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Effect of Polysorbate 80 Through Rabbit's Skin Using Transdermal Patch Loaded with Bisoprolol Fumarate as Model Drug

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ABSTRACT

An optimized matrix transdermal patch of bisoprolol fumarate was formulated with different concentrations of polysorbate 80 to evaluate the effect of permeation enhancer at different concentration through the excised rabbit's skin. PEG 400 was used as a plasticizer with Eudragit RS100 and HPMC (8:2) as polymers. The patches were prepared by solvent evaporation technique and analyzed for weight variation, thickness, drug content, swelling index, moisture content, moisture uptake, water vapor transmission rate (WVTR) and water vapor permeability (WVP). 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 F06 with 35% polysorbate 80. F06 seemed to follow zero order kinetics with super case II mechanism of drug release. Tukey's multiple comparison test suggested that there was a significant difference in steady flux, cumulative permeation rate and ER at different polysorbate 80 concentrations Thus Bisoprolol fumarate transdermal patch may give a better flux rate with 35% polysorbate 80 through model skin.

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Authors' Contributions
SA and MS designed and executed the experiments, and wrote the article. UA and IH helped in execution of experiments. NS and MR helped in interpretation of data and writing of article, respectively.

Key words
Bisoprolol fumarate, permeation enhancer, polysorbate 80, rabbit's skin, transdermal patch.

INTRODUCTION

Transdermal patch falls under 1st generation drug delivery system (Brown *et al.*, 2008, Prausnitz *et al.*, 2004). It is defined as a flexible, multi-laminated pharmaceutical preparation of varying size containing one or more drug substance which aids the passage of therapeutic quantities of drug substance through the intact skin and into the general circulation for their systemic effects or for local effect (Cai *et al.*, 2012). The barrier function of skin is essential for the protective role of stratum corneum but at the same time it may hinder the transdermal delivery of drug through it. The major route of drug is through the intracellular channels and the lipid section is a viable determinant in the first step of absorption. Penetration enhancers can temporarily diminish the barrier function of skin to enhance the drug flux (Rowat *et al.*, 2006) therefore various studies have been conducted to evaluate the optimum concentration at which the drug release may be controlled. Penetration enhancers are also known as accelerants, sorption promoter (Songkro, 2009) or permeation enhancer (Karande and Mitragotri, 2009). Polysorbate 80 is a non-ionic surfactant and contains ethyleneoxide and long chain hydrocarbon chain that imparts both hydrophobic and hydrophilic characteristics with an HBL value of 15 (Singh *et al.*, 2007). This attribute allows the partitioning between both lipophilic lipid molecules and hydrophilic protein domain. Polysorbate 80 is believed to increase the rate of drug release by penetrating into intracellular matrix followed by interaction and binding with keratin filament which causes disruption of the corneocytes (Liu *et al.*, 2007). It is generally recognized that non-ionic surfactants possesses least toxicity and skin irritation potential as compared to anionic, cationic and zwitterionic surfactants (Songko, 2009). The present study was conducted to access physicochemical properties of the transdermal patch containing different concentrations of polysorbate 80 and its effect on the permeation of drug through rabbit skin from an optimized transdermal patch.

MATERIALS AND METHODS

Bisoprolol fumarate (donated by Mass Pharma, Lahore, Pakistan), Eudragit RS100 (Merck, Germany), Hydroxypropyl methylcellulose E5 (Merck, Germany), Polyethylene glycol 400 (Merck, Germany), Polysorbate 80 (Daejung, Korea), Polyvinyl alcohol (Merck, Germany), Sodium chloride (Merck, Germany),

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Table I.- Formulation of optimized transdermal patch of Bisoprolol fumarate using polysorbate 80 as permeation enhancer.

Formulation code	ERS 100:HPMC	Bisoprolol fumarate (mg)	PEG 400 (40% w/w) (mg)	Penetration enhancer	Methanol (ml)
Control	8:2	10	400	--	20
F01	8:2	10	400	10%	20
F02	8:2	10	400	15%	20
F03	8:2	10	400	20%	20
F04	8:2	10	400	25%	20
F05	8:2	10	400	30%	20
F06	8:2	10	400	35%	20

Potassium chloride (Aldrich Chemical Co Ltd.), Potassium dihydrogen phosphate (Fluka, Germany), Disodium hydrogen phosphate (Fluka, Germany), Sodium hydroxide (Riedel-de Haen), Silica beads (Uni-chem), Calcium chloride (Uni-chem), Methanol (BDH, England), Hydrochloric acid (BDH, England).

All the chemicals used were of analytical grade.

Preparation of matrix patch without permeation enhancer

Weighed amount of polymers (Table I) were added in 15ml of methanol and stirred on a magnetic stirrer till completely dissolved. The stated amount of drug was mixed in 5ml of methanol and added to the polymer-matrix solution. Plasticizer was added and mixed for 1 h for complete homogenization of the casting solution. After said time the solution was sonicated for 20 minutes and poured on polyvinyl alcohol (PVA) backing layer (4% w/v). The films were completely dried by inverted funnel method and stored in aluminium foil in the presence of a desiccator till further analysis (Ren *et al*., 2009).

Preparation of matrix patch with permeation enhancer

Weighed amount of polymers were added in 15ml of methanol and stirred on a magnetic stirrer till completely dissolved. The stated amount of drug was mixed in 5ml of methanol and added to the polymer-matrix solution. Plasticizer and permeation enhancer were added and mixed for 1 h for complete homogenization of the casting solution. After said time the solution was sonicated for 20 minutes and poured on PVA backing layer. The films were completely dried by inverted funnel method and stored in aluminium foil in the presence of a desiccators till further analysis.

Physicochemical tests

Weight variation

The weight variation test was done by randomly selecting three patches of each formulation. The patches were weighed individually on digital weighing balance with a sensitivity of 0.0001 g (El-Gendy *et al*., 2008).

Thickness

The thickness of the patches was estimated by using digital vernier caliper (SH.0281, China). Three random patches of each formulation were selected for the test. The thickness was noted from the center and edges of patch (El-Gendy *et al*., 2008).

Content uniformity test

A film of 2x2 cm of each formulation was cut from a patch and completely dissolved in 100 ml phosphate buffer saline pH 7.4 with an aid of magnetic bead on a magnetic stirrer for 12 h. The temperature was fixed at 32°C. After 12 h, the solution was sonicated for 20 minutes. Sample of 3 ml was taken and filtered through whatman filter paper. The filtrate was diluted with equal volume of phosphate buffer saline pH 7.4 and analyzed spectrophotometrically (T-80 UV/vis spectrophotometer, PG instrument Ltd) at 223 nm (Prabhakar *et al*., 2012). Blank solution was prepared by same procedure but the film did not contain any drug.

Swelling index and percentage weight increase

A film of 1x1 cm was cut from a patch. They were dried at 40±2°C overnight before experiment. The films were than fixed on pre-weighed cover slips and completely immersed in water. After an interval of 5, 10 and 30 minutes 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 is calculated from the following equations (Pichayakorn *et al*., 2012):

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

Percentage weight increase due to swelling = $(W_2 – W_1) / (W_1) \times 100$

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 2x2 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 incubator (LIB-030M, LabTech) at 25°C containing silica beads as desiccant. The films were weighed for five days. The percentage moisture content was calculated by the following equation (Janardhanan *et al*., 2008):

Percentage moisture content = (Initial weight – final weight) / (Final weight) × 100

Percentage moisture uptake

A film of 1x1 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 incubator at 25°C (Limpongsa and Umprayn, 2008) containing 200 ml saturated solution of KCl for 84% RH. The films were weighed for five days of storage. The percentage moisture uptake was calculated by following equation (Janardhanan *et al*., 2008):

Percentage moisture content = (Final weight – initial weight) / (Final weight) × 100

Water vapor transmission test

A film of 1x1 cm with known weight was cut from a patch. The films were fixed in 5 ml vials and 1g of $CaCl_2$ was placed in each vial. The vials were weighed individually and then kept in incubator at 25°C containing 200 ml saturated solution of KCl for 84% RH. The vials were weighed for 24 h and weight was noted. The water vapor transmission was calculated by the following formula (Jaydatt and Sreenivas, 2013):

Water vapor transmission rate = $W / (S \times t)$

Where, W is grams of water transmitted per 24 h, t is total time (24 h) and S is surface area in cm^2

Water vapor permeability

A film of 1x1 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 kept in an incubator containing saturated solution of KCl, for 84% RH, at 30°C. The vials were weighed for 24 h. The water vapor permeability was calculated using the following formula (Xiangrong *et al*., 2007):

$$P = (Q \times d) / A \times T \times S \times (R_1 – R_2)$$

Where, P is permeability, Q is 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).

Preparation of rabbit skin

The hair on abdominal area of the rabbit was trimmed with an aid of hair clipper. The skin was made hairless by applying hair removal cream (Veet® depilatory cream, Reckitt Benckiser), wiped and washed off completely with warm water (Xi *et al*., 2010). The rabbit was sacrificed by cervical dislocation and abdominal region was obtained. The skin was prepared by soaking the skin in water at 60°C for 45 s (Limpongsa and Umprayn, 2008). The sub-dermal tissues were removed with forceps and dermis side was wiped for 1 min with a cotton swab dipped in Isopropyl alcohol (IPA) to remove adhering fats from the surface (Limpongsa and Umprayn, 2008). The skin was washed with warm distilled water, kept in saline solution and stored in refrigerator. It was used within one week of preparation. Before starting the experiment the skin was allowed to reach room temperature for at least 10 h and equilibrated for 1 h in phosphate buffer saline pH 7.4 (Prabu *et al*., 2012).

In vitro skin permeation

The *in vitro* skin permeation study of films across rabbit skin was conducted in Franz diffusion cell which had a diffusion area of 1.2 cm^2. The receptor compartment had a total volume of 12 ml. A circular transdermal patch was pressed on the membrane with backing layer side facing away from the skin. After securing cell assembly with a clamp the receptor compartment was filled with phosphate buffer saline pH 7.4. The buffer was stirred with an aid of magnetic bead on hot plate magnetic stirrer to maintain sink conditions (Shah *et al*., 2013). 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 (Xi *et al*., 2010). 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 (Prabhakar *et al*., 2012). A blank patch was treated similarly to obtain blank solution for UV analysis.

Data analysis

Physicochemical tests

The tests including weight variation, thickness, folding endurance, flatness, drug content, swelling index, percentage weight increase due to swelling, percentage erosion, moisture content, moisture uptake, WVTR, WVP, in vitro dissolution studies and in vitro skin permeation studies were done in triplicate. SPSS and MS Excel were used to calculate average, standard deviation and standard error for each formulation.

Statistical approach

In vitro skin permeation studies such as percentage drug release, flux, permeability coefficient and enhancement ratio (ER) for Bisoprolol fumarate across rabbit skin were estimated. One way ANOVA by Tukey's multiple comparison tests (at confidence interval of 95%) for these parameters were carried out using MiniTab® 17.1.0. Tukey's post hoc pair wise comparisons were performed to compare which factors led to significant differences (Gupta *et al*., 2011).

Calculations for in vitro *skin permeation studies*

The *in vitro* skin permeation studies were analyzed for cumulative amount of drug permeated, flux and permeability coefficient.

Cumulative amount of drug permeated in $\mu g/cm^2$ was plotted against time. According to Fick's second law of diffusion, drug flux in $\mu g/cm^2$.hr at steady state was calculated by dividing the slope of linear portion of curve by the area of the exposed skin surface *i.e*., 1.2 cm^2. The permeability coefficient in cm/hr was deduced by dividing the flux with initial drug amount (Gannu *et al*., 2007).

Calculation of enhancement ratio

Permeation enhancement ratio which is also known as enhancement factor or enhancement index or enhancement ratio (ER) is determined by (Mutalik *et al*., 2009):

ER = Drug permeability coefficient after enhancer treatment/ Drug permeability coefficient before enhancer treatment

Kinetic models

In vitro skin permeation study was further analyzed by model dependant 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: $M_t / M_\infty = k2 \sqrt{t}$

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 the 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 (Costa and Sousa Lobo, 2001; Siepmann and Peppas, 2001).

RESULTS AND DISCUSSION

Physiochemical tests

The weight variation of patches varied between 1.61±0.04 to 1.62±0.006 g (Table II). The low value of standard deviation (S.D.) ensures that the variability of weight within a patch (n=3) was low (Muzib and Lavanya, 2012). The thickness of formed patches was between 0.42±0.02 to 0.49±0.02 cm (Table II). The result depicts that S.D. value of a patch was low (n=3) thus patches of similar thickness may be achieved with negligible variance (Muzib and Lavanya, 2012).

All the patches had acceptable drug content which 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 0.53 to 0.57 and the percentage weight increase ranged from 53.20% to 57.80% (Table II). The addition of plasticizers increases the flexibility of Eudragit molecules and renders the patch more permeable to water molecule (El-Gendy *et al*., 2008). Moisture content should be between 2% to 10% in the transdermal patches (Arora and Mukherjee, 2002) thus all the formulated patches had acceptable moisture content (Table III). It affects both the mechanical properties and drug release pattern (El-Gendy *et al*., 2008). Hence lower moisture content is required to maintain the stability, reduce brittleness, prevent bulkiness and reduce susceptibility to microbial contamination (Ammar *et al*., 2009). As polysorbate 80 is hydrophilic in nature (Aizawa, 2011) thus an increase in moisture uptake was observed as the concentration of polysorbate 80 increased. For transdermal patches, moisture uptake up to 15% w/w is claimed not to cause

Table II.- Weight variation, thickness, drug content, swelling index and percentage weight increased due to swelling results of matrix type transdermal patch of Bisoprolol fumarate.

Formulation code	Weight ± S.D. (g)	Thickness ± S.D. (cm)	Drug content (%)	Swelling index	Percentage weight increase due to swelling (%)
Control	1.61±0.04	0.47±0.02	99.88±0.01	0.55±0.0002	55.20
F01	1.62±0.04	0.48±0.02	99.85±0.01	0.53±0.0002	53.20
F02	1.62±0.01	0.43±0.01	99.78±0.01	0.54±0.0005	54.50
F03	1.61±0.01	0.43±0.01	98.92±0.02	0.53±0.0005	53.12
F04	1.62±0.01	0.44±0.01	99.42±0.01	0.57±0.0005	57.80
F05	1.62±0.01	0.42±0.02	99.36±0.01	0.54±0.0006	54.50
F06	1.61±0.01	0.44±0.01	99.79±0.01	0.53±0.0003	53.90

Table III.- 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 ($g/m^2.hr$) x 10^{-6}	WVP ($mg.Pa^{-1}.cm^{-1}.hr^{-1}$) x 10^{-7}
Control	2.56±0.02	3.98±0.01	3.42±0.02	2.21±0.01
F01	2.63±0.02	3.90±0.01	3.82±0.02	2.19±0.01
F02	2.23±0.02	4.12±0.02	3.42 ±0.01	2.11±0.01
F03	2.11±0.02	4.75±0.02	3.62±0.01	2.15±0.01
F04	2.15±0.02	4.83±0.02	3.58±0.02	2.21±0.01
F05	2.47±0.02	4.76±0.01	3.42±0.01	2.16±0.01
F06	2.34±0.02	4.81±0.02	3.67±0.01	2.19±0.01

Table IV.- Percentage drug release of formulation after 12 h (n=3).

Formulations	Concentration	Mean of percentage drug release
Control	0	45.74±0.12
F01	10	29.07±0.05
F02	15	49.65±0.12
F03	20	59.44±0.19
F04	25	73.19±0.07
F05	30	90.16±0.04
F06	35	98.29±0.03

any discomfort as it prevent bulkiness of the film (De and Biswas, 2013; Arora and Mukherjee, 2002) therefore the patches complied with the limit (Table III). WVTR (Table III) was used to measure the passage of vapors through a patch, per unit area per unit time, to ensure its integrity during storage. WVP is a phenomenon which determines the onset of drug release and drug release rate during dissolution (Xiangrong *et al*., 2007).

In vitro *skin permeation study without permeation enhancer*

A control patch containing no permeation enhancer was prepared to check the cumulative drug release, flux and estimate ER through the rabbit's abdominal skin. After 12 h only 29.07% (Table IV) of drug had released *i.e.* 2907.0 $\mu g/cm^2$ (Fig. 1) from the initial dose. The patch followed zero order kinetics and a flux of 183.07 $\mu g/cm^2.hr$ was obtained. PEG is a hydrophilic compound which is capable of increasing transdermal drug release (Sonjoy *et al*., 2011). However its use as plasticizer in film formation of Eudragit RS 100 patch was the reason for lower permeation profile (Ammar *et al*., 2009).

In vitro skin permeation study with Polysorbate 80

It was observed from Figures 1 and 2 that as the percentage of polysorbate 80 increased from 10% to 35% the amount of drug released from the matrix patch also increased. The percentage drug permeated through the rabbit's skin was fitted in different kinetic models like zero order, first order, Higuchi model and Korsmeyer-Peppas model. The R^2 values, n and k are given in Table V. These values showed that all formulation followed zero order drug release kinetics. When drug is released from matrix in such a way that rate of release remains constant then release rate kinetics is believed to follow zero order (Garala *et al*., 2009). Mixed polymer system

Table V.- Kinetic models for *in vitro* permeation profile.

	Control	F01	F02	F03	F04	F05	F06
Zero order							
k_1 (% hr^{-1})	219.68	382.59	416.04	499.80	682.66	765.67	876.99
R^2	0.98	0.9918	0.95	0.99	0.97	0.98	0.99
First order							
k_2 (% hr^{-1})	0.18	0.28	0.31	0.18	0.25	0.29	0.2
R^2	0.91	0.80	0.94	0.88	0.78	0.66	0.84
Higuchi							
k_2 ($hr^{-1/2}$)	8.13	13.93	14.59	18.74	25.58	28.44	32.13
R^2	0.90	0.88	0.78	0.93	0.91	0.90	0.89
Peppas							
n	0.96	0.23	0.07	1.75	1.24	0.66	1.37
R^2	0.98	0.98	0.98	0.98	0.97	0.90	0.99

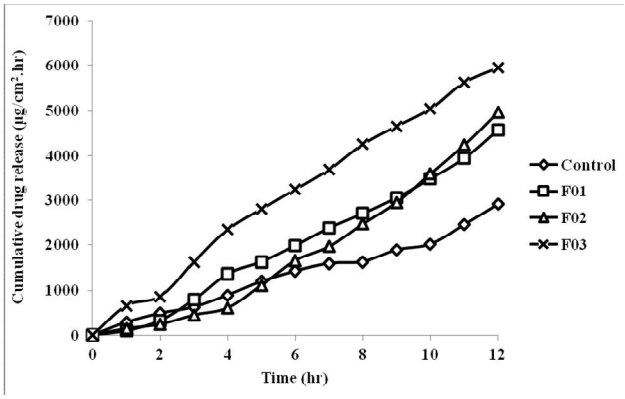

Fig. 1. Cumulative drug release from control patch, F01, F02 and F03.

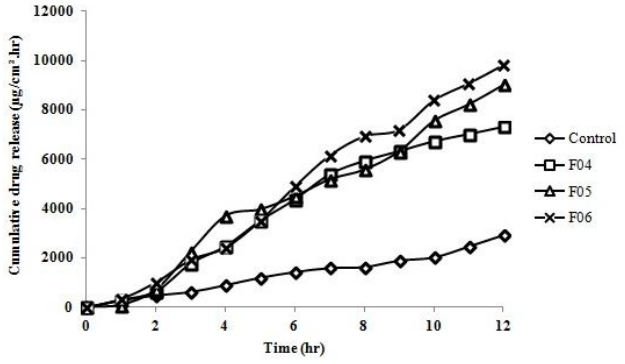

Fig. 2. Cumulative drug release from control patch, F04, F05 and F06.

and alteration of cross linking between polymeric chains together with the modulation of structural arrangement provokes different release and permeation profiles (Ammar *et al*., 2009). The value of n for F01 and F02 illustrated that the drug release mechanism from the matrix patch was Fickian diffusion (n<0.5) where as that of control and F05 signified that the formulation had an anomalous drug release (0.5<n<1.0) *i.e.,* a combination of both diffusion and erosion controlled drug release phenomena. F03, F04 and F06 had n value greater than 1.0 that indicated that drug release was due to erosion and patches followed super case II mechanism (Costa and Sousa-Lobo, 2001). When the concentration of Polysorbate 80 increases it decreases interfacial tension and increases wetting of polymer to a greater extent thus erosion of polymer occur (Sonjoy *et al*., 2011).

Statistical analysis using the one way ANOVA indicated that there was a significant difference between the formulations with P<0.001. Tukey's multiple comparison test suggest that there was a significant difference in steady flux, cumulative permeation rate and ER with P<0.001 at different polysorbate 80 concentrations (Lakshami *et al.*, 2014).

Flux and ER

There was an increase in flux and ER when concentration was increased from 10% to 35% (Table VI). The plot of ER versus concentration is depicted in Figure 3. The figure showed that the greatest enhancement occurred at 35% concentration.

At higher concentration, surfactant tends to form micelles above their CMC (≈0.01% w/w in water for Polysorbate 80). The micelles can also cause interaction with the drug. The micelles and drug entrapped within do not penetrate the skin due to bulkiness thereby it

decreases the thermodynamic activity; but at the same time they may solubilize specific components within the intercellular lipid matrix thus counteracting this effect by increasing the thermodynamic activity. Therefore, the overall effect of polysorbate 80 on the rate of drug permeation will be combination of the influence of these two opposing effects. It is also reported that glycols tend to increase the CMC of non-ionic surfactant up to 10 times where the concentration of glycol is 40% v/v (Nokhodchi *et al*., 2003).

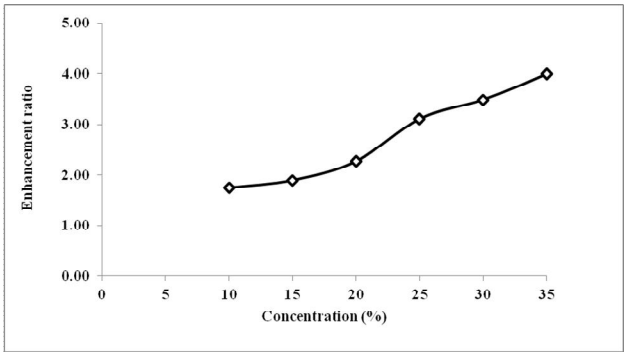

Fig. 3. The effect of surfactant concentration on the ER of Bisoprolol fumarate through rabbit's skin.

Table VI.- Flux, permeability coefficient and enhancement ratio (ER) of bisoprolol fumarate matrix patch containing permeation enhancers.

Formulation code	Flux (µg/cm²hr)	Permeability coefficient (cm/hr)	ER
Control	183.07	0.018	-
F01	318.83	0.032	1.74
F02	346.70	0.035	1.89
F03	416.50	0.042	2.28
F04	568.88	0.057	3.11
F05	638.06	0.064	3.49
F06	730.83	0.073	3.99

CONCLUSIONS

A better sustained drug release effect was obtained from F06 with 98% drug permeation through the rabbit's skin after 12 h. The patch had desirable physicochemical properties and drug loading into the patch was optimal. The formulation followed zero order kinetics with Super case II drug release mechanism. A maximum flux and ER was obtained in F06 as compared to the control patch and other formulation containing polysorbate 80. Thus it can be reasonably concluded that polysorbate 80 at 35% concentration can be used as a potential permeation enhancer to aid in passage of drug through the rabbit skin.

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