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FORMULATION DEVELOPMENT AND EVALUATION OF BRISOPROLOL FUMERATE TRANSDERMAL DRUG DELIVERY SYSTEM

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ABSTRACT

Transdermal drug delivery system is a new era of pharmaceutical dosage forms along with various features to provide successful drug delivery. Transdermal drug delivery system establishes itself as an integral part of novel drug delivery system. In the present study, an attempt was made to formulation and evaluation transdermal patches of Brisoprolol Fumerate in order to overcome first pass metabolism in GIT, drug deactivation by lever and better patient complaints and to reduce adverse effect and frequency of administration. Each of the proposed transdermal patches is composed of using different polymers, anticipating thwacking drug permeation and drug release. Controlled released transdermal preparation of Brisoprolol Fumerate prepared to give sustained effect as compared to conventional multiple oral dose. A 2^3 factorial design was applied for preparing Bisoprolol Fumarate transdermal patch and to study the effect of independent variables i.e. HPMC K4M and carbapol 934 on various responses like *In vitro* drug release, % cumulative drug release moisture content, moisture uptake, tensile strength and water vapour transmission rate. The patches were transparent, smooth and flexible. The results of weight variation, thickness, moisture content, moisture uptake, folding endurance, tensile strength, drug content. The formulation F1 to F6 shows uniform weight ranging from 153.18mg to 248.50mg and thickness of F1 to F6 are ranging from 0.033 to 0.044mm. All the formulations (F1 to F6) exhibited fairly uniform drug content ranging from 91.06 to 98.05 % respectively. The moisture content was found to be in the range of 1.9 to 4.8% and moisture uptake was found to be in the range of 2.9 to 5.2%. Folding Endurance of the developed formulations F1 to F6 varied from 224 to 283. Maximum was noted for formulation F6. Batch F1 showed highest drug release (98.36%) and the lowest release was from batch F3 (93.54%). After 1month

stability there is no change in prepared patch. A transdermal patch is defined as medicated adhesive patch which is placed above the skin to deliver a specific dose of medication through the skin with a predetermined rate of release to reach into the blood stream.

KEYWORDS: Brisoprolol Fumerate, Transdermal Drug Delivery System, Carbapol 934, Folding Endurance, Tensile Strength.

1. INTRODUCTION

Transdermal delivery not only provides controlled, constant administration of the drug, but also allows continuous input of drugs with short biological half-lives, and eliminates pulsed entry into systemic circulation which often causes undesirable side effects. Transdermal therapeutic system are defined as self-contained, discreate dosage form which, when applied to intact skin, deliver the drugs through at controlled rate to the systemic circulation.

Transdermal patches are delivered the drug through the skin in controlled and predetermined manner in order to increase the therapeutic efficacy of drug and reduced side effect of drug. Controlled drug release can be achieved by transdermal drug delivery systems (TDDS) which can deliver the drug via the skin portal to systemic circulation at a predetermined rate over a prolonged period of time. For effective Transdermal drug delivery system, the drug are easily able to penetrate the skin and easily reach the target site.[1]

1.1 Advantages of transdermal drug delivery system:[1-4]

- Transdermal medication delivers a steady infusion of a drug over an extended period of time.
- Transdermal delivery can increase the therapeutic value of many drugs by avoiding specification due to hepatic first effect with drug, e.g: gastrointestinal irritation.
- The simplified medication regimen leads to improved patience compliance and reduced inter and intra patience variability.
- The time of the maintenance of constant drug concentration within the bi phase is not desired.
- The application and removal of transdermal patches produce the optimal sequence of pharmacological effect.
- Self-administration is possible with these system
- The drug input can be terminated at any point of time by removing transdermal patches.

1.2 Dis advantages of transdermal drug delivery system[5]

- The drug must have some desirable physiochemical properties for penetration through stratum corneum and if drug dosage required for therapeutic value more than 10mg/day, the transdermal delivery will be very difficult if not impossible, daily dose less than 5mg/day are preferred.
- Skin irritation or contact dermatitis due to drug, excipient and enhancer of the drug used to increase percutaneous absorption is another limitation
- Clinical need is another area that has examined carefully before a decision is made to develop a transdermal product.
- The barrier function of the skin changes from to one another site to another on same person, from person to person and with age.
- Many drug especially drug with hydrophilic structure permeate the skin too slowly may not achieve therapeutic level.
- The drug. Adhesive and other excipient in the patch formulation can causes erythema, itching and local oedema.

Table 1: Ideal properties of transdermal drug delivery system.[6,7]

Sr. no.	Parameter	Properties
1	Shelf life	Should be upto 2 years
2	Patch size	Should be less than 40 cm2
3	Dose frequency	Once
4	Appearance	Should be clear, white
5	Packaging	Easily removable at release linear
6	Skin reaction	Non irritating
7	Dose	Should be low
8	Half life	Less than 10 hrs
9	Mol. weight	Less than 500
10	Partition coefficient	Log P between 1 and 3
11	Skin permeability coefficient	Less than 0.5x10-3cm/hr
12	Oral bioavailability	Should be low
13	Therapeutic index	Should be low
14	Concentration	Minute
15	pH saturated aqueous solubility	5-9
16	Dose deliverable	Less than 10mg/day

1.3 Factors that influence transdermal delivery[8-10]

1.3.1 Biological parameters

- **Skin condition**

The skin is a tough barrier to penetration, but only if it is intact. Vesicants such as acid,

alkalis injure barrier cells and there by promote penetration. In disease characterized by defective stratum corium, percutaneous absorption increases.

- **Blood flow**

Theoretically, changes in peripheral circulation, or blood flow through the dermis, could affect percutaneous absorption. Thus an increased blood flow could reduce time for which a penetrant remain in the dermis and also raise the concentration gradient across the skin.

- **Regional skin sites**

Variation in cutaneous permeability around the body depends on the thickness and the nature of stratum corneum and the density of skin appendages. However rate of absorption at identical skin sites in different healthy volunteers varies.

- **Skin metabolism**

It has been recently reviewed the role which the skin plays in metabolism of drugs and steroidal hormones. The topical bioavailability should account for not only skin permeation but also cutaneous drug metabolism.

- **Species differences**

Mammalian skin differs widely in characteristics such as horny layer thickness, sweat gland and hair follicle densities, and pelt condition, the capillary blood supply and the sweating ability from species to species, so affect the permeation.

1.3.2 Physicochemical parameters

- **Hydration of skin**

When water saturates the skin; tissue swells, softens and wrinkles and its permeability increases markedly. In fact, hydration of stratum corneum is one of important factor in increasing the penetration rate of most substances that permeate the skin.

- **Temperature**

The penetration rate of material through the human skin can change tenfold for large temperature variation, as the diffusion coefficient decreases as the temperature falls. Occlusive vehicles increase skin temperature by few degrees, but any consequent increased permeability is small compared to effect of hydration.

- **Diffusion coefficient**

The diffusional speed of molecule depends mainly on state of matter in the medium. In gases and air, diffusion coefficients are large because the void space available to the molecules is large as compared to their size.

- **Drug concentration**

The drug permeation usually follows the ficks law. The flux of solute is proportional to the concentration gradient across the entire barrier phase.

- **Partition coefficient**

Partition coefficient is important in establishing the flux of the drug through the Stratum corneum. The balanced partition coefficient is required for drug permeation.

- **Molecular size**

Absorption is apparently inversely related to molecular weight. Small molecule penetrates faster than large once.

1.4. Types of transdermal patches[11-16]

- **Single layer drug in adhesive**

In this type of adhesive layer contain drug, the adhesive layer not only serves to adhere the various layer together and also responsible for releasing drug to the skin. The adhesive layer is surrounded by temporary linear and backing layer.

- **Multilayer drug in adhesive**

This type also similar to the single layer but it contain immediate drug release layer and outer layer will be controlled released drug with adhesive layer. This patch also has temporary layer and permanent backing layer.

- **Vapour patch:**

In this type of patch the role of adhesive layer not only serves to adhere the various layer together also serve release vapour. The vapour patch are new to market, it commonly used for releasing essential oil in decongestion. The various are various vapoue patch are also available in the market are use to improve the quality of sleep and release to cigarette smoking condition.

- **Reservoir system**

In this system the drug reservoir is embedded between an impervious backing layer and rate controlling membrane. The drug release only through the rate controlling membrane, the drug reservoir compartment to drug can be in form of solution, suspension, gel, dispersed in solid polymer matrix.

- **Matrix adhesive system**

In this type the drug reservoir is formed by dispersing the drug is an adhesive polymer and then spreading the medicated adhesive polymer by solvent casting and melting pan an impervious backing layer.

- **Matrix dispersion system:**

In this type of drug dispersed homogenously in hydrophilic and lipophillic polymer matrix. This drug contains polymer disk is fixed on to occlusive base plate in compartment fabricated from drug impermeable backing layer.

- **Micro reservoir system:**

In this type of drug delivery system is combination of reservoir and matrix dispersing system. The drug reservoir is formed by first suspending the drug is an aqueous solution of water-soluble polymer and then dispersing the solution homogenously in lipophilic polymer to formed thousands of unreachable, microscopic sphere of drug reservoir.

2. MATERIALS AND METHODS

2.1 List of chemicals

Table 2.1: List of Drug, Excipients/Polymer and Solvent.

Sr. No.	Drug/ Excipient/ Polymer	Manufacturer
1	Bisoprolol Fumarate	IPCA Pharma Ltd, Mumbai.
2	HPMC K4M	Concept Pharma Ltd, Aurangabad.
3	Carbapol (934,974,940)	Research lab fine industry, Mumbai.
4	Polyethylene glycol (200,400,600)	Research lab fine industry, Mumbai.
5	Methanol	Research lab fine industry, Mumbai.
6	Glycerin	Research lab fine industry, Mumbai.

2.2 List of equipments

Following equipments were used for the preparation and evaluation of transdermal patch.

Table 2.2: List of equipments.

Sr. No	Instruments	Manufacturer
1	Electronic balance	Swastik Systems and Services, Delhi
2	UV spectrophotometer	Shimadzu UV-Chemito-2600
3	Magnetic stirrer	Remi Lab
4	Digital pH meter	Sunshine Instruments
5	Micro pipette	Swastik Scientific Company
6	Hot air oven	J. S. Enterprises
7	Sonicator	Analab Scientific Instruments Private Limited
8	Franz diffusion cell	Orchid Scientific

2.3 Preformulation study of drug

2.3.1 Identification of drug

- **Colour, Odour and Appearance:**

The drug sample was evaluated for its colour, odour and appearance.

- **Melting point determination:**

Melting point of the drug sample was determined by capillary method by using Melting point apparatus and can also be recorded from DSC graph.

- **Determination of solubility[17]**

A solvent under consideration was saturated with the drug powder and the vials were allowed to stand at room temperature (25°C) for 7 days with frequent shaking. The solution was filtered using Whatman filter paper. The filtrate was analysed for drug content using Ultraviolet (UV) spectroscopy.

- **Fourier Transformation Infra-red (FTIR) analysis:**

Infra-red spectroscopy analysis was performed by Fourier Transform Infrared Spectrophotometer.

- **Differential scanning calorimetry (DSC):**

DSC was performed in order to assess the thermotropic properties and thermal behaviour of drug. About 5 mg of the sample were sealed in the aluminium pans and heated at the rate of 10^0C/min, covering a temperature range of 40^0C to 300^0C under nitrogen atmosphere of flow rate 100 ml/min.

- **Ultraviolet (UV) spectroscopy:**

UV spectrum of 100 µg/ml solution of the drug powder in 0.1 N aqueous hydrochloric acid solution, water and Phosphate buffer pH 6.8 was recorded in the range of wavelengths from 200 nm to 400 nm using UV-visible Double beam Spectrophotometer (UV-Chemito-2600).

- **Preparation for calibration curve of bisoprolol fumarate**

Weigh accurately about 10mg drug dissolving in distilled water, 0.1 HCl and Phosphate buffer pH 6.8 in 100ml volumetric flask and then make up to volume distilled water. Take into different concentration from 10 -50ug/ml. Observation was recorded tables and calibration curve was prepared by plotting absorbance v/s concentration of Bisoprolol Fumarate.

2.3.2 Compatibility study of drug with polymers

The successfully formulation of suitable and effective transdermal patches depends upon carefully selection of excipient. Excipient are added to facilitate the administration, promote the consistent release and bioavailability of drug. It is necessary to study the compatible of drug and excipient.

The thermal analysis and IR spectroscopy was used to investigate and predict any

physiochemical interaction between component of formulation and its selection of suitable compatibility excipient.

- **Differential scanning calorimetry**

DSC measure the heat losers gain resulting from physical and chemical changes from within sample as function of temperature. The measurement of these pressure has many applications in Preformulation studies including purity, polymorphism, solvation degradation and excipient compatibility. DSC was performed an drug and mixture of drug and polymer. The physical mixture of drug with polymer for compatibility studies were prepared by titration drug and polymer (1:1) in dried mortar for 5 min and kept as it is 24 hr. the sample of drug and mixture. The drug and polymer (1:1) were weighing and sealed aluminium foil. The sealed aluminium pan was heated at scanning rate of 20oC/min over temperature ranges from 90 to 300^{o}C. Empty aluminium pan was used as reference. The heat flow as function of temperature was measured for drug and drug polymer mixture.

- **Infrared spectroscopy:**

IR of drug sample was recorded by using KBr pellet method. The drug was triturated porcelain mortar pestle with dry potassium bromide in ratio (1:1). The pellet was prepared KBr press at pressure 8 tones. The pellet was scanned over ranges 4000-600cm-1 and IR spectra recorded. IR spectroscopy were conducted and spectrum were recorded in wavelength region 4000 to 400 cm- The procedure consists of dispersing sample drug and drug polymer mixture (1:1) ratio in KBr and compressing into disc applying pressure 7 to 8 tons for 5 min in KBr press. The pellet was placed in light pan and spectrum was recorded.

2.3.4 Determination of powder charactertics

- **Angle of repose:**

It is minimum angle possible between the surface of pile and the horizontal plane. The lesser the angle of repose more is free flowing property. The angle of repose for for granules of each formulation was determined by funnel method. The fixed amount of granules mass was allowed to flow out the funnel orifice fixed the height at 2.5 cm from surface of the plane kept on the horizontal platform. The gradual addition of granules from mouth of funnel a pile of granule at surface.It is continued until pile of touches to the stem of tip of funnel. The base of the pile is marked and radius of powder core(r) and height (h) was measured. The angle of repose when calculated with use of following formula,

θ =tan-1(h/r)

Where, h: height of pile r: radius of the pile base

Table 2.3: Relationship between angle of Repose and Flow property.

Sr. no.	Angle of repose (θ)	Flow
1	<25	Excellent
2	25-30	Good
3	30-40	Possible
4	>40	Poor

- **Bulk density:**

Bulk density was determined by pouring blend into graduated cylinder. The bulk volume (Vb) and weight of powder (m) was determined. The bulk density was calculated following formula,

Bulk density = weight of drug/ bulk volume.

- **Tapped density:**

The measuring cylinder contain a known mass of blend (M) was tapped for fixed time (100 tapping). The minimum volume (Vt) occupied in the cylinder and weight of blend was measured. Tapped density was calculated following formula,

Tapped density = weight of drug/tap volume.

- **Carr's index:**

It is expression that shows the compressibility of powder. It is calculated by following formula.

Carr's index = tapped density - bulk density/tapped density x100.

Carr's index is frequently used as flow ability characteristics.

Table 2.4: Carrs index flow properties.

Sr. no.	Carr's index	Flow
1	5-15%	Excellent
2	12-16%	Good
3	18-25%	Fair
4	21-35%	Poor
5	35-38%	Very poor
6	40>	Extremely poor

- **Hausner ratio:**

It is indicated flow ability of powder. It calculated by formula,

H=Pt/Pb.

Hausner ratio less than 1.25 it indicates that good flow and greater than 1.25 is indicated poor flow.

2.4. Formulation and Development

2.4.1 Method of preparation of transdermal patches[18]

The Bisoprolol Fumarate patches were formulated by solvent casting method, by dissolving weighed quantity of drug in required volume of water in a beaker. The selected concentration of polymers are added to the above beaker containing Bisoprolol Fumarate in water and make up the volume up to 10 ml by adding distilled water. Keep the beaker on thermostatically controlled magnetic stirrer which is maintained at 32±0.5^{0}C. Initially stirring is at low rpm and later at higher speed. The required quantity of plasticizer is added drop wise to the beaker while stirring is continued until the drug is dispersed with polymer. The solution was poured into transdermal mould; an inverted funnel was placed over the transdermal mould to prevent fast evaporation of the solvent and dried at 40- 50°C in an air circulation dryer for 12 hrs. Patches of 2.0 cm diameter were prepared by cutting with borer and packed in an aluminium foil and stored in desiccators for further use.

Table 2.5: Formulation of bisoprolol fumarate patch.

Sr. no.	Ingredient	F1	F2	F3	F4	F5	F6
1	Bisoprolol Fumarate	0.4g	0.4g	0.4g	0.4g	0.4g	0.4g
2	HPMC K4M	30ml	20ml	20ml	-	10ml	20ml
	HPMCK15M	-	-	-	20ml	-	-
4	CP-934	10ml	-	-	-	-	-
5	CP-974	-	20ml	-	-	30ml	20ml
6	CP-940	--	-	20ml	20ml	-	-
7	GLYCERINE	-	-	80ul	-	-	-
8	PEG-200	-	-	-	-	80ul	-
9	PEG-400	100ul	115ul	-	-	-	-
10	PEG-600	-	-	-	70ul	-	90ul

2.4.2 Evaluation of bisoprolol fumarate transdermal patchesi.[19-21]

- **Thickness of the patch:** The thickness of the drug loaded patch is measured in different points by using a digital micrometre and determines the average thickness and standard deviation for the same to ensure the thickness of the prepared patch.
- **Weight uniformity:** The prepared patches are to be dried at 60°C for 4 h before testing. A specified area of patch is to be cut in different parts of the patch and weighed in digital balance. The average weight and standard deviation values are to be calculated from the

individual weights.

- **Folding endurance:** A specific area of strip is cut and repeatedly folded at the same place till it broke. The number of times the film could be folded without breaking gave the value of folding endurance.
- **Percentage moisture uptake:** The prepared patches are to be weighed individually and to be kept in a desiccator containing saturated solution of potassium chloride in order to maintain 84% Rhesus factor (RH). After 24 h, the films are to be reweighed and the percentage moisture uptake determined by the formula.

% moisture uptake (%) = (Final weight - Initial weight / initial weight) × 100

- **Percentage moisture lost:** To check the extent of moisture loss from freshly prepared film, accurately weighed films were placed in a desiccator containing fused anhydrous Calcium chloride for 72 hrs. After 72 hrs, the films were reweighed and percentage moisture loss is calculated using the following formula:

Percentage moisture loss = Initial weight – Final weight/ initial weight ×100

- **Drug content uniformity:** Patch is cut into pieces and put in 100 ml dissolution or diffusion medium used respectively and stirred continuously using a mechanical stirrer and the sample is withdrawn at the end of three hours and the drug content is determined spectrophotometrically at 223 nm.
- **Swelling Index of patches:** the determination of swelling index the reweighed (W1) three patches 10mm diameters from each formulation were placed in Petri dishes (containing 20 ml of water). After 5, 10, up to 30min. intervals, the patches were removed and the excess water on their surface was carefully removed using filter paper. The swollen patches were weighed (W2) accurately. The percentage of swelling index calculated by,

% swelling index = W2-W1/ W1 X100.

- **Surface pH of patches:** For determination of surface pH three patches of each formulation were allowed in contact with 1ml of distilled water for 1 hr at room temperature. The surface pH was measured by bringing the electrode in contact with the surface of the patch and allowing.

- ***In-vitro* release studies of Bisoprolol Fumarate patches in pH 6.8 phosphate buffer**

For *In-vitro* release study, cellophane membrane was used as a barrier membrane with pH 6.8 phosphate buffer as a medium. The cellophane membrane was soaked for 24 hrs in pH 6.8 phosphate buffer. The patches were evaluated for drug release using diffusion cells;

cellophane membrane was attached between the donor and receptors compartments. The prepared transdermal patches containing drug was placed inside donor compartment and maintained at 37 ± 2°C. The receptor compartment was filled with 100 ml pH 6.8 phosphate buffers and hydrodynamics was maintained by stirring with a magnetic bead at 100 rpm/min. 2 ml sample was withdrawn and replaced with 2 ml fresh pH 6.8 phosphate buffer to maintain the sink condition. The drug release was analysed in UV/ visible spectrophotometer at 223nm.

2.4.3. Franz diffusion cell: *In vitro* membrane permeation study[24]

In vitro membrane permeation across rat cellophane membrane was conducted with the help of Franz diffusion cell. The capacity of the receiver compartment was 10mL. Diffusion cells were mounted on base of magnetic stirrer with the use of clips. A battery of 6 cells was assembled in series with back-to-back connection of silicon pipes to water jacket of diffusion cells. Assembly of 3 diffusion cells is shown below. Prior to experimentation, the membrane was equilibrated with the receptor medium i.e. Phosphate buffer (pH 6.8). This was done by putting the membrane in a beaker containing receptor medium, for about 2 hours.

The membrane was mounted on the donor compartment. The receptor medium was introduced into the receptor compartment. It was magnetically stirred continuously at sufficient and constant speed so as to keep the receptor medium moving. The temperature of receptor chamber was maintained at 37 ˚C by circulating water of 37 ˚C through water bath around it. The donor compartment was maintained at the ambient temperature of 25±2 ˚C. A blank sample of 1m was withdrawn from the receptor compartment and analysed to ensure that the diffusion cells did not have any residual absorbance at 223 nm. This withdrawn sample was replaced with fresh buffer solution.

The patch was cut accurately so as to get the area of 1cm^2. Readymade pieces of adhesive were brought from market and cleaned; they were used to place the patch on the membrane firmly till the study continues. The patch was first adhered to the adhesive and that put on the membrane1 mounted on diffusion cell. After placing the patch, stopwatch watch started and 1 ml samples were withdrawn at specific time intervals. The receptor volume was immediately replaced with fresh buffer each time. The samples were diluted appropriately and analysed UV spectrophotometrically. Then the amount of drug permeated per centimetre square at each time interval was calculated.

The cell is composed of two compartments: donor and receptor. The receptor compartment has a volume of 5–12 ml and an effective surface area of 1.0–5.0 cm^2.The diffusion buffers is continuously stirred at 100 rpm by a magnetic bar. The temperature in the bulk of the solution is maintained by circulating thermo stated water through a water jacket that surrounds the receptor.

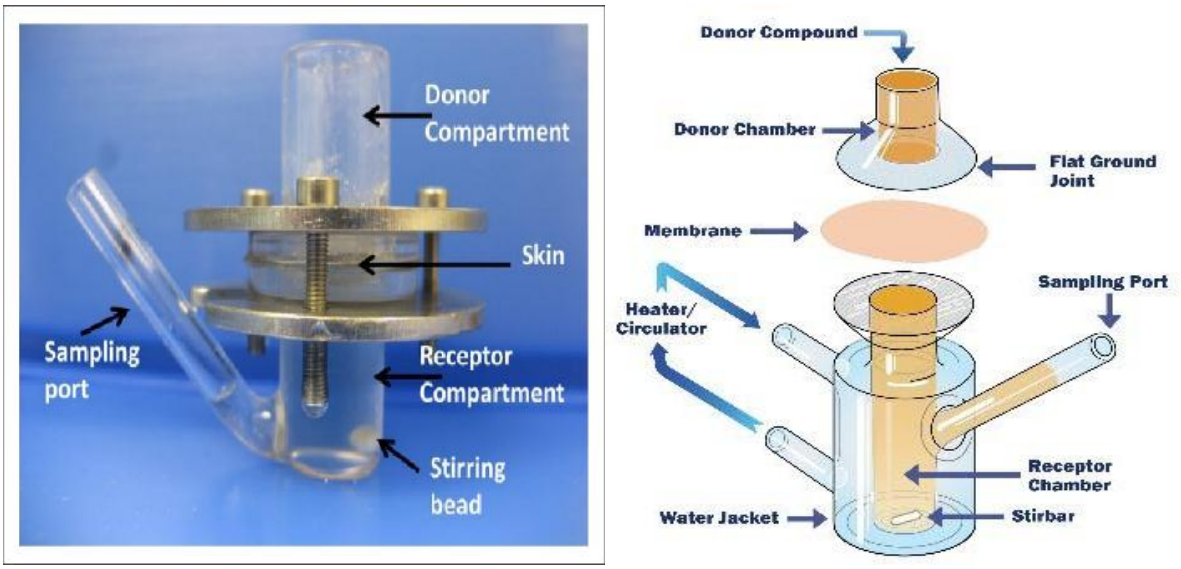

Figure 2.1: Modified franz diffusion cell.

2.4.4 Accelerated stability study of optimized batch

Stability of a drug has been defined as the ability of a particular formulation in a specific container, to remain within its physical, chemical, therapeutic andtoxicological specifications. The purpose of stability study is to provide evidence on the quality of a drug substance or drug product which varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Recommended storage conditions, re-test periods and shelf-lives are to be established. The International Conference of Harmonization (ICH) Guidelines titled, "stability testing of New Drug substance and products" (QIA) describes the stability test requirements for drug registration application in the European Union, Japan and the United States of America. ICH specifies the length of study and storage conditions

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

Accelerated Stability studies were carried out at 40^0 C / 75 % RH for the best formulations for 1 month.

3. RESULT

3.1 Preformulation study of drug

Formulation development requires in depth understanding of physical, chemical, engineering and biological principles and application of these principles. The design of controlled release dosage forms should take into account three important criteria viz. drug, delivery and destination of the delivery system. Preformulation studies help in studying the physicochemical properties of drug.

3.1.1 Identification of drug

The received drug sample was identified by using various tests. The results are presented in Table 3.1.

Table 3.1: Identification tests of drug sample.

Identification test	Results of sample obtained	Reported standards
Appearance	crystalline powder	crystalline e powder
Colour	white	white
Odor	odorless	Odorless
Melting point	101°C	101-104°C
Solubility	Readily soluble in water, methanol, ethanol, and chloroform	Readily soluble in water, methanol, ethanol, and chloroform

3.1.2. UV Spectroscopy of bisoprolol fumarate:

- **Determination of λ max:**

The λ max of Bisoprolol Fumarate was found 223 nm which is nearly same as reported in literature (225 nm)

- **Preparation for calibration curve of Bisoprolol Fumarate in water, 0.1 N HCL[ii] and** Phosphate buffer pH6.8**:**

Calibration curve of Bisoprolol Fumarate was carried out at λmax 223 nm water. Regression coefficient of Bisoprolol Fumarate was found to be R^2 0.998.the standard linear equation was found to be y=0.018x+0.077.

Calibration curve of Bisoprolol Fumarate was carried out at λ max 223 nm in 0.1 N Hydrochloride. Regression coefficient of Bisoprolol Fumarate was found to be R^2 0.997.the standard linear equation was found to be y=0.22x-0.115.

Calibration curve of Bisoprolol Fumarate was carried out at λ max 223 nm in phosphate buffer of pH 6.8. Regression coefficient of Bisoprolol Fumarate was found to be R^2 0.997.the

standard linear equation was found to be y= 0.022x-0.107.

- **Differential scanning calorimetry:**

The thermal behavior of Bisoprolol Fumarate was examined by DSC, using a SHIMADZU DSC- 60 differential scanning calorimeter. The system was calibrated with a high purity sample of Indium. Bisoprolol Fumarate were scanned at the heating rate 20°C /min over a temperature range of 100-200°C.The melting point of Bisoprolol Fumarate was found to be 101°C by DSC which is same as reported in literature (101-104°C).

- **Drug-Excipient compatibility study by FTIR:**

The FT-IR analysis of the Bisoprolol Fumarate. was carried out for qualitative compound identification. The FT-IR spectrum for pure drug was carried out by KBr disc method for pure drug Bisoprolol Fumarate, physical mixture of Bisoprolol Fumarate + HPMC K4M, Bisoprolol Fumarate + HPMCK15M. Bisoprolol Fumarate + CP-934, Bisoprolol Fumarate + CP-940 and Bisoprolol Fumarate + CP-974 the spectrum was recorded in the range of 4000 cm-1 and 450cm-1.

3.2 Determination of powder characteristics

Powder characteristics of drug affect formulation of tablets. Results shown in Table 3.2 indicated that the drug powder was fairly good flowing. Values of angle of repose revealed that the drug powder was passable.

Table 3.2: Powder characteristics of bisoprolol fumarate.

Sr. No	Parameters	Specifications	
		Range	Observed
1	Bulk density (g/cc)	0.38-0.43	0.41 g/cc
2	Tapped Density (g/cc)	0.43-0.50	0.45 g/cc
3	Carrs index	18-21	21.00%
4	Hausner's ratio	1.25-1.50	28.650 to 33.6 28. 68-33.60^{o}
5	Angle of repose (□')	>40^{o}	46.74^{o}

The Preformulation studies angle of repose was found to be 46.74^{o,} but range observed to be >40, that it is extremely poor flow. Carr's index was found to be 21.00% this may also range for 18-21, so it's performance is fair. The Hausner's ratio was found 1.28, and it ranges 1.25-1.5, The bulk and tapped density of all the formulations were in the range of (0.38 to 0.43) and (0.43 to 0.50) respectively The bulk and tapped density of all the formulations were in

the range of (0.38 to 0.43) and (0.43 to 0.50) respectively The bulk and tapped density of all the formulations were in the range of (0.38 to 0.43) and (0.43 to 0.50) respectively The bulk and tapped density of all the formulations were in the range of (0.38 to 0.43) and (0.43 to 0.50) respectively.

3.3 Evaluation of transdermal patchesiii[19-23]

- **Thickness of the patch:** All the patches have uniform thickness throughout and patch thickness in the range of 0.307 to 0.284 mm. comparatitive table no: 22 show that F1 formulation highest thickness of patch i.e. (0.307mm).
- **Folding endurance:** The folding endurance was measured manually, patches were folded repeatedly till it broke, and it was considered as the end point. Folding endurance was found to be in the range of 224 to 283. The folding endurance was found to be highest for F6 and the lowest for F1 formulation.
- **Percentage moisture uptake:** All patches of formulation % moisture absorbed are highest from 40% F5 and lowest from F1.it and % of moisture lost which f1 formulation which lost 52.50% as compared to other formulation.

% moisture uptake (%) = (Final weight - Initial weight / initial weight) × 100

- **Percentage moisture lost:** To check the extent of moisture loss from freshly prepared film, accurately weighed films were placed in a desiccator containing fused anhydrous Calcium chloride for 72 hrs. After 72 hrs, the films were reweighed and percentage moisture loss is calculated using the following formula:

  Percentage moisture loss = Initial weight – Final weight/ initial weight ×100
- **Drug content uniformity:** Drug content result was found to be F1 (98.05%) and lowest F4(91.18%). the comparative sequence are following F1>F6>F3>F5>F2>F1.
- **Weight uniformity:** The weight of the patches was found uniform weight and the weight patches are ranges from 0.042 to 0.033.the weight of uniformity are following sequence F1>F3>F4>F5>F2>F6.as table no: 25 highest weight of uniformity was found to be F1 (0.042+- 0.0016).
- **Swelling Index of patches:** the determination of swelling index the reweighed (W1) three patches 10mm diameters from each formulation were placed in Petri dishes (containing 20 ml of water). After 5, 10, up to 30min. intervals, the patches were removed and the excess water on their surface was carefully removed using filter paper. The swollen patches were weighed (W2) accurately. The percentage of swelling index calculated by,

- **% swelling index = W2-W1/ W1 X100.** The comparative percentage swelling of various formulations was in order ofF2>F6>F5>F3>F4>F1 percentage swelling was highest for F2 and the Lowest for F1Formulation. Triplicate for each batch
- **Surface pH of patches:** The surface ph of prepared transdermal patches was found to be 6.16 to 7.20. normally pH of the skin between 6-7.so that transdermal patch would not show any irritation.

Table 3.3: Evaluation of transdermal patches.

F C	Thickness of patch mm	Folding endurance	% moisture lost	% moisture uptake	Drug content	Weight of uniformity	% swelling index	Surface pH
F1	0.307mm	224	52.50%	19.10 %	98.05%	0.042± 0.0016	29.54 %	6.16
F2	0.294mm	275	29.54 %	40.00%	91.18%	0.033±0.0016	52.50%	7.20
F3	0.278mm	255	40.74%	36.70%	96.15%	0.038 ± .0016	40.74%	6.73
F4	0.291mm	260	38.97%	24.00%	91.06%	0.033± 0.0016	38.97%	6.23
F5	0.280mm	255	45.30%	48.70 %	95.55%	0.033±0.0016	45.30%	6.42
F6	0.284mm	283	49.80%	22.31%	97.75%	0.033±0.0016	49.80%	6.71

n=3

- ***In-vitro* release studies of Bisoprolol Fumarate patches in pH 6.8 phosphate buffer[22,24]**

For *in-vitro* release study, cellophane membrane was used as a barrier membrane with pH 6.8 phosphate buffer as a medium. The cellophane membrane was soaked for 24 hrs in pH 6.8 phosphate buffer. The patches were evaluated for drug release using diffusion cells; cellophane membrane was attached between the donor and receptors compartments. The prepared transdermal patches containing drug was placed inside donor compartment and maintained at 37 ± 2°C. The receptor compartment was filled with 100 ml pH 6.8 phosphate buffers and hydrodynamics was maintained by stirring with a magnetic bead at 100 rpm/min. 2 ml sample was withdrawn and replaced with 2 ml fresh pH 6.8 phosphate buffer to maintain the sink condition. The drug release was analysed in UV/ visible spectrophotometer at 223nm.

Table 3.4: *In-vitro* release of drug.

Formulation Code	Time in hrs			
	2hrs	4hrs	6hrs	8hrs
F1	46.80%	59.51%	73.62%	89.30%
F2	36.99%	47.03%	58.18%	70.58%

F3	34.24%	43.54%	53.86%	68.33%
F4	35.80%	45.52%	56.32%	71.31%
F5	34.74%	44.17%	54.65%	70.29%
F6	40.67%	51.71%	63.97%	77.60%

In the above table shows that *in- vitro* drug release studies are performed for all prepared formulation by using phosphate buffer 6.8 as dissolution medium and measuring drug concentration of UV spectrophotometrically at 223nm. The study was performed for all formulation up to 8 hrs. All patches are design by considering a well understandable fact that if a patch gives sustainable release profile. It means that the patches should kept in contact with skin for prolonged period of time which comfortable to patient. The data obtained from *in-vitro* drug release profile desired for transdermal drug delivery system, other hand F3 formulation shows lowest release profile at 8hrs 68.33%% but among all formulation shows that more than 70% drug release at 8hrs.

3.4.4 Accelerated stability study of optimized batch[25]

Accelerated stability study was performed as per ICH Guideline Q1 (A) for formulation i.e., F1 was selected on the basis of 89.30% cumulative drug release and also results drug content98.05%. From these results it was concluded that, formulation F1 is stable and retained their original properties with minor differences. The *in vitro* release profile of F1 at 40^{O}C/75% RH conditions after 1 month was 96.78 % and 87.67% respectively, has indicated that there is no or minor alteration after storage.

Table 3.4: Accelerated stability F1 batch.

Batch	Appearance	%Drug Content
Initial	Transparent	98.05%
After 1 Month	Transparent	96.78%

Table 3.5: Accelerated stability *In-vitro* release study F1 batch.

Time (hr)	Initial	After 1 month
2	46.80%.	42.75%%
4	59.51%	57.34%
6	73.62%	71.45%
8	89.30%	87.67%

4. CONCLUSION

In order to investigate the possible interaction between drug and selected polymers, IR and DSC spectroscopy study was carried out. IR spectrum for pure drug and physical mixture of drug-polymers were obtained and characterized.

It was observed that there were no changes in these main peaks in IR spectra of mixture of drug and polymers, which show there were no physical interactions because of some bond formation between drug and polymers.

A 2^3factorial design was applied for preparing Bisoprolol Fumarate transdermal patch and to study the effect of independent variables i.e. HPMC K4M and carbapol 934 on various responses like In vitro drug release, % cumulative drug release moisture content, moisture uptake, tensile strength and water vapors transmission rate.

The 6 batches of Bisoprolol Fumarate were prepared by solvent casting method using polymers, like HPMC K4M and Carbopol 934 and other necessary excipients. It was observed that there is efficiency drying of transdermal patches at room temperature. Inverted glass funnels were put on them for uniform drying. This maintained uniform drying rate of patches.

The patches were transparent, smooth and flexible. The results of weight variation, thickness, moisture content, moisture uptake, folding endurance, tensile strength, drug content. The formulation F1 to F6 shows uniform weight ranging from 153.18mg to 248.50mg and thickness of F1 to F6 are ranging from 0.033 to 0.044mm. Among the various batches, the uniformity weight and thickness indicate that the polymeric solution of the drug is well dispersed in the patches. All the formulations (F1 to F6) exhibited fairly uniform drug content ranging from 91.06 to 98.05 % respectively.

Moisture content of patch may affect the release of drug from it. Relatively “dry” patches may need more time to release the drug. Hence, these tests were performed to evaluate the ability of patch to lose and to gain the moisture. The moisture content was found to be in the range of 1.9 to 4.8%it shows that as the concentration of hydrophilic polymer increased moisture content were increases i.e. HPMC K4M. Moisture content in the films were found to be low, low moisture content helps them to remain stable and from being completely dried and brittle. Moisture uptake was found to be in the range of 2.9 to 5.2% Low moisture uptake protects the film from microbial contamination and bulkiness. The increase in the concentration HPMC K4M increases the moisture absorption of the film.

Folding endurance is parameter to measure the ability of patch to withstand the conditions of folding when it is applied to skin. If patch withstand these conditions and does not break, then

burst release can be avoided. Folding endurance of patches was found to be increase with increasing concentration of plasticizer Folding Endurance of the developed formulations F1 to F6 varied from 224 to 283. The highest folding endurance was noted for formulation F6.

Drug release from polymer matrix ensured sustained reproducibility of rate and duration of drug release. *In vitro* drug dissolution studies of the transdermal patch were conducted using a Franz diffusion cell in phosphate buffer (pH6.8) as dissolution fluid. From results it was found that that batch F1 showed highest drug release (98.36%) and the lowest release was from batch F3 (93.54%). After 1 month stability there is no minor change in prepared patch.

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