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EFFECT OF HYDROPHILIC AND HYDROPHOBIC POLYMER ON *IN VITRO* DISSOLUTION AND PERMEATION OF BISOPROLOL FUMARATE THROUGH TRANSDERMAL PATCH

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Abstract: A matrix transdermal patch of bisoprolol fumarate was formulated with different concentrations of Eudragit RS100 and Methocel E5 with PEG 400 as plasticizer by solvent evaporation technique. Tween 80 was added to the optimized patch to evaluate the effect of permeation enhancer at different concentration through the excised rabbit's skin. The patches were analyzed for weight variation, drug content, swelling index, erosion studies, moisture content, moisture uptake, water vapor transmission rate (WVTR) and water vapor permeability (WVP). *In vitro* dissolution test was carried out in USP dissolution apparatus V to select the optimized formulation. *In vitro* skin permeation studies were done in Franz diffusion cell using rabbit skin as a model membrane. The cumulative drug release and flux were determined to compare the result of test patches with a control patch. The greatest enhancement ratio (ER) was obtained in F03-PE with 30% Tween 80. F03-PE seemed to follow zero order kinetics with super case II mechanism of drug release. Statistical ANOVA suggested that there was a significant difference in formulations, steady flux and cumulative permeation rate at different Tween 80 concentrations.

Keywords: Eudragit RS100, kinetics, Methocel E5, Tween 80, transdermal patch

Oral drug delivery has been considered the most suitable method of drug administration for decades. The drugs that cannot be given by the oral route have been alternated through injection by hypodermic needles. However, it has several drawbacks such as pain at the site of administration and removal of drug once it has been administered (1). These problems have thus pilot to the development and advancement in alternate means of drug delivery. One of such technique is transdermal drug delivery system. This method operates by delivering the drug through the skin using a patch (2).

Over the past two decades, the challenge of transdermal drug delivery has been recognized by pharmaceutical scientists. The intensity of interest in the potential biomedical applications of transdermal controlled drug administration is verified by increasing research activity in the development of various types of transdermal therapeutic systems for long-term continuous delivery of therapeutic agents, including antihypertensive, antianginal, analgesic, steroidal, and contraceptive drugs. Bisoprolol fumarate belongs to the class of β-blockers. In comparison with other β-blockers like atenolol and metoprolol, bisoprolol has proved to be a compound with highest β_1-selectivity. It only shows low affinity to the β_2-recpetor of the smooth muscles of bronchi and vessels as well as to the β_2-receptors concerned with metabolic regulation.

The present study was undertaken to evaluate the effective permeation of bisoprolol from transdermal patch through the skin. The formulations were evaluated by altering the concentration of Methocel E5 (hydrophilic polymer) and Eudragit RS 100 (hydrophobic polymer) to select an optimized formulation based on physicochemical and *in vitro* dissolution studies. A permeation enhancer (Tween 80) was added in optimized formulation to check the permeation rate of drug through the skin by disruption of the stratum corneum.

MATERIALS AND METHODS

Material

Bisoprolol fumarate (donated by Mass Pharma, Lahore, Pakistan), Methocel E5 (Merck,

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Germany), Eudragit RS 100 (Merck, Germany), polyethylene 400 (Merck, Germany), polyvinyl alcohol (Merck, Germany), sodium chloride (Merck, Germany), potassium dihydrogen phosphate (Fluka, Germany), disodium hydrogen phosphate (Fluka, Germany), potassium chloride (Aldrich, Germany), calcium chloride (Uni-chem, Pakistan), sodium hydroxide (Riedel-de Haen, Germany), methanol and hydrochloric acid (BDH, UK).

Construction of calibration curve of bisoprolol fumarate

A calibration curve of drug was prepared by stock solution dilution method in phosphate buffer saline (PBS) pH 7.4. The dilutions were made at 1, 2, 4, 6, 8 and 12 µg/mL from the stock solution (100 mg drug in 100 mL of PBS pH 7.4). The samples were withdrawn, filtered and analyzed spectrophotometrically at 223 nm (T-80 UV/Vis Spectrophotometer, PG Instrument, Midland, Canada).

Preparation of matrix transdermal patch of bisoprolol fumarate

Transdermal patch of bisoprolol fumarate was prepared by solvent evaporation technique according to the formulation depicted in Table 1. Weighed amount of Methocel E5 and Eudragit RS 100 were added in 15 mL of methanol and homogenously mixed on hot plate magnetic stirrer (DHPS-1, Galvano Scientific and HJ-5) for 60 min. After the stated time, drug solution (bisoprolol fumarate in 5 mL of methanol) and PEG 400 (as a plasticizer) were added and further mixed for 30 min to ensure complete mixing. The casting solution was sonicated (Supersonic X-3, AFD Instruments, Lahore, Pakistan) for 20 min to remove air bubbles and poured on PVA backing layer (4% w/v) in a Petri dish. A funnel was placed on it in an inverted manner to control rate of evaporation of methanol. The patches were dried at 35°C for 48 h and stored till further analysis.

Preparation of matrix transdermal patch of bisoprolol fumarate

Weighed amount of Methocel E5 and Eudragit RS 100 were added in 15 mL of methanol and homogenously mixed on hot plate magnetic stirrer for 60 min. After the stated time, drug solution, PEG 400 and Tween 80 (as permeation enhancer) were added and further mixed for 30 min to ensure complete mixing. The casting solution was sonicated for 20 min to remove air bubbles and poured on PVA backing layer (4% w/v) in a Petri dish. A funnel was placed on it in an inverted manner to control rate of evaporation of methanol. The patches were dried at 35°C for 48 h and stored till further analysis.

Physicochemical properties of transdermal patch

Weight variation

The patches were randomly selected (n = 3) and weighed on digital weighing balance (3) with a sensitivity of 0.0001 g (DV215CD, Ohaus, New Jersey, USA).

Table 1. Formulation of matrix transdermal patch of bisoprolol fumarate.

Formulation code	ERS 100 : Methocel E5	Drug (mg)	PEG 400 (40% w/w)	Tween 80 (w/w)	Methanol (mL)
F01	10 : 0	10	400	-	20
F02	9 : 1	10	400	-	20
F03	8 : 2	10	400	-	20
F04	7 : 3	10	400	-	20
F05	6 : 4	10	400	-	20
F06	5 : 5	10	400	-	20
F07	4 : 6	10	400	-	20
F08	3 : 7	10	400	-	20
F09	2 : 8	10	400	-	20
F10	1 : 9	10	400	-	20
F11	0 : 10	10	400	-	20
F01-PE	8 : 2	10	400	10%	20
F02-PE	8 : 2	10	400	20%	20
F03-PE	8 : 2	10	400	30%	20

Content uniformity test

A film of 1 × 1 cm was completely dissolved in PBS pH 7.4 on a magnetic stirrer for 12 h (4). After the stated time, solution was sonicated for 20 min and filtered through Whatman filter paper. A filtrate of 3 mL was withdrawn, appropriately diluted and analyzed spectrophotometrically at 223 nm. A blank (patch without drug) solution was prepared by the same method. The amount of drug was determined with reference to calibration curve.

Swelling index, percentage weight increase and erosion studies

A film of 2 × 2 cm was cut from the patch and dried overnight at 40 ± 2°C. The films were fixed on preweighed cover slips and weighed. They were properly labeled and dipped in distilled water to ensure complete immersion. For swelling index and percentage weight increase the cover slips were taken out after 30 min, blotted to remove excess of water and weighed. The films that had disintegrated prior to time stated were discarded. The procedure was continued for up to 60 min for percentage erosion studies. The results were calculated by following equations (5):

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

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

$$\text{Percentage weight decrease due to erosion} = (W_1 - W_2) / (W_1) \times 100$$

where W_1 is initial weight of the film before swelling; W_2 is weight of the film after time 't'.

Percentage moisture content

A film of 2 × 2 cm was weighed and placed in a properly labeled Petri dish. The Petri dishes were placed in an incubator (LIB-030M, LabTech, Namyangju, Korea), containing silica beads as a desiccant, at 25°C. The films were weighed for five days of storage. The percentage moisture content was calculated by the following equation (6):

$$\text{Percentage moisture content} = (\text{Initial weight} - \text{Final weight}) / (\text{Final weight}) \times 100$$

Percentage moisture uptake

A film of 2 × 2 cm was weighed and placed in a properly labeled Petri dish. They were placed in an incubator containing 200 mL of saturated solution of potassium chloride (KCl) (84% RH) at 25°C. The moisture uptake was calculated by the following equation (7):

$$\text{Percentage moisture uptake} = (\text{Final weight} - \text{Initial weight}) / (\text{Final weight}) \times 100$$

Water vapor permeability (WVP)

A film of 1 × 1 cm, with known thickness and weight, was fixed in 5 mL vial containing silica beads. The vials were weighed and kept in an incubator containing saturated solution of KCl (84% RH) at 30°C. The vials were weighed for 24 h and WVP calculated by following equation (8):

$$P = (Q \times d) / (A \times T \times S \times [R_1 - R_2])$$

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

Water vapor transmission rate (WVTR)

A film of 1 × 1 cm was fixed in a 5 mL vial containing 1 g of calcium chloride ($CaCl_2$). The vials were weighed and kept in an incubator at 25°C containing 200 mL of saturated solution of KCl (84% RH). The vials were weighed for 24 h and WVTR was calculated by equation (9):

$$WVTR = (W / S \times t)$$

where W is grams of water transmitted per 24 h, S is surface area in cm^2, t is total time.

In vitro dissolution studies

The *in vitro* dissolution studies were done in USP apparatus V, paddle method (Curio 2020+, Pakistan). The disk assembly was prepared by using a watch glass, a synthetic mesh (120 µm) and plastic coated stainless steel clips. The patch was placed against the watch glass such that the backing layer was on the surface of watch glass and release surface facing upward and parallel to the bottom of the paddle blade. The patch was retained in position with synthetic mesh net using plastic coated stainless steel clips. The vessels were filled with 500 mL of PBS pH 7.4 at 32 ± 2°C and stirring speed was fixed at 50 rpm (7). A samples of 3 mL was withdrawn after suitable interval of time over a period of 12 h, filtered, diluted and analyzed spectrophotometrically at 223 nm. The percentage drug release was estimated with reference to calibration curve.

In vitro skin permeation studies through animal membrane

The rabbit was sacrificed by cervical dislocation and hair on abdominal region was trimmed with an aid on hair clipper. The abdominal skin was prepared by soaking the skin in water at 60°C for 45 s and sub-dermal tissues were removed with forceps. The dermis was wiped for 1 min with a cotton swab dipped in isopropyl alcohol (IPA) to remove adher-

ing fats. The skin was washed with warm water and kept in saline solution. It was stored in refrigerator and used within one week of preparation. Before experimentation, the skin was allowed to reach at room temperature for at least 10 h and equilibrated for 1 h in PBS pH 7.4 (7).

The *in vitro* skin permeation study was conducted in Franz diffusion cell which had a diffusion area of 1.2 cm^2 and receptor compartment volume of 12 mL. The rabbit's membrane was soaked in PBS pH 7.4 for 1 h before experimentation. The membrane was mounted on the surface of the receptor compartment and a circular patch was pressed on it such that the backing layer was facing away from the skin. After securing the cell assembly with clamp, the receptor compartment was filled with PBS pH 7.4 and placed on hot plate magnetic stirrer. The system was connected to thermostatically controlled water bath to maintain temperature at 32 ± 2°C (10). A sample of 0.5 mL was withdrawn after every hour over a period of 24 h and analyzed spectrophotometrically at 223 nm. The cumulative percentage drug released was estimated with reference to calibration curve.

Data analysis

Kinetic model

The *in vitro* dissolution and permeation study were analyzed by model dependent approach by fitting the data in zero order, first order, Higuchi model and Korsmeyer-Peppas model.

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 is amount of drug dissolved in time t, Q_0 is initial amount of drug in the solution, K_0 is zero order release constant, K_1 is first order release constant, M_t is cumulative amount of drug released at time t, M_∞ is absolute cumulative amount of drug released at infinite time, k_2 is constant reflecting the design variable of the system, k_3 is constant incorporating structural and geometric characteristics of the device, *n* is release exponent indicative of the mechanism of drug release (11).

Calculation for permeation studies

The targeted flux was estimated by the following equation

$$J \times A = Cl \times C_p \times W$$

where J is flux in µg/cm^2 h, A is the area in cm^2, Cl is clearance of bisoprolol fumarate (0.214 L/h×kg), C_p is plasma concentration of bisoprolol fumarate (50 µg/L) (bisoprolol, Merck, Germany 2001), W is average weight (70 kg) (12).

Cumulative amount of drug permeated (µg/cm^2) was plotted against time. Drug flux (µg/cm^2×h) at steady state was calculated by dividing the slope of linear portion of curve by area of the exposed skin surface (1.2 cm^2). The permeability coefficient (cm/h) was deduced by dividing the flux with initial dose (13).

Permeation enhancement index was determined by the equation

ER = (Drug permeability coefficient after enhancement treatment) / (Drug permeability coefficient before enhancement treatment)

Statistical data analysis

For statistical analysis of data, MiniTab® 17.1.0 was used to interpret data. Analysis of vari-

Table 2. Weight, drug content, swelling index and percentage erosion of matrix transdermal patch.

Formulation code	Weight ± S.D. (g)	Drug content (%)	Swelling index	Erosion (%)
F01	1.6946 ± 0.0851	99.45 ± 0.01	0.4184 ± 0.0002	18.60 ± 0.15
F02	1.6180 ± 0.0065	99.89 ± 0.01	0.5411 ± 0.0005	25.51 ± 0.55
F03	1.6654 ± 0.0166	98.85 ± 0.02	0.8201 ± 0.0005	34.19 ± 0.52
F04	1.6566 ± 0.0296	99.02 ± 0.01	1.1831 ± 0.0005	32.05 ± 0.39
F05	1.6600 ± 0.0570	100.0 ± 0.02	1.7526 ± 0.0003	35.08 ± 0.29
F06	1.6919 ± 0.0120	97.25 ± 0.01	1.7595 ± 0.0006	32.95 ± 0.88
F07	1.6925 ± 0.0090	98.24 ± 0.02	2.2147 ± 0.0003	62.77 ± 0.47
F08	1.6370 ± 0.0070	99.62 ± 0.02	2.2595 ± 0.0003	67.11 ± 0.47
F09	1.6193 ± 0.0249	98.98 ± 0.01	2.5439 ± 0.0001	72.51 ± 0.26
F10	1.6403 ± 0.0412	99.45 ± 0.01	Disintegrated after 10 min	
F11	1.6788 ± 0.0583	97.87 ± 0.01		

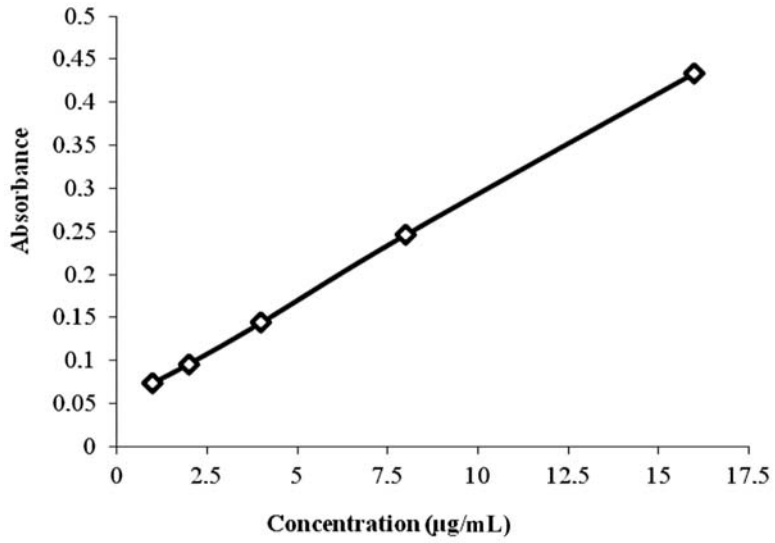


Figure 1. Calibration curve of bisoprorol fumarate in phosphate buffer saline pH 7.4, where linear line equation: y = 0.0241x + 0.0492, R^2= 0.9998

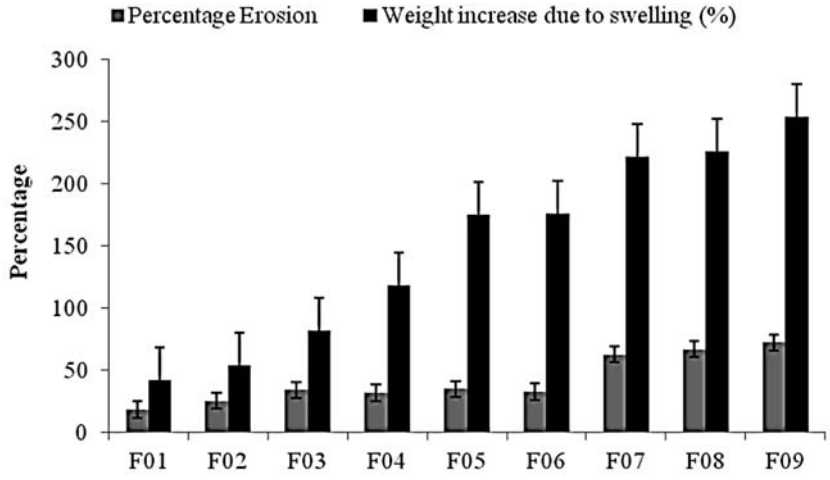


Figure 2. Effect of increasing hydrophilic polymer on percentage weigh increase and percentage erosion of matrix transdermal patch

ance (ANOVA) by Tukey's multiple comparison test with $p < 0.05$ as a minimal level of significance was used to determine statistical difference between dissolution and permeation studies (14).

RESULTS AND DISCUSSION

The formulation F01 and F02 were colorless as compared to other formulations which had higher concentration of Methocel E5. It was observed during preparation that a slightly cloudy solution was formed during mixing and casting as Methocel E5 concentration was increased. Drying was a crucial element during preparation of transdermal patch as higher temperature (< 50°C) lead to cloudy appearance whereas backing layer was separated when patches were dried at room temperature. Thus the drying was done at an optimum temperature of 35°C. It was observed that formulations containing higher concentration of Eudragit RS100 to Methocel E5 ratio had higher strength and flexibility (15).

The weight variation of patches varied between 1.6180 ± 0.0065 to 1.6946 ± 0.08510 g (Table 2). The low value of standard deviation (S.D.) ensures that the variability of weight within a patch (n = 3) was low, therefore the formed patches are reproducible with negligible variance (16). As backing layer was part of the matrix system thus increased

weight was obtained with total polymer weight of 1000 mg.

The minimum content uniformity was detected in F06 with 97.25 ± 0.01% and maximum value was obtained for F05 with 100% drug content in a 22 cm patch. The low value of S.D. 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 solvent evaporation technique.

The swelling index varied from 0.42 to 2.54 and the percentage weight increase ranged from 41.84 to 254.39% (Table 2). The formulation M09-A showed the maximum swelling index and percentage weight increase. The results reveal that increasing the amount of Methocel E5 increased the swelling index and percentage weight due to hydrophilic nature of the polymer. F01 had the minimum change because of the hydrophobic nature of Eudragit RS 100. The patches F10 and F11 disintegrated after 5 min and did not retain their shape because of the maximum concentration of Methocel E5. The hydration of polymers in transdermal patch may affect the sustained release profile of the matrix film as higher rate of swelling leads to the formation of empty spaces and structure becomes less resistant to mechanical stresses (17). The addition of plasticizers increases the flexibility of Eudragit molecules and renders the patch more permeable to water molecule (3). The percentage erosion ranged from 18.61 to 72.51% in F01 and F09, respectively (Table 2). F10 and F11 disintegrated after 10 min, therefore they were excluded from further analysis. These formulations contained maximum amount of Methocel

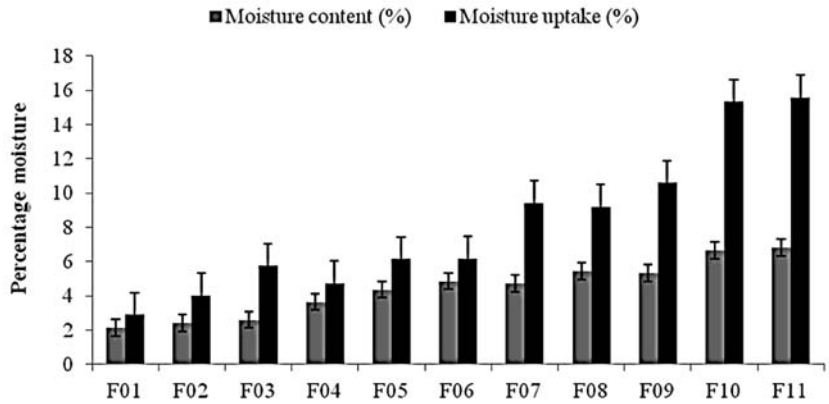


Figure 3. Percentage moisture content and percentage moisture uptake in matrix transdermal patch

Table 3. Moisture content, moisture uptake, WVTR and WVP of matrix transdermal patch.

Formulation	Moisture content (%)	Moisture uptake (%)	WVTR ($g/m^2.h$) × 10^{-6}	WVP ($mg.Pa^{-1}.cm^{-1}.h^{-1}$) × 10^{-7}
F01	2.13 ± 0.02	2.90 ± 0.01	2.82 ± 0.02	2.09 ± 0.01
F02	2.43 ± 0.02	4.02± 0.02	3.48 ± 0.01	2.16 ± 0.02
F03	2.61 ± 0.02	5.75± 0.03	3.52 ± 0.01	2.21 ± 0.02
F04	3.65 ± 0.02	4.73± 0.02	3.88 ± 0.02	2.31 ± 0.02
F05	4.37 ± 0.01	6.15± 0.01	4.35 ± 0.02	2.31 ± 0.02
F06	4.86 ± 0.02	6.16± 0.01	4.27 ± 0.01	2.42 ± 0.01
F07	4.74 ± 0.02	9.41± 0.02	4.32 ± 0.01	2.47 ± 0.01
F08	5.46 ± 0.01	9.21± 0.01	4.30 ± 0.01	2.52 ± 0.01
F09	5.34 ± 0.02	10.60± 0.01	4.37 ± 0.01	2.44 ± 0.01
F10	6.65 ± 0.03	15.32± 0.02	4.24 ± 0.02	2.67 ± 0.01
F11	6.81 ± 0.02	15.56± 0.02	4.44 ± 0.02	3.00 ± 0.02

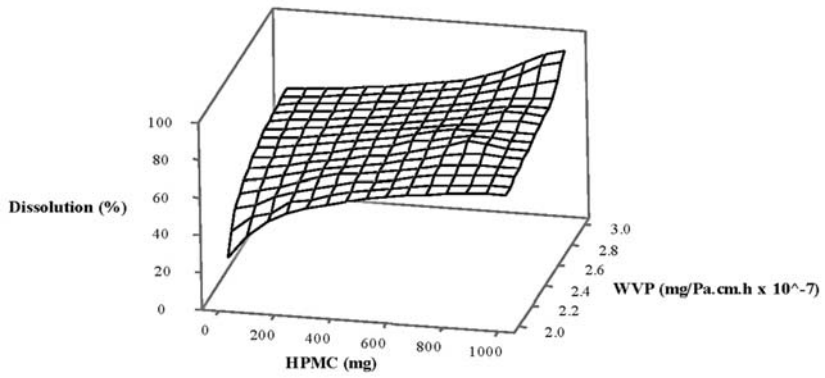
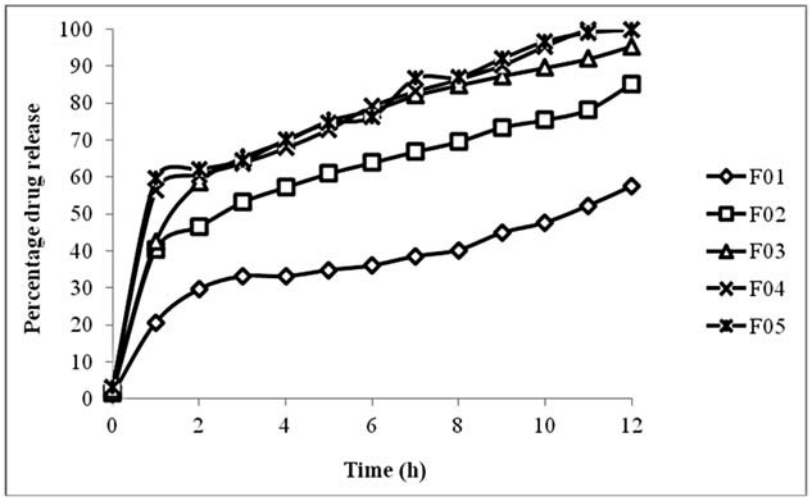


Figure 4. Effect of Methocel E5 concentration and WVP on dissolution

A



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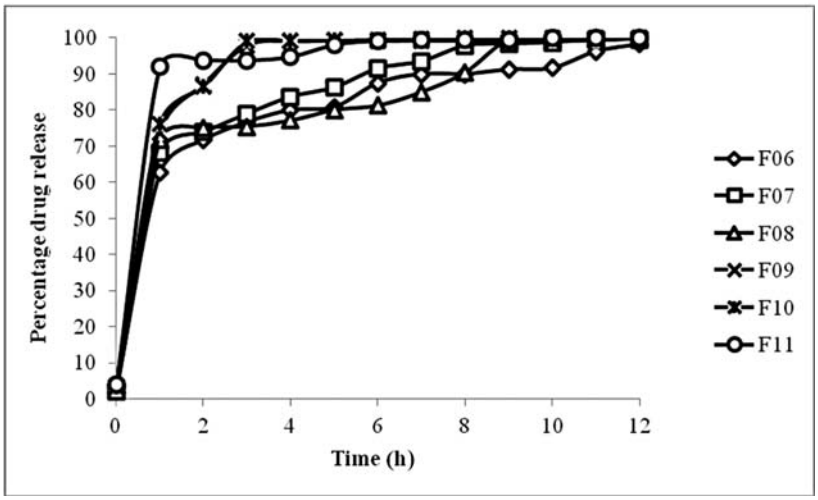

Figure 5. Dissolution profile of (**A**) F01, F02, F03, F04, F05 and (**B**) F06, F07, F08, F09, F10 and F11

E5 which imbibed water owing to hydrophilic nature of polymer and disintegrated in shorter life-span as compared to other formulation which contained greater amount of hydrophobic polymer Eudragit RS 100 (Fig. 2).

The percentage moisture content varied from 2.13 to 6.81% (Table 3). Films containing higher amount of Methocel E5 showed more moisture content as compared to the films containing higher amount of Eudragit RS 100 due to the hydrophilic nature of Methocel E5. Moisture content should be between 2 to 10% in the transdermal patches (18). Moisture content studies were used to estimate presence of moisture in the formulated patches after complete drying. It affects both the mechanical properties and drug release pattern (3). The lower moisture content is required to maintain the stability, reduce brittleness, prevent bulkiness and reduce susceptibility to microbial contamination (19).

The moisture uptake of formulated patches varied from 2.90 to 15.56%, F01 showing the lowest moisture uptake while F11 showing the maximum (Table 3). For transdermal patches, moisture uptake up to 15% w/w is claimed not to

Table 4. Kinetic model of bisoprolol transdermal patch.

Formulation	Zero order kinetics		First order kinetics		Higuchi model		Korsmeyer-Peppas	
	R^2	K_1	R^2	K_2	R^2	K_3	R^2	n
F1	0.8523	3.4082	0.4544	0.1698	0.9509	13.9600	0.9243	0.35
F2	0.7950	4.9284	0.3956	0.1669	0.9547	20.8660	0.9845	0.29
F3	0.7397	5.4545	0.3855	0.1516	0.9319	23.6440	0.9975	0.26
F4	0.5222	4.9898	0.3074	0.1376	0.7824	23.5990	0.9364	0.16
F5	0.7058	5.2009	0.3690	0.1415	0.8749	22.3730	0.8480	0.20
F6	0.5657	4.7298	0.3177	0.1318	0.8408	24.1780	0.9627	0.15
F7	0.5994	5.2837	0.3216	0.1518	0.8024	21.7650	0.9866	0.19
F8	0.3670	4.1710	0.2630	0.1217	0.6382	21.2520	0.7369	0.10
F9	0.3293	3.9491	0.2534	0.1193	0.5969	20.5420	0.5604	0.08
F10	0.6105	5.0528	0.3365	0.1290	0.8154	22.5610	0.8131	0.15
F11	0.3008	3.6752	0.2396	0.1117	0.5529	19.2520	0.9007	0.04

Table 5. Drug release (%) and cumulative amount of drug release from transdermal patch containing permeation enhancer.

Time (h)	Percentage drug release				Cumulative amount of drug released (μg/cm^2)			
	Control	F01-PE	F02-PE	F03-PE	Control	F01-PE	F02-PE	F03-PE
0	0.00	0.00	0.00	0.00	0	0	0	0
1	2.87	0.98	6.57	3.85	287	98	657	385
2	4.89	3.23	8.66	7.12	489	323	866	712
3	6.27	7.92	16.25	22.52	627	792	1625	2252
4	8.98	13.64	23.39	36.98	898	1364	2339	3698
5	12.01	16.25	28.00	39.63	1201	1625	2800	3963
6	14.25	19.85	32.39	44.98	1425	1985	3239	4498
7	15.87	23.78	36.85	51.81	1587	2378	3685	5181
8	16.25	27.02	42.52	55.75	1625	2702	4252	5575
9	18.85	30.58	46.57	63.21	1885	3058	4657	6321
10	20.14	34.85	50.45	75.56	2014	3485	5045	7556
11	24.52	39.45	56.12	82.25	2452	3945	5612	8225
12	29.07	45.74	59.44	90.16	2907	4574	5944	9016

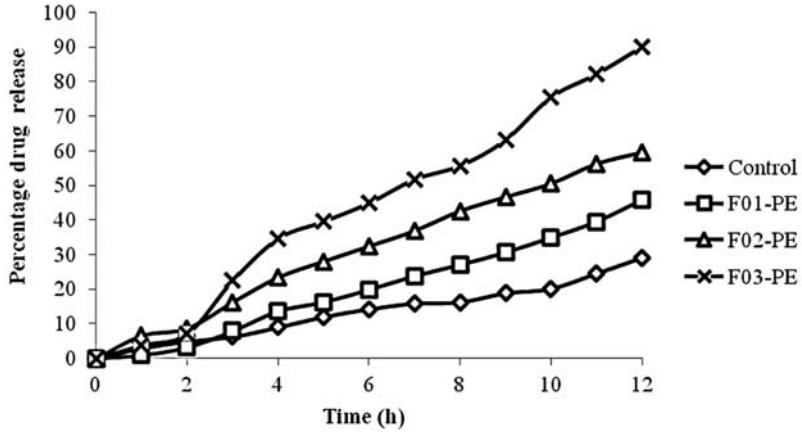

Figure 6. Percentage drug release from transdermal patch containing permeation enhancer

Table 6. Kinetic model and flux of transdermal patch containing permeation enhancer.

Time	Control	F01-PE	F02-PE	F03-PE
Zero order				
$k_{1\ (\%\ h^{-1})}$	382.59	219.68	499.80	767.42
R^2	0.9918	0.9819	0.9946	0.9853
First order				
$k_{2\ (\%\ h^{-1})}$	0.283	0.1849	0.1881	0.2414
R^2	0.803	0.9181	0.8825	0.7846
Higuchi				
$k_{2\ (h^{-1/2})}$	13.932	8.1332	18.746	28.410
R^2	0.8809	0.9017	0.9376	0.9111
Peppas				
n	0.2291	0.9661	1.7568	0.6616
R^2	0.9793	0.9879	0.9839	0.9044
*Flux (*µg/cm^2.h)				
	183.07	318.82	416.50	631.18

cause any discomfort as it prevent bulkiness of the film (18). Increased drift in percentage moisture uptake at 84% RH was seen as the amount of Methocel E5 increased (Fig. 3). Although Eudragit RS 100 possesses some hydrophilic property due to the presence of 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 of polymeric chain and network becomes less dense. This pore formation increases the permeability of film and increases film porosity (19).

The WVP was lowest in F01, 2.09×10^{-7} whereas the highest WVP was seen in F11, 3.00×10^{-7} (Table 3). WVP is a phenomenon which determines the onset of drug release and drug release rate during dissolution (8). It can be observed from Figure 4 that with an increase in Methocel E5 the WVP increased, which subsequently increased the rate of dissolution (t = 1st hour).

The WVTR after 24 h was minimal in F01 - 2.82×10^{-6} g×cm^{-2}×h^{-1}, and maximum in F09; 4.44×10^{-6} g×cm^{-2}×h^{-1} (Table 3). WVTR was used to measure the passage of vapors through a patch, per unit

area per unit time, to ensure its integrity during storage (8). As the amount of Methocel E5 increased the WVTR also increased.

The cumulative percentage drug release of F01, F02, F03, F04 and F05 is given in Figure 5 A whereas that of F06, F07, F08, F09, F10 and F11 is demonstrated in Figure 5 B. *In vitro* dissolution studies of F01 showed that 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. On the other hand, 92.03% of drug was released from the matrix system F11 within 1 h of initiation of test. This instant release of drug within an hour was an undesirable property as this could not achieve the sustain effect over 12 h. Thus, a rate controlling polymer was required to retard drug release from the matrix system. It was achieved by blending Eudragit RS 100 with Methocel E5 at different concentrations to obtain an optimum formulation of sustained drug delivery.

As the concentration of Methocel E5 increased the release rate of drug also increased. It was found that changing polymer ratio of Eudragit RS 100 and Methocel E5 from 10 : 0 to 9 : 1 increased the initial release of drug from 20.63 to 40.52% in F01 and F02, respectively, within 1 h of dissolution study. This phenomenon is known as the burst effect and occurs due to the hydrophilic nature of Methocel E5. Due to the imbibition of water inside the matrix, chain relaxation and volume expansion occurs, which causes the polymer to swell and becomes porous. This increases the diffusion coefficient and system becomes less restrictive to diffusion of drug through the matrix (20). The difference in final cumulative percentage drug release was not drastic from F03 to F11.

The cumulative percentage drug release was fitted in different models i.e., zero order kinetics, first order kinetics, Higuchi model and Korsmeyer-Peppas equation for drug release mechanism. The values of R^2, k and n are given in Table 4. It can be observed that F01, F02 and F03 follows 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 (20). All the other formulations i.e., F04 to F11 failed to follow any order as R^2 values were too low for good fitting.

The value of n for the three formulations F01, F02 and F03 was anomalous. The value of n between 0.5 and 1.0 is regarded as an anomalous transport which is an indicator of superposition of two phenomena i.e., diffusion controlled drug release and swelling controlled drug release (11).

From the above results it was observed that release characteristics from transdermal patch are restricted as *in vitro* dissolution mainly favors hydrophilicity. When these patches are applied to skin, the results may differ as *ex vivo* and *in vivo* permeation studies involve lipophilicity which plays a major role for drug transport system through the skin (21). F03 was selected as an optimized formulation with favorable physicochemical and *in vitro* dissolution characteristics and Tween 80 was added at concentration of 10 to 30%. A control patch containing no permeation enhancer gave a 29.07% drug release after 12 h of study i.e., only 2907.0 $\mu g/cm^2$ of drug was released from the initial dose (Table 5). Although the patch followed zero order kinetics, the flux (183.07 $\mu g/cm^2 \times h$) was not near the targeted flux of 624.17 $\mu g/cm^2 \times h$. With the addition of permeation enhancer, a considerable enhancement in the flux was observed. It was estimated that as the amount of Tween 80 increased from 10 to 30% the amount of drug released from the matrix patch also increased (Fig. 6). The R^2 values showed that all formulations followed zero order drug release, which signifies that the release of drug remained constant over the period of time (Table 6). The value of n for F01-PE illustrated that the drug release mechanism from patch was Fickian diffusion ($n < 0.5$) whereas control patch and F03-PE signified that the formulation had an anomalous drug release ($0.5 < n < 1.0$) which is a combination of both diffusion and erosion controlled drug release mechanism. F02-PE followed super case II mechanism ($n > 1.0$) that indicated that drug release was due to erosion. Statistical analysis using the one way ANOVA signified that there was a significant difference between the formulation, steady flux and cumulative permeation rate ($p < 0.001$) at different Tween 80 concentrations.

CONCLUSION

A better sustained release effect was obtained in F03 with 95% of drug being released after 12 h of dissolution study. The patch had desirable physicochemical properties. The optimized formulation (F03-PE) containing 30% Tween 80 had closer flux as compared to the targeted flux. The formulation followed zero order kinetics with anomalous drug

release. Thus, it can be reasonably concluded that Tween 80 can be used as a potential permeation enhancer for the development of matrix transdermal patch of bisoprolol fumarate.

Conflict of interest

The authors declare no conflict of interest.

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