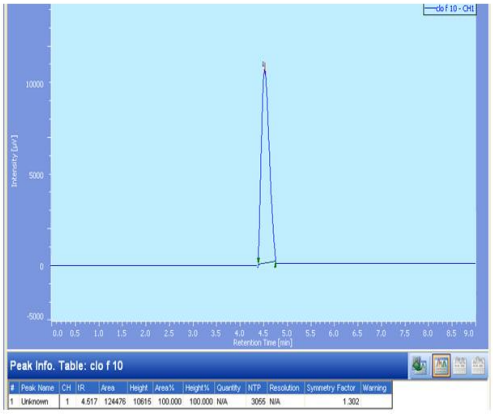


Fig. no.31 pure drug

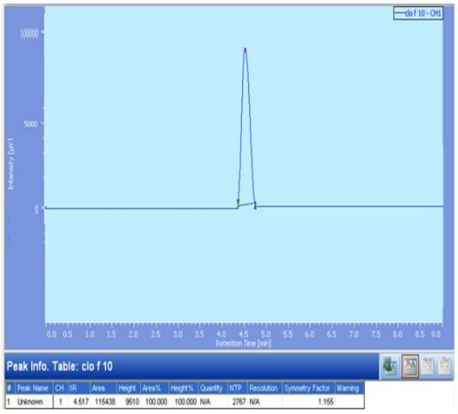


Fig. no.32 Initial drug content

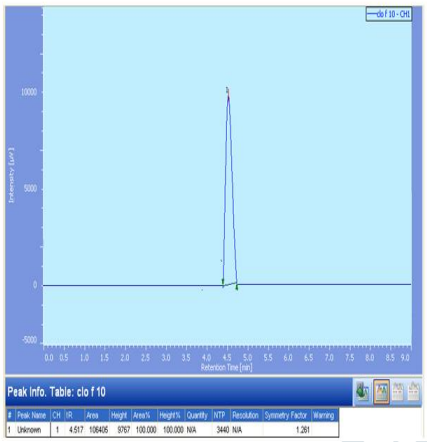


Fig. no.33 First sample (1 h)

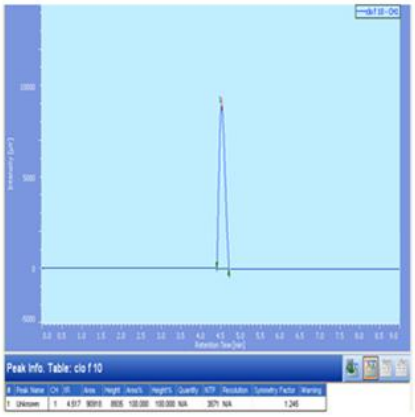


Fig.no.34 Second sample (2 h)

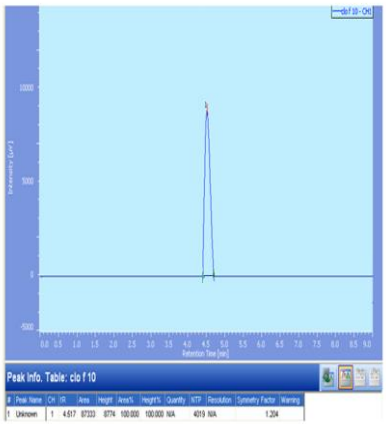


Fig. no.35 Third sample (3 h**)**

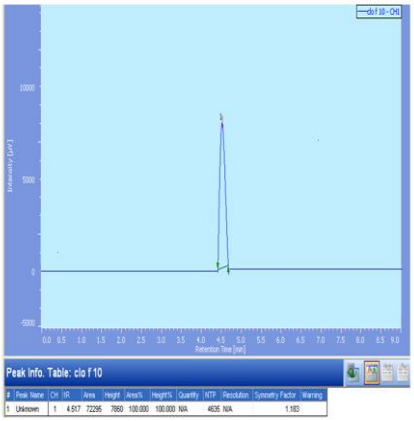


Fig. no.36 Fourth sample (4 h)

Table no. 26 Area obtained after specific intervals

Sr.no.	Pure drug	Peak area obtained after specific intervals				
		Initial	1 h	2 h	3 h	4 h
1	124476	115438	106406	90918	87333	72296

Table no. 27 % Drug content obtained after specific intervals

Sr.no.	Pure drug	% Drug content obtained after specific intervals				
1	124476	Initial	1 h	2 h	3 h	4 h
		92.73	85.48	73.08	70.16	58.08

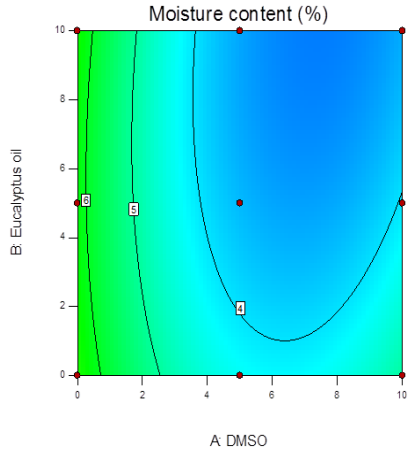
The *in-vivo* determination of drug content was carried out by using RP-HPLC method at specific time intervals. The study revealed that there was loss of drug from patch after specific intervals and it was calculated according to the peak area.

10.11 FACTORIAL DESIGN

10.11.1.1 3^3 Factorial design of batch A1

Table no. 28 3^3 Factorial design of batch A1

Batch code (A1)	Variable Levels in Coded Form			Responses				
	X1	X2	X3	Moisture Content (%)	Thickness mm	Diffusion at 1 h (%)	Diffusion at 4 h (%)	Diffusion at 6 h (%)
SN1	0	5	10	9.3	0.12	15.75	57.59	67.72
SN2	0	10	5	3.14	0.128	9.02	33.38	42.18
SN3	5	10	0	3.46	0.17	13.57	33.18	45.46
SN4	10	5	0	4.34	0.163	15.35	47.24	68.52
SN5	10	0	5	5.47	0.17	20.05	57.78	67.29
SN6	5	0	10	3.28	0.173	25.11	68.39	92.47



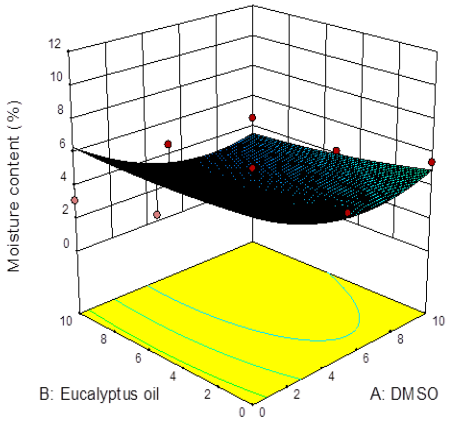
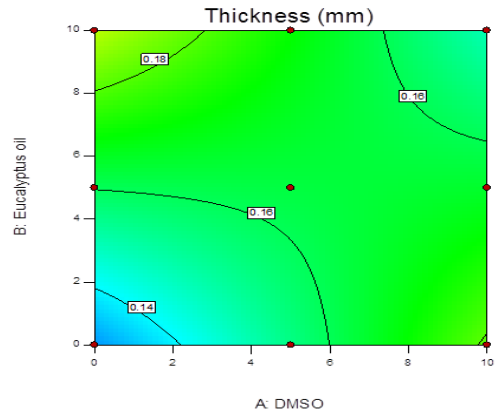


Fig.no.37

(a) Contour surface plot of moisture content (b) Three-dimensional surface plots of moisture content



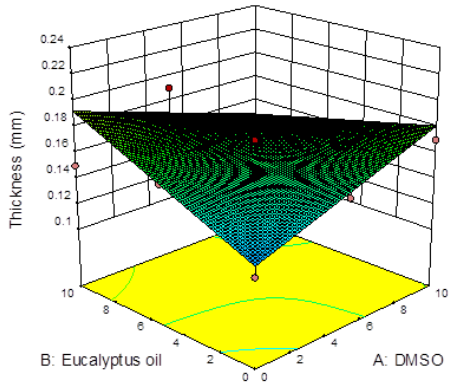
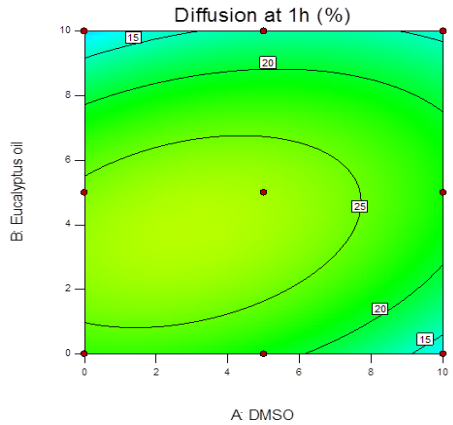


Fig.no.38

(a) Contour surface plot of thickness (b)Three-dimensional response surface plots of thickness



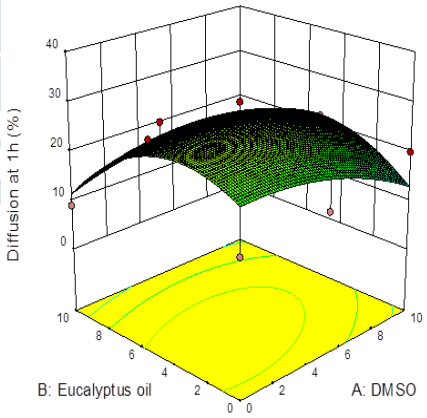
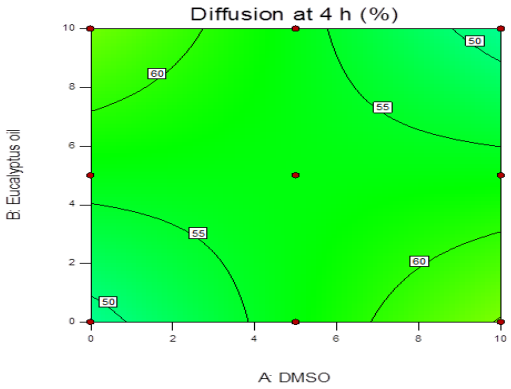


Fig.no.39

(a) Contour surface plot of diffusion in 1 h (b) Three-dimensional response surface plots of diffusion in 1 h



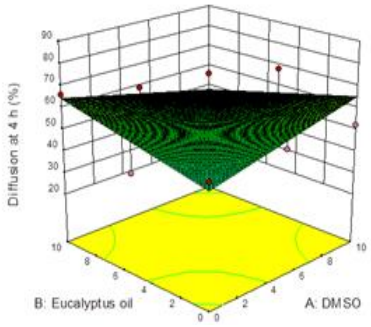
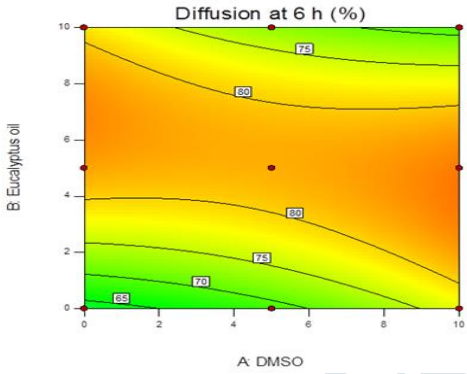


Fig.no.40

(a) Contour surface plot of diffusion in 4h

(b) Three-dimensional response Surface plot of diffusion at 4 h



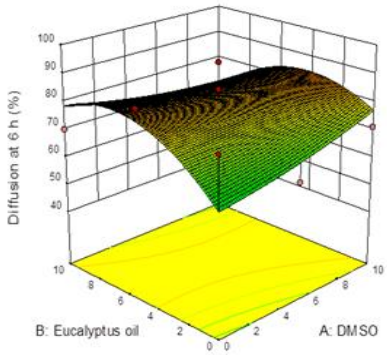


Fig.no41

(a) Contour surface plot of diffusion in 6h

(b) Three-dimensional response surface plots of diffusion in 6h

10.11.1.2 Regression analysis data of batch A1

Table no. 29 Regression analysis data of batch A1

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Suggested Model
% Moisture content (Y_1)						
Linear	2.54	0.2266	0.1257	-0.1396	218.14	-
2FI	2.37	0.4121	0.2358	-0.2619	241.56	-
Quadratic	2.19	0.5742	0.3488	-0.1242	215.19	Suggested
Cubic	2.37	0.7055	0.2342	-2.2361	619.48	Aliased
Thickness (Y_2)						
Linear	8.09	0.1160	0.0006	-0.2361	2106.73	-
2FI	8.01	0.2468	0.0208	-0.5358	2617.46	-
Quadratic	6.54	0.5735	0.3477	-0.0845	1848.30	Suggested
Cubic	3.68	0.9205	0.7934	0.1512	1446.67	Aliased
Diffusion at 1 h (Y_3)						
Linear	0.032	0.1995	0.0951	0.0951	0.034	-
2FI	0.029	0.4400	0.2720	0.2720	0.032	Suggested
Quadratic	0.029	0.5171	0.2615	0.2615	0.039	-
Cubic	0.027	0.7472	0.3427	0.3427	0.075	Aliased
Diffusion at 4 h (Y_4)						
Linear	11.59	0.2050	0.1013	-0.1511	4470.51	-
2FI	10.36	0.4477	0.2820	-0.1122	4319.66	Suggested
Quadratic	10.75	0.4941	0.2263	-0.3839	5374.82	-
Cubic	8.54	0.8121	0.5115	-1.0052	7787.76	Aliased
Diffusion at 6 h (Y_5)						
Linear	12.27	0.0610	-0.0615	-0.3751	5067.91	-
2FI	10.59	0.3918	0.2094	-0.2397	4568.83	-
Quadratic	9.22	0.6078	0.4001	-0.0806	3982.45	-
Cubic	10.46	0.7031	0.2280	-2.3578	12375.01	-

10.11.1.3 Analysis of variance (ANOVA)

Table no. 30 Analysis of variance (ANOVA) of batch A1

Source	Sum of Squares	df	Mean Square	F Value	p- value Prob.>F	
Y_1 % Moisture Content						
Model	109.92	9	12.21	2.55	0.0463	significant
X_1	21.04	1	21.04	4.39	0.0515	-
X_2	2.71	1	2.71	0.56	0.4627	-
X3	19.64	1	19.64	4.10	0.0590	-
Y_2 Thickness						
Model	0.013	6	2.150E-003	2.62	0.0487	significant
X_1	8.889E-005	1	8.889E-005	0.11	0.7455	-
X_2	1.089E-003	1	1.089E-003	1.33	0.2630	-
X3	4.672E-003	1	4.672E-003	5.69	0.0271	-
Y_3 Diffusion at 1hr						
Model	977.46	9	9	2.54	0.0468	significant
X_1	66.24	1	1	1.55	0.2301	-
X_2	130.95	1	1	3.06	0.0981	-
X_3	0.43	1	1	0.010	0.9213	-
Y_4 Diffusion at 4 h						
Model	1738.83	6	289.81	2.70	0.0436	significant
X_1	0.16	1	0.16	1.480E-	0.9697	-
X_2	2.07	1	2.07	0.019	0.8910	-
X3	794.01	1	794.01	7.40	0.0132	-
Y_5 Diffusion at 6 h						
Model	2239.92	9	248.88	2.93	0.0271	significant
X_1	16.40	1	16.40	0.19	0.6661	-
X_2	53.56	1	53.56	0.63	0.4383	-
X3	154.82	1	154.82	1.82	0.1949	-

10.11.1.4 Final equation in actual value of batch A1

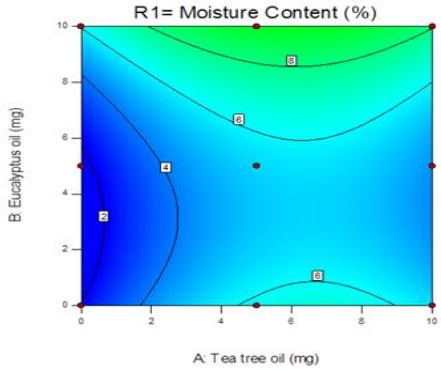
Table no. 31 Final equation in actual value of batch A1

	Actual factor equation
Y1=% Moisture content	$Y_1 = 7.46722-0.40456X_1-0.084056X_2-0.5347X_3-0.013X_1X_2-0.067X_1X_3-4.266X2X_3+0.059X_1^2+9.466X_2^2+0.0468400X_3^2$
Y2= Thickness	$Y_2= 0.14704+5.11X_1+6.055X_2-3.722X_3\ 9.66X_1X_2+3.333X_1X_3+6.666X_2X_3$
Y3= Diffusion at 1 h	$Y3=21.001940.26283X_1+2.434X_2+1.160X_3+0.12650X_1X_2+0.1041X_1X_3-0.053667X_2X_3-0.12740X_1^2-0.3380X_2^2-0.1490X_3^2$
Y4= Diffusion at 4 h	$Y_4=45.74981+1.090X_1+1.41356X_2+0.55817X_3-0.00237X_1X_2+0.06100X_2X_3$
Y5= Diffusion at 6 h	$Y_5= 43.37+1.5788X_1+7.497X_2+3.120X_3-0.2383X_1X_2-0.1633X_1X_3-0.2813X_2X_3+0.062X_1^2-0.455X_2^2-0.0311X_3^2$

10.11.2 3^3 factorial design of batch A2

Table no. 32 3^3 factorial design of batch A2

Batch code (A2)	**Variable Levels in actual Form**			**Responses**				
	X1	**X2**	**X3**	**Moisture Content (%)**	**Thickness mm**	**Diffusion at 1 h (%)**	**Diffusion at 4 h (%)**	**Diffusion at 6 h (%)**
SP1	5	5	10	4.28	0.136	15.15	43.17	70.11
SP2	5	10	5	4.95	0.148	15.41	59.11	86.21
SP3	10	5	5	7.14	0.183	23.62	70.42	76.11
SP4	0	10	10	19.62	0.117	28.02	68.61	74.73
SP5	10	10	0	2.89	0.108	7.85	27.52	33.98
SP6	10	0	10	7.62	0.132	26.54	66.06	96.67



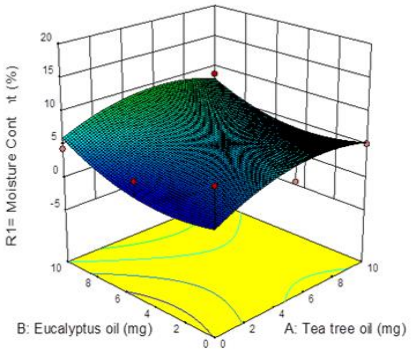
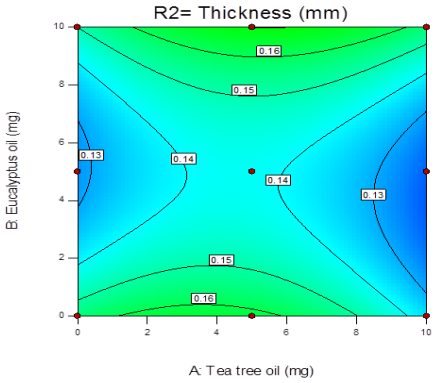


Fig.no.42

(a) Contour surface plot of %moisture content (b) Three-dimensional response surface plot of % moisture content



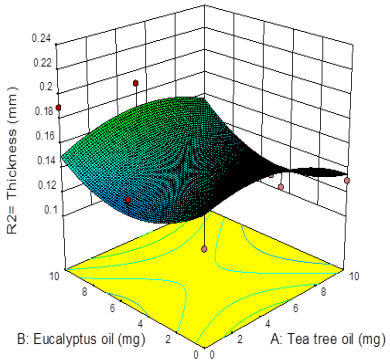
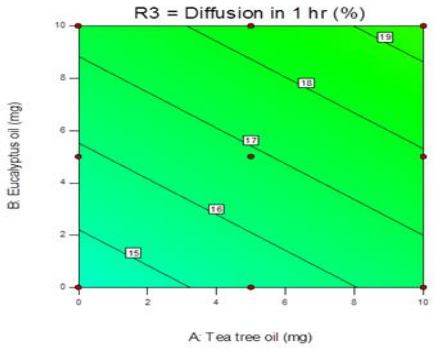


Fig.no. 43

(a) Contour surface plot of thickness (b) Three-dimensional response surface plots of thickness.



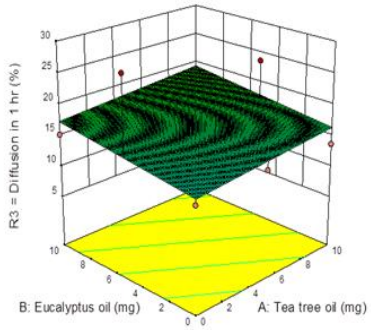
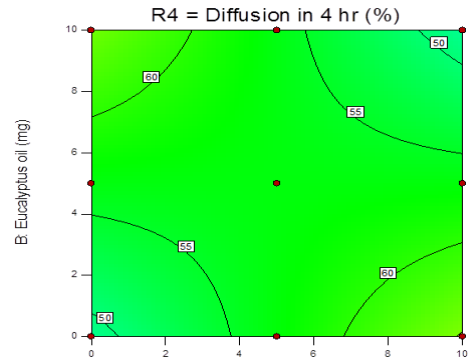


Fig. no.44

(a) Contour surface plot of diffusion in 1h (b) Three-dimensional response surface plots of diffusion in 1h



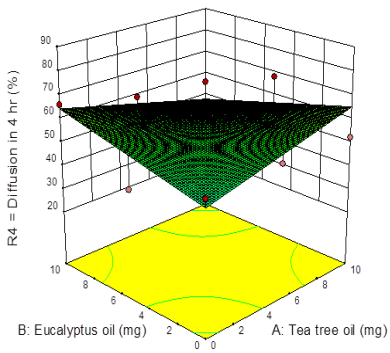


Fig.no.45

(a) Contour surface plot of diffusion in 4h (b) Three-dimensional response surface plots of diffusion in 4h

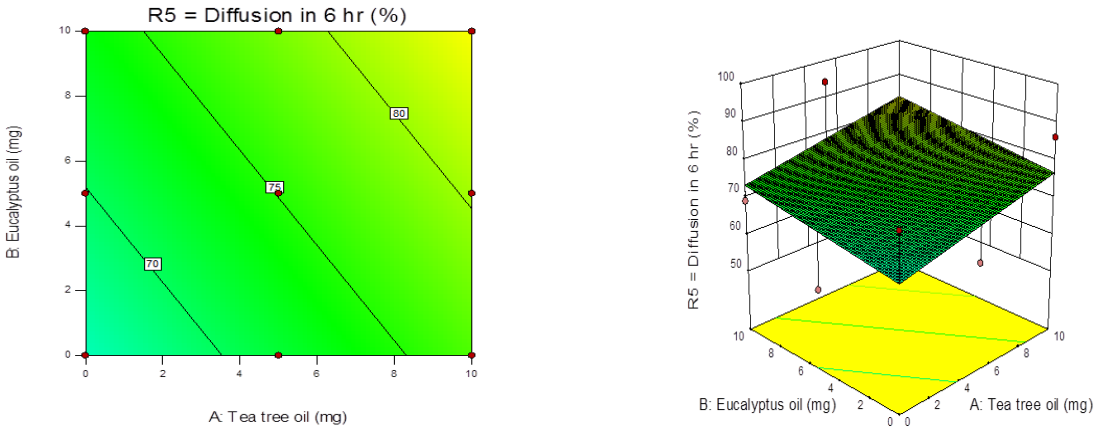


Fig.no.46

(a) Contour surface plot of diffusion in 6h (b) Three-dimensional response surface plots of diffusion in 6 h

10.11.2.1 Regression analysis data of batch A2

Table no. 33 Regression analysis data of batch A2

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Suggested Model
% Moisture content (Y1)						
Linear	4.53	0.1906	0.0850	-0.1639	679.09	-
2FI	4.25	0.3823	0.1969	-0.2826	748.32	-
Quadratic	3.83	0.5716	0.3449	-0.2740	743.34	significant
Cubic	3.02	0.8434	0.5928	-0.3339	778.28	Aliased
Thickness (Y2)						
Linear	0.033	0.0477	-0.0765	-0.3873	0.037	-
2FI	0.029	0.3802	0.1943	-0.1028	0.029	-
Quadratic	0.025	0.5858	0.3666	-0.0646	0.028	Suggested
Cubic	0.025	0.7596	0.3751	-1.8727	0.077	Aliased
Diffusion at 1 h (Y3)						
Linear	4.87	0.4019	0.3239	0.1814	746.03	Suggested
2FI	4.64	0.5279	0.3863	0.1825	745.09	-
Quadratic	4.62	0.6010	0.3898	-0.0035	914.62	-
Cubic	4.41	0.7868	0.4457	-0.2740	1161.14	Aliased
Diffusion at 4 hr (Y4)						
Linear	11.54	0.2093	0.1061	-0.1433	4431.57	-
2FI	10.36	0.4462	0.2801	-0.1148	4321.00	Suggested
Quadratic	10.74	0.4940	0.2261	-0.3833	5361.86	-
Cubic	8.43	0.8168	0.5236	-0.9493	7555.63	Aliased
Diffusion at 6 h(Y5)						
Linear	9.72	0.2685	0.1731	-0.0001	2968.74	Suggested
2FI	9.80	0.3530	0.1589	-0.1676	3465.98	-
Quadratic	10.54	0.3638	0.0270	-0.5890	4716.96	-
Cubic	11.52	0.5530	-0.1621	-2.6673	10886.63	Aliased

10.11.2.3 Analysis of variance (ANOVA)

Table no. 34 Analysis of variance (ANOVA) of batch A2

Source	Sum of Squares	df	Mean Square	F Value	p- value Prob.>F	
Y_1 % Moisture Content						
Model	333.53	9	37.06	2.52	0.0481	Significant
X_1	32.56	1	32.56	2.21	0.1550	-
X_2	50.37	1	50.37	3.43	0.0816	-
X3	28.25	1	28.25	1.92	0.1836	-
Y_2 Thickness						
Model	0.016	9	1.736E-003	2.67	0.0387	Significant
X_1	2.000E-004	1	2.000E-004	0.31	0.5862	-
X_2	2.722E-004	1	2.722E-004	0.42	0.5261	-
X3	8.000E-004	1	8.000E-004	1.23	0.2826	-
Y3 diffusion at 1h						
Model	366.29	3	122.10	5.15	0.0072	Significant
X_1	19.24	1	19.24	0.81	0.3769	-
X_2	41.01	1	41.01	1.73	0.2013	-
X_3	306.03	1	306.03	12.91	0.0015	-
Y4 Diffusion at 4 h						
Model	1729.66	6	288.28	2.69	0.0445	Significant
X_1	0.028	1	0.028	2.61	0.9873	-
X_2	2.81	1	2.81	0.026	0.8731	-
X3	808.29	1	808.29	7.53	0.0125	-
Y5 Diffusion at 6 h						
Model	842.46	3	280.82	3.07	0.0478	Significant
X_1	494.55	1	494.55	5.42	0.0291	-
X_2	228.77	1	228.77	2.50	0.1272	-
X3	119.15	1	119.15	1.30	0.2651	-

10.11.2.4 Final equation in actual value of batch A2

Table no. 35 Final equation in actual value of batch A2

	Actual factor equation
Y1=% Moisture content	$Y_1 = 2.76341 + 1.3322X_1 + 0.03194X_2 - 0.64450X_3 - 0.01383X_1X_2 - 1.000E\text{-}004X_1X_3$
Y2= Thickness	$Y_2 = 3.2022 + 1.66E\text{-}003X_1 - 0.0148X_2 - 1.000E\text{-}004X_3 - 2.667E\text{-}004X_1X_2 - 6.66E\text{-}004X_1X_3 + 8.66E\text{-}004X_2X_3 - 6.667E\text{-}004X_1^2 + 1.000E\text{-}003X_2^2 + 1.33E\text{-}004X_3^2$
Y3= Diffusion at 1 h	$Y_3 = 10.20704 + 0.2067X_1 + 0.030189X_2 + 0.82467X_3$
Y4= Diffusion at 4 h	$Y_4 = 45.76093 + 1.07939X_1 + 1.40217X_2 + 0.60289X_3 - 0.3290X_1X_2 + 0.11470X_1X_3 + 0.037X_2X_3$
Y5= Diffusion at 6 h	$Y_5 = 63.72130 + 1.04833X_1 + 0.7130X_2 + 0.51456X_3$

XI. CONCLUSION

Transdermal patches were prepared by using polymers like hydroxyl propyl methyl cellulose (HPMC E5), and Eudragit RS-100 by using solvent evaporation technique. Depending upon the solubility of drug and polymer, the solvent system of Ethanol: Chloroform (1:1) was chosen. Polyethylene glycol 400 (PEG 400) was used as a plasticizer and varying concentration of penetration enhancers such as DMSO, eucalyptus oil, clove oil, tea tree oil, and sweet basil oil were used as a penetration enhancers.

Physical and mechanical factors such as thickness, weight homogeneity, folding endurance, percentage moisture loss, percentage moisture absorption, water vapour transmission rate, and medication content were assessed for all patches. Formulations having suitable physical and mechanical properties were selected for in vitro drug release and diffusion through rat skin. The comparative effects of various concentrations of penetration enhancers were studied. Selected patches were studied for diffusion through rat skin for 6 h.

The concentration of polyethylene glycol 400 in patches showed effect on physical and mechanical properties of films. The patches containing combination of HPMC E5 and ERS-100 has shown good physical, mechanical, drug content, *in-vitro* drug release and drug diffusion properties. The selected formulation SN6 of batch A1 showed highest diffusion rate of 92.47±0.9, and SP5 of batch A2 showed highest diffusion rate of 96.67±0.42 from this it could be concluded that batch containing tea tree oil, eucalyptus oil and sweet basil oil showed highest diffusion rate across rat skin membrane up to 6 h. Estimation of *in-vivo* drug content were carried out by using RP-HPLC and it showed reduction in drug content after specific time intervals (1-4 h) from patch samples.

The formulation batches were subjected to factorial design in terms of actual value to study the comprehensive effect as well as the significance of variables.

XII. REFERENCE

1. Chein Y.W. Transdermal controlled systemic medication. New York and Basel. Marcel Dekker Inc. (1987), 159 – 176.
2. Transdermal patch From Wikipedia, the free encyclopaedia available: http://en.wikipedia. Org/wiki/Transdermal patch:
3. Takmaz E.A, Inal O, and Baykara T. Studies on transdermal delivery enhancement of Zidovudine. *AAPS Pharm Sci Tech*. (2009), 10(1): 89-97.
4. Vasilev A. E, Krasnyuk I, Ravikumar S, and Tockmakhchi V.N. Drug synthesis methods and manufacturing technology, Transdermal therapeutic systems for controlled drug release. *Pharmaceutical Chemistry Journal*. (2001), 35(11):613-626.
5. Robinson J. R and Lee H.L. Controlled drug delivery fundamentals and applications. 2nd edi, Marcel Dekker, New York. (1987), 524-552.
6. Aquil M. Sultana Y. and Ali A. Matrix type transdermal drug delivery systems of metoprolol tartrate: *In-vitro* characterization. *Acta Pharm*, (2003), 53: 119-125.
7. Ramesh G. Vamshi Vishnu, Y. Kishan V and Madhusudan Rao Y. Development of nitrendipine transdermal patches: *in-vitro* and *ex-vivo* characterization. *Current Drug Del*, (2007), 4: 69-76.
8. Chan M.Y, Weitz J.I, Merhi Y, Harrington R.A and Becker R.C. Catheter thrombosis and percutaneous coronary intervention: fundamental perspectives on blood, artificial surfaces and antithrombotic drugs. *J Thromb Thrombolysis*. (2009),28 (3):366-80.
9. Gurbel P.A, and Tantry U.S. Do platelet function testing and genotyping improve outcome in patients treated with antithrombotic agents: Platelet function testing and genotyping improve outcome in patients treated with antithrombotic agents. Circulation.(2012),125(10):1276-87.

10. Broos K. Feys H.B, De Meyer S F, Vanhoorelbeke K, and Deckmyn H. Platelets at work in primary haemostasis. Blood Rev. (2011), 25(4):155-67.
11. Patel D, Chaudhary S.A, Parmar B. and Bhura N. Transdermal drug delivery system: A Review, *the Pharma Innovation*, (2012), 1(4): 66-75.
12. Shingade G.M., Aamer Q, Sabale P.M, Grampurohit N.D, Gadhave M.V, Jadhav S.L. and Gaikwad D.D. Review on: Recent trend on transdermal drug delivery system. *Journal of Drug Delivery and Therapeutics*, (2010), 2(1): 66-75.
13. Sharma N, Parashar B, Sharma S.and Mahajan U. Blooming pharma industry with transdermal drug delivery system, *Indo Global Journal of Pharmaceutical Sciences*, (2012), 2(3): 262-278.
14. Arunachalam A, Karthikeyan M, Kumar V.D, Prathap M, Sethuraman S, Ashutosh kumar S, and Manidipa S. Transdermal Drug Delivery System: A Review. *Current Pharma Res.* (2010), 1(1):70-81.
15. Panchagnula R. Transdermal delivery of drugs. *Indian journal of pharmacology* (1997), 29: 140 –156.
16. Sharma N, Agrawal G, Rana A, Alibhat Z and Kumar D. A. Review: Transdermal drug delivery system: a tool for novel drug delivery system. *International Journal of Drug Development & Research. (*2011), 3(3):70-84.
17. Kurz A, Farlow M and Lefevre G. Pharmacokinetics of a novel transdermal rivastigmine patch for the treatment of Alzheimer's disease: a review. *International Journal of Clinical Practice.* (2009), 63(5): 99–805.
18. Heather A.E. Transdermal Drug Delivery: Penetration enhancement techniques. *Current Drug Delivery*. (2005), 2: 23-33.
19. Touitou E, Junginger H, Weiner N.D, Nagai T.and Mezei M. Liposomes as carriers for topical & transdermal delivery. *Journal of Pharmaceutical Science.* (1994), 83:1189- 1203.
20. Wissing S.A and Muller R.H. The influence of solid lipid nanoparticles on skin hydration & viscoelasticity-*in-vivo* study. *European Journal of Pharmaceutics and Biopharmaceutics*. (2003), 56:67-72.
21. Vijayan V, Sumanth M.H, Suman L, vinay T, Srinivasra D. and Kumar K.J. Development and physiochemical, *in-vitro* evaluation of Antihypertensive transdermal Patches. J. *Pharm. Sci. & Res*.(2010), 2(3):171-177
22. Shivaraj A, Selvam R.P, Mani, T.T. and Sivakumar, T. Design and evaluation of transdermal drug delivery of ketotifen fumarate, *Int. J. Pharm.Biomed. Res*. (2010), 1(2):42-47.
23. Ramkanth. S, Alagusundaram M, Gnanaprakash K, Rao K.M, Mohammed S.T.S, Paneer, K. and Chetty M.C, Design and characterization of matrix type transdermal drug delivery System using metoprolol tartarate, *Int. J. Pharm Res*. (2010), 1(1): 1-5
24. Shivaraj A. Selvam R.P, Mani T.T. and Sivakumar T. Design and evaluation of transdermal drug delivery of ketotifen fumarate. *Int. J. Pharm. Biomed. Res.* (2010),1(2): 42-47
25. Bharkatiya, M. and Nema, R., Design and characterization of drug free patches for transdermal application. *Int. J. Pharm, Sci*. (2010) 2 (1):35-39.
26. Ramkanth. S, Alagusundaram M., Gnanaprakash K., Rao K.M., Mohammed S.T.S. paneer, K, and Chetty M.C., Design and characterization of matrix type transdermal drug delivery System using metoprolol tartarate, *Int. J. Pharm Res.* (2010), 1(1): 1-5.
27. Shivaraj A, Selvam, R.P, Mani T.T. and Sivakumar, T, Design and evaluation of transdermal drug delivery of ketotifen fumarate, *Int. J. Pharm. Biomed. Res*. (2010), 1(2): 42-47.
28. Bharkatiya M. and Nema R.K, Design and characterization of drug free patches for transdermal application. *Int. J. Pharm, Sci*. (2010), 2 (1): 35-39.
29. Devi K.V, Saisivam S, Maria G.R, and Deepti P.U. Design and evaluation of matrix diffusion controlled transdermal patches of verapamil hydrochloride: *Drug Dev Ind Pharm* 2003; 29(5):495-503.
30. Sanjoy m, Thimmasetty, j., Ratan, G.N. and Kilarimath, B.H., Formulation and evaluation of Crvedilol transdermal patches. *Int. Res. J. Pharm*. (2011), 2 (1): 237-248.
31. Gupta R, and Mukherjee B. Development and *in-vitro* evaluation of diltiazem hydrochloride transdermal patches based on povidone-ethyl cellulose matrices. *Drug Dev Ind Pharm.* (2003), 29:1-7.
32. Magnusson B.M, Walters K.A. and Roberts M.S, Veterinary drug delivery: potential for skin penetration enhancement. *Adv. Drug Del. Rev. (*2001), 50:205-227.
33. European Commission. Scientific Committee on Consumer Products (SCCP) Opinion on basic criteria for the *in-vitro* assessment of dermal absorption of cosmetic ingredients. SCCP 0970/06. Adopted March 2006.
34. Saisivam S, Vijaya Muthu Manikandhar, and Nagarajan M. Design and Evaluation of Ciprofloxacin HCl Ocuserts: *Int J Pharm Sci.* (1999), 61:34-38.