Formulation Factors Affecting In Vitro and Ex Vivo Permeation of Bisoprolol Fumarate from a Matrix Transdermal Patch

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ABSTRACT: The present study was done to develop and evaluate a matrix transdermal patch for bisoprolol fumarate. Different combinations of Eudragit RS 100 and HPMC E5 were used with polyethylene glycol 400 as a plasticizer on a polyvinyl alcohol backing layer by the solvent evaporation technique. The patches were evaluated for organoleptic characteristics and physicochemical parameters. Initial in vitro dissolution experiments were conducted to optimize formulation parameters prior to ex vivo skin permeation studies. Eudragit RS 100 and HPMC E5 (9:1) combination was studied for skin permeation because of the sustain release effect. The effect of control patch and permeation enhancer including Tween 80, propylene glycol, and DMSO were evaluated at 10%–40% concentration in the Franz diffusion cell using excised abdominal skin of rabbit. Different kinetic models were used to interpret the release kinetics and drug release mechanism. The patch M04-PE containing 40% Tween 80 had better sustained release effect and had closer flux to the desired flux. M04-PE followed the zero-order kinetics with super case II release drug mechanism.
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KEY WORDS: Bisoprolol fumarate, Drug delivery system, Films, Flux, Kinetics, Matrix system, Permeation enhancer

Introduction

The outermost layer of the skin, the stratum corneum, is responsible for the barrier function of the skin.[1] It is also known as nonviable epidermis.[2] The stratum corneum is 10–15 μm in thickness and is made up of dead flattened corneocytes, which are surrounded by an extracellular matrix of lipid.[3] The stratum corneum is composed of approximately 40% protein, mostly keratin, and 40% water, with balance of (phospho)lipid with other cellular components has been denoted. On the surface of the skin, there is a film of emulsified material, which is composed of a complex blend of sweat, sebum, and desquamating cells of epidermis. However, this layer offers little obstruction for the drug to permeate. It is claimed that drug with molecular weight less than 600 Da easily permeate the skin membrane.[4] To cause the penetration of drug with molecular weight greater than 600, penetration enhancers are used, which cause disruption of stratum corneum.[5] The objective of the present study was to assess the effect of permeation enhancers in facilitating the passage of bisoprolol fumarate (molecular weight 767.0 Da) through the skin in terms of cumulative drug release and flux. The previous studies on bisoprolol fumarate for the drug delivery system have also confirmed the passage of drug through animal membrane using different combinations of polymers[6] and plasticizer.[7] Recently, a transdermal patch of bisoprolol by the trade name of Bisono^{TM} Tape (2013) has been launched in Japan, which has isopropyl myristate as a permeation enhancer. Thus other different classes of permeation enhancers were selected, namely nonionic surfactant (Tween 80),

sulfoxide (dimethyl sulfoxide), and polyol (propylene glycol). The present study was undertaken to identify the optimum concentration of hydrophilic and hydrophobic polymers (hydroxypropyl methylcellulose E5 (HPMC E5) and Eudragit RS 100, respectively) in conjugation with a permeation enhancer with most favorable properties and concentration for the permeation of bisoprolol fumarate through rabbit's skin.

Materials and Method

MATERIALS

Bisoprolol fumarate (donated by Mass Pharma, Lahore, Pakistan), Eudragit RS 100 (Merck, Germany), Hydroxypropyl methylcellulose (HPMC; Merck, Germany), polyethylene glycol 400 (PEG 400; Merck, Germany), dimethyl sulfoxide (DMSO; Fisher Scientific, Korea), Tween 80 (Daejung, Korea), propylene glycol (PG; Merck, Pakistan), polyvinyl alcohol (PVA; Merck, Germany), sodium chloride (Merck, Germany), potassium chloride (KCl; Aldrich, Germany), potassium dihydrogen phosphate (Fluka, Germany), disodium hydrogen phosphate (Fluka, Germany), sodium hydroxide (Riedel-de Haen, Germany), silica beads (Uni-chem, Pakistan), calcium chloride ($CaCl_2$; Uni-chem, Pakistan), methanol (BDH, UK), and hydrochloric acid (BDH, UK). All the chemicals used were of analytical grade. They were used without any further treatment.

METHODS

Preparation of PVA Backing Layer

A backing layer of 4% PVA solution was prepared by dissolving PVA in distilled water. The hot plate magnetic stirrer (DHPS-1, Galvano Scientific, Lahore, Pakistan) was preset at 80°C to maintain the temperature of distilled water. A weighed amount of PVA was added in portion in distilled water over 2 h to ensure complete mixing. Backing solution was poured on the surface of dry Petri dish with an aid of syringe and was allowed to dry completely at room temperature for 24 h.

Preparation of Bisoprolol Fumarate Matrix Transdermal Patch without a Permeation Enhancer

A weighed amount of HPMC E5 and Eudragit RS 100 was added to 15 mL of methanol followed by a plasticizer and stirred by a magnetic stirrer at 32°C for 60 min on a hot plate to ensure complete mixing (Table I). The total polymeric content was fixed at 1000 mg. The drug was dissolved in 5 mL of methanol and slowly added to the polymeric solution. The solution was further mixed for 15 min for homogeneous mixing of drug. To remove air bubbles, the casting solution was sonicated (Supersonic X-3, AFD Instruments, Lahore, Pakistan) for 20 min. The casting solution was poured on the surface of backing layer in a Petri dish, and a funnel was placed on it in an inverted manner to control the rate of evaporation of methanol. They were dried at 35°C in an oven for 48 h.

TABLE I
Formulation of Matrix Type Transdermal Patch of Bisoprolol Fumarate

Formulation code	ERS 100[a]: HPMC E5	Drug (mg)	PEG 400 (40% w/w)	Penetration Enhancer (w/w)	Methanol (mL)
M01	10:1	10	400	–	20
M02	9:1	10	400	–	20
M03	8:2	10	400	–	20
M04	7:3	10	400	–	20
M05	6:4	10	400	–	20
M06	5:5	10	400	–	20
M01-PE	9:1	10	400	Tween 80 (10%)	20
M02-PE	9:1	10	400	Tween 80 (20%)	20
M03-PE	9:1	10	400	Tween 80 (30%)	20
M04-PE	9:1	10	400	Tween 80 (40%)	20
M05-PE	9:1	10	400	PG (10%)	20
M06-PE	9:1	10	400	PG (20%)	20
M07-PE	9:1	10	400	PG (30%)	20
M08-PE	9:1	10	400	PG (40%)	20
M09-PE	9:1	10	400	DMSO (10%)	20
M10-PE	9:1	10	400	DMSO (20%)	20
M11-PE	9:1	10	400	DMSO (30%)	20
M12-PE	9:1	10	400	DMSO (40%)	20

[a]ERS 100: Eudragit RS 100.

Preparation of Bisoprolol Fumarate Matrix Transdermal Patch with Permeation Enhancer

A weighed amount of HPMC E5 and Eudragit RS 100 was added in 15 mL of methanol followed by a plasticizer and permeation enhancer. The solution was stirred with a magnetic stirrer at 32° C for 60 min on a hot plate to ensure complete mixing (Table I). The drug was dissolved in 5 mL of methanol and was slowly added to the polymeric solution. The solution was further mixed for 15 min for homogeneous mixing of drug. To remove air bubbles, the casting solution was sonicated for 20 min. The casting solution was poured on the surface of backing layer in a Petri dish, and a funnel was placed on it in an inverted manner to control the rate of evaporation of methanol. They were dried at 35°C in an oven for 48 h.

Construction of Calibration Curve of Bisoprolol Fumarate

A calibration curve of bisoprolol fumarate was constructed by the stock solution dilution method (see Fig. 1). Stock solution was prepared by dissolving 100 mg of drug in 100 mL of saline phosphate buffer pH 7.4. It was added in a 100-mL volumetric flask and dissolved in saline phosphate buffer pH 7.4 by making up the volume up to 100 mL. The solution was sonicated for 5 min for complete mixing. Now 0.1 mL of stock solution

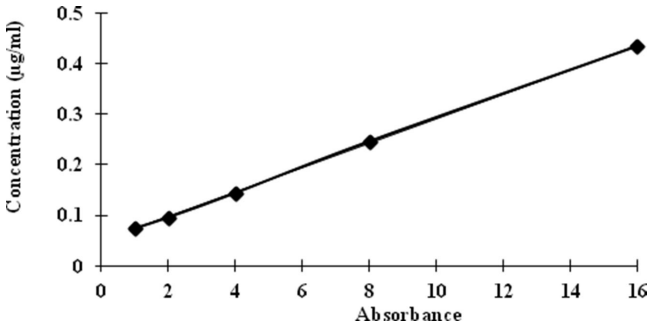

FIGURE 1. Calibration curve of bisoprolol fumarate in phosphate buffer saline pH 7.4; where linear line equation: $y = 0.0241x + 0.0492$, $R^2 = 0.9998$, slope: 0.0241, y intercept: 0.0492.

was taken with an aid of a micropipette and transferred to another 100 mL volumetric flask and volume made up to 100 mL with saline phosphate buffer pH 7.4. This made the dilution of 1 μg/mL. Similar dilutions were made containing 2, 4, 8, and 16 μg/mL. The sample was taken from each dilution, which was filtered and analyzed spectrophotometrically at 223 nm (T-80 UV/vis Spectrophotometer, PG Instrument, Midland, Canada).[6]

PHYSICOCHEMICAL PROPERTIES OF BISOPROLOL FUMARATE MATRIX TRANSDERMAL PATCH

Organoleptic Properties

The organoleptic properties of patches including color, transparency, flexibility, homogeneity, and gloss were recorded. A simple scoring system was developed to each criteria with (+++) representing the most positive and targeted characteristic and (—) representing the most negative result.[8]

Weight Variation

The weight variation test was done by randomly selecting three patches of each formulation. The patches were weighed individually on a digital weighing balance with a sensitivity of 0.0001 g (DV215CD, Ohaus, New Jersey, USA).[9]

Thickness

The thickness of the patches was estimated using a digital vernier caliper (SH-0281, Zhejiang, China). Three random patches of each formulation were selected for the test. The thickness was noted from the center and edges of patch.[9]

Folding Endurance

The folding endurance of patch was estimated by repeatedly folding a film of 2 × 2 cm from same point until it broke. The 2× 2 cm films were taken from the center and from the edge of the patch. The test was performed on three randomly selected patches from each formulation.[10]

Content Uniformity Test

A film of 2× 2 cm was dissolved in 100 mL phosphate buffer pH saline 7.4 on a magnetic stirrer for 12 h at 32°C. After 12 h, the solution was sonicated for 20 min. A sample of 3 mL was taken and filtered through Whatman filter paper. The filtrate was diluted with an equal volume of phosphate buffer saline pH 7.4 and analyzed spectrophotometrically at 223 nm. Blank solution was prepared by dissolving a film of 2× 2 cm in 100 mL phosphate buffer saline pH 7.4 by the same procedure as stated above, but the film did not contain any drug. The absorbance was put in the calibration curve to determine the amount of drug present in the patch.

Flatness

A strip of a definite length 4 cm was cut from the center and from each side of the patch. The length of the strip was noted after cutting and variation in length noted. If no change in length occurs, then it means that there was no constriction. Zero constriction signifies 100% flatness. The percentage constriction was calculated from the following equation (1):

$$\text{Flatness} = (\text{Initial length} - \text{final length})/\left(\text{Final length}\right) \times 100 \quad (1)$$

Swelling Index and Percentage Weight Increase

A film of 1× 1 cm was cut from the patch. They were dried at 40 ± 2°C overnight before experiment. The films were than fixed on preweighed cover slips and weighed on a digital weighing balance. They were placed in appropriately labeled Petri dishes, and distilled water was poured until the films were completely immersed in water. After an interval of 5, 10, and 30 min, the cover slips were taken out, blotted to remove excess of liquid, and immediately weighed. If films showed disintegration or began to dissolve, the experiment was discontinued. The swelling index and percentage weight increase due to swelling were calculated from the following equations (2, 3):

$$\text{Swelling index} = (W_2 - W_1)/(W_1) \quad (2)$$

$$\text{Percentage weight increase due to swelling} = (W_2 - W_1)/(W_1) \times 100 \quad (3)$$

where W_1 is the initial weight of the film before erosion and W_2 is the weight of the film after time t.

Percentage Moisture Content

A film of 2× 2 cm was cut from a patch. The films were weighed individually using a digital weighing balance. They were placed in properly labeled Petri dishes and stored in an incubator (LIB-030M, LabTech, Namyangju, Korea) at 25°C containing silica beads as a desiccant. The films were weighed for 5 days. The percentage moisture content was calculated by the following equation (4):

$$\text{Percentage moisture content} = (W_1 - W_2)/(W_2) \times 100 \quad (4)$$

Percentage Moisture Uptake

A film of 1× 1 cm was cut from a patch. The films were weighed individually using a digital weighing balance. Then they were placed in properly labeled Petri dishes and stored in an incubator at 25°C containing 200 mL saturated solution of KCl for 84% RH.[14] The films were weighed for 5 days after storage. The percentage moisture uptake was calculated by following equation (5):

$$\text{Percentage moisture uptake} = (W_2 - W_1)/(W_2) \times 100 \quad (5)$$

Water Vapor Transmission Test

A film of 1× 1 cm with known weight was cut from a patch. The films were fixed in 5 mL vials, and 1 g of $CaCl_2$ was placed in each vial. The vials were weighed individually and then kept in an incubator at 25°C containing 200 mL saturated solution of KCl for 84% RH. The vials were kept for 24 h, and weight was noted. The water vapor transmission was calculated by the following formula (6):

$$\text{Water vapor transmission rate} = W/(S \times t) \quad (6)$$

where W is the quantity (g) of water transmitted in 24 h; $t$is the total time (24 h), and S is the surface area (cm^2).

Water Vapor Permeability

A film of 1× 1 cm with known thickness and weight was fixed in a 5-mL vial containing silica beads as a desiccant. The vials were weighed individually and were kept in an incubator containing saturated solution of KCl, for 84% RH at 30°C. The vials were weighed for 24 h, and weight was noted. The water vapor permeability was calculated using the following formula (7):

$$P = (Q \times d)/A\,T\,S\,(R_1 - R_2) \quad (7)$$

where P is the permeability, Q is the amount of water vapor absorbed (mg) at time t (h), d is film thickness (cm), A is area (cm^2), S is saturated water vapor pressure at test temperature (Pa), R_1 is RH in the chamber (84% RH), R_2 is RH inside the vial (0% RH).

In Vitro Dissolution Studies of Matrix Type Transdermal Patches of Bisoprolol Fumarate

The in vitro dissolution studies were done in a USP apparatus V (Curio 2020+, Lahore, Pakistan). The dissolution studies were carried out in 500 mL of phosphate buffer saline pH 7.4, stirring at 50 rpm while maintaining temperature at 32.0 ± 1°C.[14] A sample of 3 mL was retrieved at time 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12th h. The sample was filtered through Whatman filter paper and equally diluted with phosphate buffer saline pH 7.4. The sample was then analyzed using a UV-spectrophotometer at 223 nm.[6] A patch without any drug was treated similarly to obtain blank solution for UV analysis. The test was run in triplicate, and average reading was taken. The absorbance was fitted to the calibration curve to determine percentage drug release from the patch.

Ex Vivo Skin Permeation Studies

The barrier properties of stratum corneum can be assessed readily by the ex vivo technique by using fresh epidermal skin.[16]

Preparation of Rabbit Skin

The hair on abdominal area of the rabbit was trimmed with the aid of a hair clipper. The skin was made hairless by applying hair removal cream for sensitive skin, wiped and washed off completely with warm water. The rabbit was sacrificed by cervical dislocation, and the abdominal region was obtained. The skin was prepared by soaking it in water at 60°C for 45 s.[14] The subdermal tissues were removed with forceps, and dermis side was wiped for 1 min with a cotton swab dipped in isopropyl alcohol to remove adhering fats from the surface.[17] The skin was washed with warm distilled water, kept in saline solution, and stored in a refrigerator. It was used within 1 week of preparation. Before starting the experiment, the skin was allowed to reach room temperature for at least 10 h [18] and equilibrated for 1 h in phosphate buffer saline pH 7.4.[19]

Ex Vivo Skin Permeation

The ex vivo skin permeation study of films across rabbit skin was conducted in a Franz diffusion cell. The dermal side of skin was placed facing the receptor compartment. A circular transdermal patch was pressed on the skin with the backing layer side facing away from the stratum corneum. The receptor compartment was filled with phosphate buffer saline pH 7.4. The system was connected to a thermostatically controlled water bath to maintain temperature at 32 ± 2°C by circulating water through a jacket surrounding the cell body.[17] After every 1 h, a sample of 0.5 mL was withdrawn from the receptor compartment and replaced with an equal volume of phosphate buffer saline pH 7.4. The sample was diluted with appropriate volume of fresh phosphate buffer saline pH 7.4 and analyzed spectrophotometrically at 223 nm. A patch without any drug was treated similarly on a Franz diffusion cell to obtain blank solution for UV analysis. The test was run in triplicate, and average reading was taken. The absorbance was fitted to the calibration curve to determine percentage drug release from the patch.

DATA ANALYSIS

Kinetic Models

In vitro dissolution study and ex vivo skin permeation study were further analyzed by the model-dependent approach by fitting the data in following models:

Zero-order equation: $Q_t = Q_0 + K_0 t$

First-order equation: $\log Q_t = \log Q_0 + K_1 t/2.303$
Higuchi equation: $M_t/M_\infty = k_2 \sqrt{t}$
Korsmeyer–Peppas equation: $M_t/M_\infty = k_3 t^n$

where Q_t, amount of drug dissolved in time t; Q_0, initial amount of drug in the solution; K_0, zero-order release constant; K_1, first-order release constant; M_t, cumulative amount of drug released at time t; M_∞, absolute cumulative amount of drug released at infinite time; k_2, constant reflecting the design variable of the system; k_3, constant incorporating structural and geometric characteristics of the device; n, release exponent indicative of the mechanism of drug release.[20,21]

Calculation of Targeted Flux

Target flux was calculated by the following formula:

$$J \times A = Cl \times C_p \times W \quad (8)$$

where J, flux in $\mu g/cm^2 \cdot h$; A, area in cm^2; Cl, clearance of bisoprolol fumarate (0.214 L/h kg); C_p, plasma concentration of bisoprolol fumarate (50 μg/L) (Bisoprolol; Merck, Germany); W, average weight of patient (70 kg).[22]

Calculations for Ex Vivo Skin Permeation Studies

The ex vivo skin permeation studies were analyzed for a cumulative amount of drug permeated, flux, and permeability coefficient.

A cumulative amount of drug permeated in $\mu g/cm^2$ was plotted against time. Drug flux in $\mu g/cm^2 \cdot h$ at steady state was calculated by dividing the slope of a linear portion of the curve by the area of the exposed skin surface, i.e. 1.2 cm^2. The permeability coefficient in cm/h was deduced by dividing the flux with initial drug amount.[23]

The steady-state flux can also be calculated by following equation (9):

$$J_{ss} = P_s C_d = (K \times D_{ss} C_d)/L \quad (9)$$

where J_{ss}, steady-state flux; P_s, permeability coefficient; C_d, concentration of drug in donor compartment; K, partition coefficient from the transdermal drug delivery system onto stratum corneum; D_{ss}, apparent diffusivity through skin; L, thickness of skin.

Statistical Data Analysis

In the present study, MiniTab® 17.1.0 was used to interpret statistical data. Analysis of variance (ANOVA) with $p < 0.05$ as a minimal level of significance was used to determine statistical difference between formulation's in vitro dissolution study. A three-dimensional (3D) surface plot at the 10th h was constructed to check the effect of addition of HPMC E5 on the release rate of drug from matrix patch.

Similarly ANOVA ($p < 0.05$) and Tukey's multiple comparison tests (at confidence interval of 95%) were used to determine statistical difference between the formulations containing a permeation enhancer in the optimized matrix patch.[24]

Results and Discussion

PHYSICOCHEMICAL EVALUATION OF MATRIX TRANSDERMAL PATCH OF BISOPROLOL FUMARATE WITHOUT PERMEATION ENHANCER

Organoleptic Characteristics

The organoleptic properties of patches including color, transparency, gloss, flexibility, smoothness, and homogeneity are given in Table II. The formulation M01 and M02 were colorless as compared to other formulations. The slightly opaque color of M03 to M06 was attributed to the presence of HPMC E5 as it was observed during preparation that a slightly cloudy solution was formed during mixing and casting when the HPMC E5 concentration was increased. HPMC E5 is a white powder or granular polymer, which forms a clear or slightly opalescent solution. On the other hand, Eudragit RS 100 beads were clear, colorless, and solution prepared for casting of M01 and M02 was translucent. M01 and M02 had maximum glossy appearance and smooth surface as compared to other formulations. Gloss is an optical property and is based on the interaction of light with physical characteristic of a surface. Patches with smooth surfaces appear glossy, whereas very rough surfaces do not reflect light and therefore patches appear to be matte.[25] It was observed that formulations containing a higher concentration of Eudragit RS 100 to HPMC E5 ratio had higher strength and flexibility as compared to those formulations that had higher HPMC E5 concentration.[26] Incorporation of PEG 400 as a plasticizer at 40% w/w polymer concentration formed smooth and flexible films. Plasticizers are low molecular weight resins or liquids. According to the lubricating theory of plasticization, the plasticizer molecules diffuse into the polymeric solution and weaken the polymer–polymer interactions. Plasticizers with low molecular weight act by reducing the secondary bonds (e.g., hydrogen bonding) of the polymer chains and themselves form secondary bond with polymers. The reduction in intermolecular or van der Waals forces along the polymer chains decreases tensile strength and glass transition temperature, whereas it increases the elongation of the polymer and flexibility and prevents film cracking.[25]

Weight Variation

The weight variation varied between 1.6180 ± 0.0065 and 1.6946 ± 0.0851 g (Table III). The low value of standard deviation (SD) ensures that the variability of weight within a patch ($n = 3$) was low.[27] As the backing layer was part of the matrix system, thus increased weight was obtained with total polymer weight of 1000 mg.

Thickness

The thickness of formed patches was between 0.433 ± 0.020 and 0.493 ± 0.031 cm (Table III). The result depicts that the SD value of a patch was low ($n = 3$); thus patches of similar thickness may be achieved with negligible variance.[27]

TABLE II
Organoleptic Characteristics of Bisoprolol Fumarate Transdermal Patch

Formulation code	Color	Transparency	Gloss	Flexibility	Smoothness	Homogeneity
M01	Colorless	+++[a]	+++	+++	+++	+++
M02	Colorless	++	+++	+++	+++	+++
M03	Slightly opaque	++	++	+++	+++	++
M04	Slightly opaque	++	++	+++	++	++
M05	Slightly opaque	++	++	+++	++	+++
M06	Slightly opaque	+++	++	++	++	++

[a]+++ represents the most positive or targeted characteristic.

TABLE III
Physicochemical Results of Matrix Transdermal Patches of Bisoprolol Fumarate

Formulation code	Weight (g)	Thickness (cm)	Folding Endurance	Drug Content (%)	Flatness (%)	Swelling Index	Weight Increase Due to Swelling (%)
M01	1.6946 ± 0.0851	0.467 ± 0.011	>100	99.45 ± 0.01	100.00 ± 0.00	0.4184 ± 0.0002	41.84
M0A	1.6180 ± 0.0065	0.447 ± 0.020	>100	99.89 ± 0.02	99.58 ± 0.71	0.5411 ± 0.0005	54.11
M03	1.6654 ± 0.0166	0.453 ± 0.011	>100	98.85 ± 0.02	100.00 ± 0.00	0.8201 ± 0.0005	82.01
M04	1.6566 ± 0.0296	0.463 ± 0.015	>100	99.02 ± 0.01	98.34 ± 0.74	1.1831 ± 0.0005	118.31
M05	1.6600 ± 0.0571	0.433 ± 0.035	>100	100.01 ± 0.03	99.58 ± 0.72	1.7526 ± 0.0003	175.26
M06	1.6919 ± 0.0120	0.493 ± 0.031	>100	97.25 ± 0.01	99.60 ± 0.69	1.7595 ± 0.0006	175.95

Folding Endurance

The formulated patches showed folding endurance greater than 100 (Table III). The folding endurance of patches was increased when the concentration of Eudragit RS 100 was increased.[28] The addition of Eudragit RS 100 makes cross-linking with HPMC E5 effective, which increases tensile strength.[29]

Drug Content and Content Uniformity

The minimum content uniformity was detected in M06 with 97.25% ± 0.01%, and the maximum value was obtained for M05 with 100% drug content in a 2× 2 cm patch (Table III). The low value of SD illustrates that the distribution of drug within the patch was uniform, and variability within different formulations was also negligible. This assures that rheological properties of the casting solution were suitable and assures homogeneity of drug by the solvent evaporation technique.

Flatness

The formulations M01 and M03 showed 100% flatness (Table III). A negligible constriction illustrates that the patches prepared by the solvent evaporation technique is reproducible, and they can maintain satisfactory surface smoothness.[30]

Swelling Index and Percentage Weight Increase Due to Swelling and Erosion Studies

The swelling index varied from 0.4184 to 1.7595, and the percentage weight increase ranged from 41.84% to 175.95% (Table III). The results reveal that increasing the amount of HPMC E5 increased the swelling index, percentage weight, and erosion of patches due to the hydrophilic nature of the polymer. The hydration of polymers in a transdermal patch may affect the sustained release profile of the matrix film as a higher rate of swelling leads to the formation of empty spaces and structure becomes less resistant to mechanical stresses. The addition of plasticizer increases the flexibility of Eudragit molecules and renders the patch more permeable to water molecule.[10]

Moisture Content

The percentage moisture content varied from 2.13% to 4.86% (Table IV). Films containing a higher amount of HPMC E5 showed more moisture content as compared to the films containing a higher amount of Eudragit RS 100 due to the hydrophilic nature of HPMC E5. Moisture content should be between 2% and 10% in the transdermal patches.[31] Moisture content studies were used to estimate the presence of moisture in the formulated patches after complete drying. It affects both the mechanical properties and drug release pattern.[10] The lower moisture content is required to maintain the stability, reduce brittleness, prevent bulkiness, and reduce susceptibility to microbial contamination.[32]

Moisture Uptake Capacity

The moisture uptake of formulated patches varied from 2.90% to 6.16% (Table IV). For transdermal patches, moisture uptake up to 15% w/w is claimed not to cause any discomfort as it prevents bulkiness of the film.[31,33] Eudragit RS 100 possesses some hydrophilic property due to the presence of the quaternary ammonium group, thus it may uptake some amount of moisture. The moisture capacity was further increased due to the presence of PEG 400 as a plasticizer, which is hydrophilic in nature. PEG 400 increases the free volume of Eudragit RS 100 by spacing out the polymeric chain from one another. This increases the mobility

TABLE IV
Moisture Content, Percentage Moisture Uptake at 84% RH, Water Vapor Transmission Rate, and Water Vapor Permeability Results of Matrix Type Transdermal Patch of Bisoprolol Fumarate

Formulation	Moisture Content (%)	Moisture Uptake (%)	WVTR ($g/cm^2 \cdot h$) x 10^{-6}	WVP ($mg/(Pa \cdot cm \cdot h)$) x 10^{-7}
M01	2.13 ± 0.02	2.90 ± 0.01	2.82 ± 0.02	2.09 ± 0.01
M02	2.43 ± 0.02	4.02 ± 0.02	3.48 ± 0.01	2.16 ± 0.02
M03	2.61 ± 0.02	5.75 ± 0.03	3.52 ± 0.01	2.21 ± 0.02
M04	3.65 ± 0.02	4.73 ± 0.02	3.88 ± 0.02	2.21 ± 0.02
M05	4.37 ± 0.01	6.15 ± 0.01	4.35 ± 0.02	2.31 ± 0.02
M06	4.86 ± 0.02	6.16 ± 0.01	4.27 ± 0.01	2.42 ± 0.01

of polymeric chain, and the network becomes less dense. This pore formation increases the permeability of film and increases film porosity.[32]

Water Vapor Transmission Rate

The water vapor transmission rate (WVTR) after 24 h was minimal in M01 (2.82×10^{-6} $g/cm^2 \cdot h^1$) and maximum in M05 (4.35×10^{-6} $g/cm^2 \cdot h^1$). WVTR was used to measure the passage of vapors through a patch, per unit area per unit time, to ensure its integrity during storage.[15] As the amount of HPMC E5 increased, the WVTR also increased.

Water Vapor Permeability

The water vapor permeability (WVP) was lowest in M01 (2.09×10^{-7}), because of the hydrophobic nature of Eudragit RS 100. The highest WVP was calculated in M05 (2.42×10^{-7}). WVP is a phenomenon, which determines the onset of drug release and drug release rate during dissolution.[15] A higher value of WVP signified that the dissolution rate would be greatest in M06 because of the hydrophilic nature of HPMC E5 (Table IV).

IN VITRO DRUG RELEASE STUDY

The dissolution studies were done to select an optimized formulation for ex vivo skin permeation studies. The in vitro dissolution studies were conducted for a period of 12 h.

In Vitro Drug Release Study of Formulation Containing 100% Eudragit RS 100

The cumulative drug release is shown inFig. 2. After 12 h, 57.45% of drug was released. This low release profile is due to the hydrophobic nature of Eudragit RS 100. The polymer has lower affinity for water and imbibes water at a lesser rate, thus it retards the release of drug from the matrix. As acquired cumulative drug release was too low, therefore a copolymer was added in the formulation.

In Vitro Drug Release Study of Formulation Containing Combination of Eudragit RS 100 and HPMC E5

The cumulative percentage drug release is shown in Fig. 2. It was found that changing polymer ratio of Eudragit RS 100 and

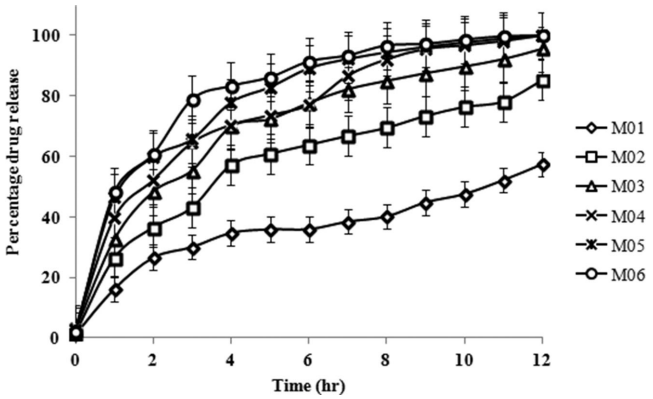

FIGURE 2. In vitro dissolution of M01, M02, M03, M04, M05, and M06 (n = 3).

HPMC E5 from 10:0 to 9:1 increased the initial release of drug from 16.63% to 26.52% in M01 and M02, respectively, within 1 h of dissolution study. This phenomenon is known as the burst effect and occurs due to the hydrophilic nature of HPMC E5. Owing to the imbibitions of water, chain relaxation and volume expansion occur that cause the polymer to swell and it becomes porous. This increases the diffusion coefficient, and the system becomes less restrictive for diffusion of drug through the matrix.[21] The percentage drug release was lowest in M02 and maximum in M04, M05, and M06 with 85.23% and 99.99% drug release, respectively.

The values of R^2, k, and n are given in Table V. All R^2 values better fitted in the Higuchi model. This signified that the main drug release mechanism from polymeric matrix was diffusion as proportionality between cumulative percentage drug release and square root of time is commonly regarded as an indicator of diffusion-controlled drug release.[21] The drug release mechanism of the formulations favored Fickian diffusion as the values of n were less than 0.5.[20,21]

Statistical analysis using the one-way ANOVA indicated that there was a significant difference between the formulation with $p < 0.001$. 3D plot (Fig. 3) at the 10th h signified that with the increase in hydrophilic polymer (HPMC E5) the drug release from the matrix patch also increased. From the above results, it was observed that release characteristics from the transdermal patch are restricted as in vitro dissolution mainly favors hydrophilicity. When these patches are applied to the skin, results may differ as ex vivo skin permeation studies involve lipophilicity, which plays a major role for the drug transport system.[7] Thus

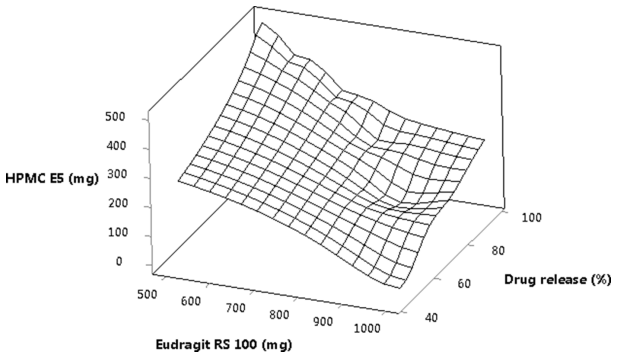

FIGURE 3. 3D surface plot of dissolution profile at the 10th h.

further studies were performed on rabbit's skin to analyze the effect of polymers and permeation enhancers on the release rate of drug.

The cumulative percentage drug released from M02 was 85.23% after 12 h, which achieved the desired sustain effect as compared to other formulations which experienced either the burst effect or complete release of drug over the 12-h period. Thus M02 was selected for further analysis for skin permeation studies through the excised rabbit's abdominal skin.

EX VIVO SKIN PERMEATION STUDIES OF BISOPROLOL FUMARATE MATRIX TYPE TRANSDERMAL PATCH

Ex Vivo Skin Permeation Study of Bisoprolol Fumarate Control Patch Containing No Permeation Enhancer

A control patch containing no permeation enhancer was made to check the cumulative drug release through the rabbit's abdominal skin. After 12 h, only 42.2% of drug had released, i.e. 3518.17 $\mu g/cm^2$ of the initial dose. Although the patch followed the zero-order kinetics (Table VI) but it showed a flux of only 246.51 $\mu g/cm^2 \cdot h$ and failed to achieve the targeted flux of 624.17 $\mu g/cm^2 \cdot h$ (Table VII). Thus it was necessary to use permeation enhancers to increase the skin permeation of bisoprolol fumarate. PEG 400 is a hydrophilic compound, which was capable of increasing transdermal drug release.[30,34] However, its use as a plasticizer in film formation of Eudragit RS 100 patch was the reason for lower permeation profile.[32]

Ex Vivo Skin Permeation Study of Bisoprolol Fumarate Containing Tween 80 as Permeation Enhancer

The permeation of drug from the formulations containing Tween 80 was minimum in M01-PE and maximum in M04-PE with cumulative drug release of 3772.00 $\mu g/cm^2$ and 8400.8 $\mu g/cm^2$, respectively (Fig. 4). The R^2 values (Table VI) showed that M01-PE and M04-PE followed the zero-order drug release kinetics. When drug is released from matrix in such a way that the rate of release remains constant, then the release rate kinetics is believed to follow zero order.[26] M02-PE and M03-PE better fitted in the Higuchi model, which indicated diffusion-controlled drug release.[35] The value of n for M02-PE signified that the formulation had an anomalous drug release, i.e. a combination of both diffusion- and erosion-controlled drug release phenomena. M01-PE, M03-PE, and M04-PE had the n value greater than 1.0, which indicated that the drug release was due to erosion, and patches followed the super case II mechanism.[20]

The flux increased with an increase in the Tween 80 concentration (Table VII). The targeted flux was calculated to be 624.17 $\mu g/cm^2 \cdot h$, which denotes that M04-B containing 40% Tween 80 had better cumulative drug profile as compared to other formulation containing the same permeation enhancer. The ANOVA test and Tukey's multiple comparison test suggested that there was a significant difference ($p < 0.001$) between percentage drug release at t_{10} and flux from the formulations containing different concentrations of Tween 80. Tween 80 is a nonionic surfactant and contains ethylene oxide and a long-chain hydrocarbon chain that imparts both hydrophobic and hydrophilic characteristics. This attribute allows the partitioning between both lipophilic lipid molecules and hydrophilic protein domain. Tween 80 is believed to increase the rate of drug release by penetrating into intracellular matrix followed by interaction and binding with the keratin filament, which causes disruption of the corneocytes.[36] It is generally recognized that nonionic surfactants possesses least toxicity and skin irritation potential as compared to anionic, cationic, and zwitterionic surfactants.[37]

Ex Vivo Skin Permeation Study of Bisoprolol Fumarate Containing Propylene glycol as Permeation Enhancer

As the concentration of PG increased, the cumulative amount of drug increased from 5148.05 $\mu g/cm^2$ to 8468.8 $\mu g/cm^2$ in M05-PE and M08-PE, respectively (Fig. 5). The R^2 value of M05-PE, M06-PE, M07-PE, and M08-PE implied that

TABLE V
Kinetic Models for Dissolution Profile

	Zero-Order Kinetics		First-Order Kinetics		Higuchi Model		Korsmeyer–Peppas	
Formulation	R^2	K_1	R^2	K_2	R^2	K_3	R^2	n
M01	0.8897	13.809	0.4982	2.2631	0.9708	2.6578	0.9561	0.4371
M02	0.8733	22.608	0.4869	2.6700	0.9864	4.0828	0.9829	0.4587
M03	0.8303	30.295	0.4776	2.9829	0.9758	8.5300	0.9761	0.4206
M04	0.8340	33.413	0.4669	2.7753	0.9783	10.855	0.9896	0.3775
M05	0.7489	40.015	0.4120	2.8847	0.9411	16.758	0.9777	0.3165
M06	0.6789	43.978	0.3701	2.8471	0.9000	20.105	0.9398	0.2903

TABLE VI
Kinetic Models for Ex Vivo Skin Permeation Studies

	Zero-Order Kinetics		First-Order Kinetics		Higuchi Model		Korsmeyer–Peppas	
Formulation	R^2	K_1	R^2	K_2	R^2	K_3	R^2	n
M01-PE	0.9715	1.8916	0.9478	0.2088	0.8472	9.4668	0.9808	1.0134
M02-PE	0.9631	4.3723	0.8342	0.1759	0.9822	20.387	0.9717	0.9058
M03-PE	0.9537	7.0925	0.5048	0.3782	0.9684	32.999	0.7835	1.1478
M04-PE	0.9663	9.0104	0.7063	0.2225	0.9586	41.211	0.9146	1.2080
M05-PE	0.9433	4.0117	0.9380	0.1398	0.9000	18.092	0.9470	0.6701
M06-PE	0.9382	4.3422	0.8895	0.0859	0.9168	19.818	0.9333	0.4200
M07-PE	0.9752	9.0924	0.7808	0.2482	0.9415	41.250	0.9479	1.3047
M08-PE	0.9515	10.138	0.8988	0.2339	0.9318	46.322	0.9664	1.1568
M09-PE	0.9765	4.6367	0.9420	0.1661	0.9340	20.938	0.9763	0.8069
M10-PE	0.9261	8.5666	0.5794	0.2793	0.8796	38.547	0.7901	1.5560
M11-PE	0.9708	8.8798	0.9545	0.1975	0.9163	41.250	0.9582	0.9432
M12-PE	0.8780	6.9228	0.5736	0.1446	0.9410	33.089	0.8164	0.8227
Control	0.9731	3.5498	0.7985	0.2716	0.9362	16.075	0.9715	1.4292

TABLE VII
Slope, Flux, and Permeability Coefficient of Bisoprolol Fumarate Matrix Patch Containing Permeation Enhancers

Formulation	Slope	Flux (μg/cm^2·h)	Permeability Coefficient (cm/h)
M01-PE	298.13	248.44	0.0248
M02-PE	364.36	303.63	0.0337
M03-PE	591.04	492.53	0.0547
M04-PE	752.84	627.37	0.0697
M05-PE	334.30	278.58	0.0310
M06-PE	361.85	301.54	0.0335
M07-PE	910.97	759.14	0.0843
M08-PE	844.04	703.03	0.0782
M09-PE	386.39	321.99	0.0358
M10-PE	713.88	594.90	0.0661
M11-PE	739.99	616.66	0.0685
M12-PE	576.90	482.30	0.0536
Control	295.81	246.51	0.0274

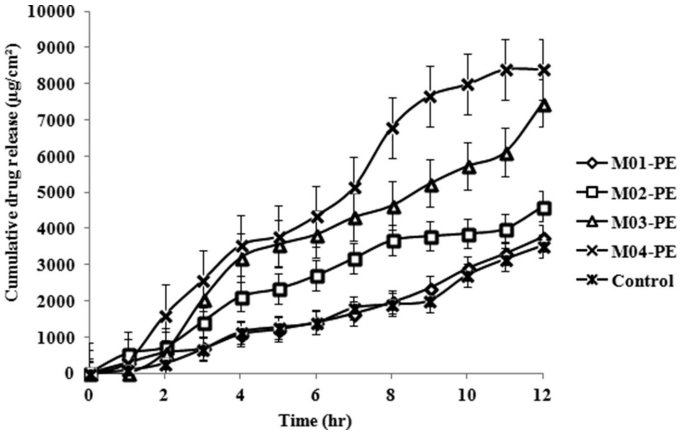

FIGURE 4. Ex vivo cumulative drug release from transdermal patches containing Tween 80 as compared to control ($n = 3$).

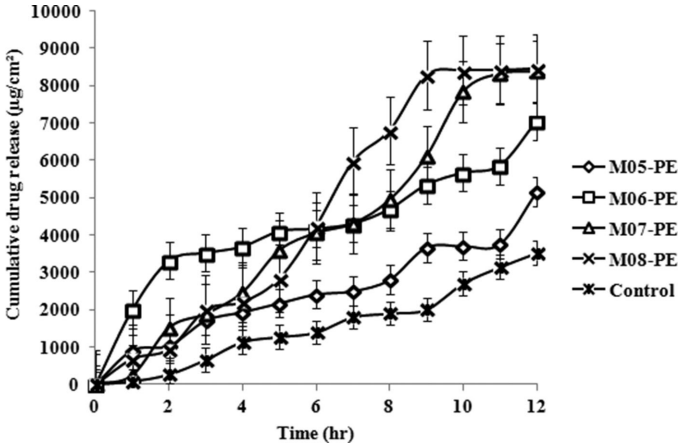

FIGURE 5. Ex vivo cumulative drug release from transdermal patches containing PG as compared to control ($n = 3$).

formulations followed the zero-order kinetics. The value of n of Korsmeyer–Peppas equation was greater than 0.5 for M05-PE, which signified that the drug release mechanism was anomalous, favoring both diffusion and erosion whereas the n value of M06-PE indicated the Fickian diffusion release mechanism.[20] The formulation M07-PE and M08-PE showed the super case II transport, i.e. erosion-controlled drug release mechanism (Table VI). In case of HPMC E5–based system, the value of n gives limited insight to the exact release mechanism, i.e. if a formulation is following the Higuchi model it would not necessarily be based on a simple diffusion control mechanism ($n < 0.5$). This is because HPMC E5 swells and dissolves more or less rapidly, whereas the Higuchi equation and Korsmeyer–Peppas equation assumes constant diffusivity and constant dimensions of the device during drug release. Thus release kinetics may result from superposition of various effects.[21]

The flux values indicated that as the concentration of PG increased from 10% to 30% flux through the skin also increased by 2.7 fold. The one-way ANOVA test and Tukey's multiple comparison test suggested that there was a significant difference ($p < 0.001$) between percentage drug release at t_{10} and flux

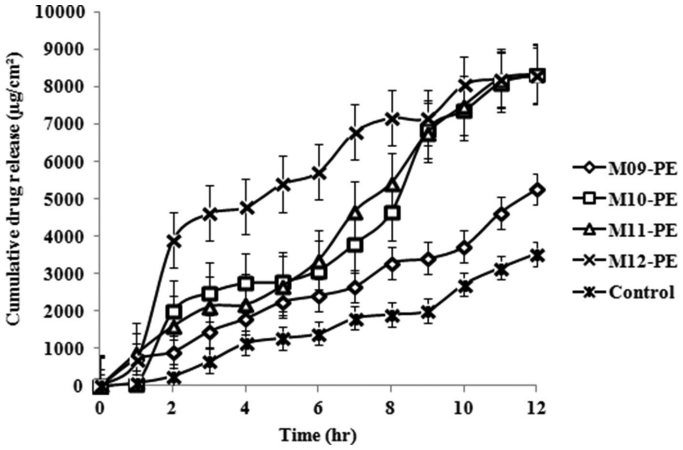

FIGURE 6. Ex vivo cumulative drug release from transdermal patches containing DMSO as compared to control ($n = 3$).

from the formulations at different concentrations of PG. The permeation of PG alters thermodynamic activity of drug in the system and modifies driving force for diffusion. It partitions into the tissue, facilitating uptake of drug into skin and implements some minor disturbance to intercellular lipid packing within the stratum corneum.[38] PG acts as a penetration enhancer by the solvent drag mechanism, i.e. it carries a drug into the tissues rather than fluidizing the lipids.[39]

Ex Vivo Skin Permeation Study of Bisoprolol Fumarate Containing DMSO as Permeation Enhancer

The data for the cumulative amount of drug permeated through matrix patches containing DMSO as penetration enhancer is shown in Fig. 6. The study depicts that the drug permeation through skin increased from 5252.61 to 8335.0 $\mu g/cm^2$ in M09-PE and M12-PE, respectively, when the concentration of DMSO was increased from 10% to 40%. The formulations M09-PE, M10-PE, and M11-PE showed best fitting in the zero-order kinetic model. On the other hand, M12-PE followed the Higuchi model. The n values of M09-PE, M11-PE, and M12-PE signified that the mechanism of drug release was non-Fickian or anomalous, favoring both diffusion and erosion (Table VI). As the n value of M10-PE was greater than 1, thus it had the super case II transport drug release mechanism, which favored an erosion-based release pattern.[20,21] From the given data, M11-PE had a closer flux to that of the targeted flux of 624.17 $\mu g/cm^2 \cdot h$. Although a significant difference was depicted in flux by the one-way ANOVA test and Tukey's multiple comparison test ($p < 0.001$), the drug release at t_{10} from patches containing 20% and 30% DMSO was not significantly different ($p > 0.05$). DMSO acts as a permeation enhancer by denaturing intercellular structural proteins of the horny layer and promoting lipid fluidity by disruption of lipid chains in the skin. DMSO also alters the physical structure of stratum corneum by extraction of lipids, lipoprotein, and nucleoproteins from the skin structure.[40]

COMPARISON OF BISOPROLOL FUMARATE MATRIX TRANSDERMAL PATCH HAVING FLUX CLOSER TO THE TARGETED FLUX

The addition of the permeation enhancer in matrix films of bisoprolol fumarate greatly increased flux as compared to patches having no permeation enhancer (246.51 $\mu g/cm^2 \cdot h$). The matrix patches having closer flux to that of the aimed flux included M04-PE (616.93 $\mu g/cm^2 \cdot h$), M08-PE (704.03 $\mu g/cm^2 \cdot h$) and M11-PE (616.66 $\mu g/cm^2 \cdot h$). The formulations contained 40% Tween 80, 40% PG, and 30% DMSO, respectively. These three formulations showed sustained and 100% drug release after 12 h.

Conclusions

Based on the study, it can be reasonably concluded that the addition of hydrophilic polymer increases the release rate of drug from a hydrophobic matrix system. The blend of Eudragit RS 100–HPMC E5 (9:1) and PEG 400 with 40% Tween 80 can be used to enhance the permeation of bisoprolol fumarate from a matrix transdermal patch. The study can be further assisted by in vivo drug profile and bioequivalence studies for better conclusion of the present work.

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