Influence of different formulation variables on the performance of transdermal drug delivery system containing tizanidine hydrochloride: *in vitro* and *ex vivo* evaluations

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The present study was aimed at preparation of transdermal patches of tizanidine HCl, evaluation of the effect of polymers on *in vitro* release pattern of the drug, and the effect of permeation enhancers on the penetration of the drug through the rabbit skin. Various proportions of hydrophilic (HPMC) and hydrophobic (Eudragit L-100) polymers were used with PEG 400 as film-forming agent, and Span 20 or DMSO as permeation enhancer. The formulations were assessed for physicochemical characteristics and *in vitro* drug release studies using USP paddle over disc method in phosphate buffered saline (pH 7.4) at 32.0±1°C. On the basis of *in vitro* studies and physicochemical evaluations, S03-A and S04-A were selected at Eudragit : HPMC ratios of 8 : 2 and 7 : 3, respectively, for further *ex vivo* analysis. The effects of different concentrations of Span 20 and DMSO were evaluated on excised rabbit skin using Franz diffusion cell. Cumulative drug permeation, flux, permeability coefficient, target flux, and enhancement ratio were calculated and compared with the control formulations. Kinetic models and Tukey's multiple comparison test were applied to evaluate the drug release patterns. Formulation SB_{03}-PE containing Eudragit L-100:HPMC (7:3) with Span 20 (15% w/w) produced the highest enhancement in drug permeation, and followed zero order kinetic model with super case-II drug release mechanism.

Keywords: *Ex-vivo* permeation. Eudragit L100. Permeation enhances. Transdermal matrix patch. Monolithic system.

INTRODUCTION

Transdermal drug delivery systems (TDDS) are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s) through the skin at controlled rates to the systemic circulation (Shirsand *et al*., 2012). Transdermal drug products are intended to deliver therapeutic quantities of drugs systemically for the treatment or prevention of disorders at locations distant from the site of topical application. They have numerous merits over conventional drug delivery systems, including avoidance of hepatic first pass metabolism, reduction of pain, reduction in fluctuations in plasma drug levels, reduction of dosing frequency, ease of termination of therapy, and sustained release of drugs (Ali *et al*., 2016; Kumar, Philip, 2007). Notwithsatnding these advantages, transdermal passage of molecules is tedious due to the low permeability of the *stratum corneum*, the outermost layer of the skin. Indeed, a passive barrier is set up by different skin layers, a process which controls the permeation of drugs via the skin. *Stratum corneum* has a very high resistance to permeation and is the rate-controlling factor in percutaneous absorption. To overcome this passive barrier system, chemical penetration enhancers (CPEs) are used to lower the impermeability of the skin for a limited time, thereby facilitating the permeation of drugs through the skin (Williams, Barry, 2012; Pathan, Setty, 2009).

Tizanidine hydrochloride (TZD HCl) is a centrally-acting skeletal muscle relaxant. It is an α_2-adrenergic agonist, acting mainly at spinal and supraspinal levels to inhibit excitatory interneurones. It is used for the

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Braz. J. Pharm. Sci. 2018;54(4):e00130

Page 1 / 12

symptomatic relief of spasticity associated with multiple sclerosis or with spinal cord injury, and also in the symptomatic treatment of painful muscle spasm (Kamen, Henney, Runyan, 2008). The oral bioavailability of TZD (Figure 1) is about 34 - 40%, with a half-life of 2 - 2.5 h. It undergoes rapid and extensive first-pass metabolism in the liver (about 95%) which leads to reduction in its bioavailability (Shanker *et al.*, 2009). Studies on the effect of CPEs and sonophoresis on the transdermal flux of TZD HCl buffer solution across mouse skin revealed that most permeation enhancers, as well as low frequency ultrasound, were able to increase the permeation of TZD HCl through the skin (Mutalik *et al.*, 2009). Moreover, Sintov and Botner (2006) evaluated the potential transdermal delivery of diclofenac sodium using microemulsion system both *in vivo* and *in vitro*. It was found that topical administration of diclofenac microemulsion to rats resulted in 8-fold increase in plasma drug levels when compared to commerecially available Voltral gel®. In another study, Gratieri *et al.* (2014) demonstrated the iontophoretic delivery of Ketorolac® (a peripherally-acting muscle relaxant) from hydroxymethyl cellulose gel using porcine and human skin. Their study demonstrated that electrotransport of Ketorolac® was propotional to the current density and drug concentration in the gel. Many attempts have been made to increase the trandermal flux of different drugs using different techniques. Some of these have been mentioned in this section, and there are a number of available studies aimed at increasing the permeation of TZD HCl through the skin. However, there are no extant studies based on the use of transdermal patch formulations of TZD HCl.
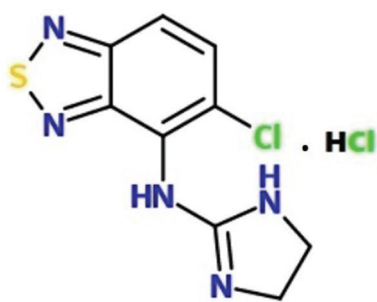

FIGURE 1 - Chemical structure of tizanidine HCl (MW = 290.17 Da, pKa = 7.4, Log p = 2.02, $t_{1/2}$ = 2.25 hour).

The present study was carried out to prepare formulation of monolithic matrix type, sustained-release transdermal patch containing TZD HCl and CPEs. This was with a view to controlling drug release with increased drug permeation through the skin, and to achieve higher flux in order to improve patient compliance with reduced dosing frequency. The patches were evaluated for the principal effects of different concentrations of Eudragit (EL-100) and hydroxypropyl methylcellulose (HPMC) on drug release. The optimum formulations from *in vitro* drug release studies were further evaluated for the binary effect of dimethyl sulfoxide (DMSO) and sorbitan monolaurate (Span 20) as permeation enhancers in the permeation of TZD HCl through rabbit skin.

MATERIAL AND METHODS

Material

Tizanidine hydrochloride (TZD HCl) was generously donated by English Pharma, Lahore Pakistan, while HPMC E-5, EL-100, polyvinyl alcohol (PVA) and PEG-400 were purchased from Merck, Germany. Methanol was obtained from BDH, England. Span 20 and DMSO were procured from Daejung (Korea) and Fisher Scientific (Waltham, USA), respectively. All other chemicals and solvents used were of analytical grade, and were used without any need for further purification.

Methods

Preparation of Matrix type Transdermal Patches of TZD HCl

Transdermal patch of TZD HCl was formulated by solvent-casting technique. Accurately weighed amounts of HPMC and EL-100 were dissolved in 15 mL of methanol and mixed thoroughly for 30 minutes using a hot plate magnetic stirrer at 250 rpm. The drug was dissolved in 5 mL of methanol and added to the polymeric solution with 40% w/w of PEG 400 as plasticizer. After complete mixing, the solution was sonicated for 20 min at 35 ℃ to remove any bubbles. The formulation was then cast onto a petri-dish containing PVA (4% w/v) backing layer. An inverted funnel was placed over the petri-dish to control the rate of evaporation. The solvent was allowed to evaporate completely at room temperature for 48 h, and the separated patch was wrapped in aluminium foil, labeled properly and stored in a desiccator until further use (Table I). For the preparation of patches containing permeation enhancers, the casting solution was prepared by keeping the amount of polymer and drug constant and varying the amount of permeation enhancers. Permeation enhancer was added along with plasticizer in the drug-polymer solution (Table II). A calibration curve was constructed from serial dilution of stock solution. Different dilutions were prepared, filtered and read at 319 nm in a

UV spectrophotometer. A regression line equation with a correlation coefficient (R^2) was then constructed using Graph Pad Prism®:

$$y = mx + b$$

where m (slope) = 0.0367, b (y-intercept) = 0.0288 and R^2 (regression coefficient) = 0.9995.

Physicochemical evaluation of transdermal patches containing TZD HCl

Weight variation

Patches (n = 3) were weighed individually on digital weighing balance and the mean weight and standard deviation were calculated using MS Excel (El-Gendy *et al.*, 2008).

TABLE I - Composition of different transdermal formulations containing TZD HCl without permeation enhancers

Formulation	EL 100 (mg)	HPMC E-5 (mg)	TZD HCl (mg)	PEG 400 (40%w/w)	Methanol (mL)
S01-A	1000	-	4	400	20
S02-A	900	100	4	400	20
S03-A	800	200	4	400	20
S04-A	700	300	4	400	20
S05-A	600	400	4	400	20
S06-A	500	500	4	400	20
S07-A	400	600	4	400	20
S08-A	300	700	4	400	20
S09-A	200	800	4	400	20
S10-A	100	900	4	400	20
S11-A	-	1000	4	400	20

EL100: Eudragit L100; HPMC: Hydroxypropyl methyl cellulose E-5; TZD HCl: Tizanidine hydrochloride; PEG 400: Polyethylene glycol 400

TABLE II - Composition of different transdermal formulations of TZD HCl containing permeation enhancers

Formulation	EL 100: HPMC	TZD HCl (mg)	Methanol (mL)	Penetration enhancer
SA$_{01}$-B (Control)	8:2	2	20	-
SB$_{01}$-B (Control)	7:3	2	20	-
SA$_{01}$-PE	8:2	2	20	Span 20 (5%)
SA$_{02}$-PE	8:2	2	20	Span 20 (10%)
SA$_{03}$-PE	8:2	2	20	Span 20 (15%)
SA$_{04}$-PE	8:2	2	20	DMSO (5%)
SA$_{05}$-PE	8:2	2	20	DMSO (10%)
SA$_{06}$-PE	8:2	2	20	DMSO (15%)
SB$_{01}$-PE	7:3	2	20	Span 20 (5%)
SB$_{02}$-PE	7:3	2	20	Span 20 (10%)
SB$_{03}$-PE	7:3	2	20	Span 20 (15%)
SB$_{04}$-PE	7:3	2	20	DMSO (5%)
SB$_{05}$-PE	7:3	2	20	DMSO (10%)
SB$_{06}$-PE	7:3	2	20	DMSO (15%)

*PEG 400 (40% w/w) as plasticizer. Span 20: Sorbitan monolaurate; DMSO: Dimethylsulfoxide; PE: Permeation enhancer

Braz. J. Pharm. Sci. 2018;54(4):e00130

Page 3 / 12

Thickness

Thickness of the transdermal patch (n = 3) was estimated with a vernier caliper. The average thickness and standard deviation were calculated using MS Excel (El-Gendy *et al*., 2008).

Swelling index, percentage weight increase and erosion studies

Films of 1x1 cm were cut from the patch (n=3), fixed on pre-weighed cover slips and weighed on digital weighing balance. They were then placed in appropriately labeled petri-dishes and completely immersed in distilled water. At specified time intervals, the cover slips were taken out, blotted to remove excess of liquid and immediately weighed. If the 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 formula (Pichayakorn *et al*., 2012):

Swelling Index = (W$_2$ – W$_1$)/ (W$_1$)
Weight increase due to swelling (%) = (W$_2$- W$_1$)/ (W$_1$) x 100

where *W$_1$* is initial weight of the film before swelling/erosion, and *W$_2$* is weight of the film after time *t*.

The percentage erosion was estimated after 60 min of study and calculated using the formula (Pichayakorn *et al*., 2012):

Weight decrease due to erosion (%) = (W$_1$ – W$_2$)/ (W$_1$) x 100

Content uniformity test

A film of 2 x 2 cm of each formulation was cut from a patch (in triplicate), and completely dissolved in 100 mL of PBS (pH 7.4) on a hot plate magnetic stirrer preset at 32 °C. A sample of 3 mL was taken through syringe filters, diluted with an equal volume of fresh PBS and the absorbanace was read spectrophotometrically at 319 nm. The amount of drug was estimated by extrapolation from the calibration curve.

Percentage moisture content

Triplicate samples of 1 x 1 cm film were weighed individually and stored in a desiccator containing silica beads at 25 °C. The films were weighed for five days until constant weights resulted. The percentage moisture content was calculated as outlined previously (Janardhanan, Ramachandra, Rajappan, 2007):

Moistue content (%) = (W$_1$ – W$_2$)/ (W$_1$) x 100

Percentage moisture uptake

Triplicate samples of 1 x 1 cm film were weighed individually and stored in a desiccator containing 200 mL of saturated solution of potassium chloride (KCl) at 25 °C for 84% RH. The films were weighed for five days till constant weight was achieved. The percentage moisture uptake was calculated viz:

Moistue uptake (%) = (W$_2$ – W$_1$)/ (W$_2$) x 100

(Limpongsa, Umprayn, 2008).

Water vapor transmission rate (WVTR)

A 1x1 cm film was cut in triplicate and placed separately in 5-mL vials containing 1 g of calcium chloride (CaCl$_2$). The vials were weighed individually and kept in a desiccator at 25 °C containing 200 mL saturated solution of KCl for 84% RH. The vials were weighed for five days until constant weights were obtained. Then, WVTR was calculated using the formula of Jaydatt, Sreenivas (2013):

WVTR = (W$_2$ – W$_1$)/ (Time x Area) x 100

Water vapor permeability (WVP)

Triplicate samples of 1 x 1 cm of known thickness and weight were individually fixed in 5-mL vials containing silica beads as desiccant. The vials were weighed individually and kept in an incubator containing saturated solution of KCl at 30 °C. Then, they were were weighed for 24 h, and the WVP was calculated as indicated by Xiangrong *et al*. (2007):

P = (Q x d)/ [A x R x S (R$_1$–R$_2$)]

where *P* is permeability, *Q* is amount of water vapor absorbed (in mg) at time *t* (h), *d* is film thickness (cm), *A* is area (cm^2), *S* is saturated water vapor pressure at the test temperature (Pa), *R$_1$* is relative humidity (RH) in the chamber (84%), and *R$_2$* is relative humidity (RH) inside the vial (0%)

Page 4 / 12

Braz. J. Pharm. Sci. 2018;54(4):e00130

In vitro drug release studies of matrix transdermal patches of TZD HCl

In vitro drug release studies (n = 3) were done in USP apparatus V, paddle over disc, in 500 mL of PBS (pH 7.4) at 32.0 ± 1 °C at 50 rpm. The sample (3 mL) was taken at suitable time intervals over a period of 12 h, filtered, diluted appropriately and read spectrophotometrically at 319 nm (Prabhakar *et al*., 2012). The percentage drug release was estimated with reference to the calibration curve.

Ex vivo skin permeation studies

Preparation of rabbit skin

Approval for the *ex vivo* permeation studies on rabbit skin was obtained from Animal Ethics Committee (Ref. No. IAEC-2015-06A), Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan. The rabbit was sacrificed by cervical dislocation and a hairless abdominal region was obtained. The epidermis was prepared by soaking the skin in water at 60 °C for 45 sec. The sub-dermal tissues were removed with forceps and the inner dermis side was wiped with a cotton swab dipped in isopropyl alcohol to remove adhering fats from its surface (Xi *et al*., 2010). The skin then 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 attain room temperature for at least 10 h, and equilibrated for 1 h in PBS (pH 7.4) (Prabu *et al*., 2012).

Ex vivo permeation studies using Franz diffusion cell

The *ex vivo* permeation study was conducted in a Franz diffusion cell with a diffusion area of 1.2 cm^2 using a receptor compartment volume of 12 mL. The dermal side of the skin was placed facing the receptor compartment. A circular transdermal patch (n = 3) was pressed on the skin, with backing layer side facing away from the *stratum corneum*. After securing the cell assembly with a clamp, the receptor compartment was filled with PBS (pH 7.4) maintaining sink conditions. The system was connected to a thermostatically controlled water bath to maintain the temperature at 32 ± 2 °C by circulating the water through a jacket surrounding the cell body (Xi *et al*., 2010). After every hour, a sample of 0.5 mL was withdrawn from the receptor compartment and replaced with an equal volume of fresh PBS. The sample was diluted with appropriate volume of fresh buffer and the absorbance was read spectrophotometrically. The

percentage drug release was estimated with reference to the calibration curve.

DATA ANALYSIS

Kinetic modeling of drug release from TDZ HCl transdermal patches

After *in vitro* drug release and *ex vivo* permeation of TZD HCl, mathematical models were used to determine the kinetics of drug release from the transdermal patches. The various kinetic models applied to the data were zero-order, first order, and Higuchi and Korsmeyer-Peppas model. These models are described below (Siepmann, Peppas, 2012):

Zero-order model

To study the zero-order release kinetics, data obtained from *in vitro* and *ex vivo* drug release studies were plotted as cumulative amount of drug release against time. The release of the drug from the dosage form after the dissolution can be expressed as:

$$Q_o - Q_t = K_o t$$

where Q_t is the amount of the drug dissolved in time *t*, Q_o is the initial amount of drug in the solution, and K_o is zero order release constant expressed as concentration/time

First order model

To study the first order release kinetics, data obtained from the *in vitro* drug release and Franz cell diffusion studies were plotted as log cumulative percentage of drug remaining against time. The release of the drug which follows first order release kinetics can be expressed as:

$$log\ C = log\ C_o - kt/\ 2.303,$$

where *k* is the 1st order rate constant expressed in time^{-1}., C_o is initial concentration of the drug, and *t* is time.

Higuchi model

To study the Higuchi model, data obtained from *in vitro* drug release study and *ex vivo* permeation were plotted as cumulative percentage drug release against the square root of time. A simplified Higuchi model can be described as:

$$Q = K_{Hx} \, t^{1/2}$$

where K_H is Higuchi dissolution constant, and Q refers to amount of drug release in time t.

Korsmeyer Peppas model

Data obtained from *in vitro* drug release study and skin permeation studies were plotted as log cumulative percentage drug release against log time. This can be represented vis:

$$M_t / M_\infty = Kt^n$$

where M_t / M_∞ refers to the fraction of drug released at time t, M_t is total amount released at time t, M_∞ is total amount of drug present in the patches, T is release time in hours, K is kinetic constant, and n is the release exponent indicative of the operating release mechanism.

Analysis of data obtained from *ex vivo* permeation studies

The *ex vivo* skin permeation studies were fitted into kinetic models as stated above. Data was also analyzed for cumulative amount of drug permeated ($\mu g/cm^2$), target flux ($\mu g/cm^2 h$) for TZD HCl, drug flux ($\mu g/cm^2 h$) and permeability coefficient (cm/h).

The cumulative amount of drug permeated ($\mu g/cm^2$) through the skin was plotted against time, and drug flux ($\mu g/cm^2 h$) at steady state was calculated by dividing the slope of the linear portion of the curve with the area of exposed skin surface. The permeability coefficient (cm/h) was derived by dividing the flux obtained from initial drug load. The enhancement ratio was estimated by dividing the flux of patch containing permeation enhancer to the flux of control patch (Shabbir *et al*., 2016).

STATISTICAL ANALYSES

One-way ANOVA by Tukey's multiple comparison tests (at 95% confidence interval) for drug permeated (%) and flux ($\mu g/cm^2 h$) was estimated by Minitab® 17.1.0. Tukey's post hoc pairwise comparisons were performed to compare factors which led to significant differences (Shabbir *et al*., 2016).

RESULTS AND DISCUSSION

Physicochemical Evaluations

Weight variation
The weight of the formulated patches varied from 1.6031 ± 0.0311 to 1.6909 ± 0.0153 g (Table III). The low values of the SD indicate that the weight variability within a formulation was minimal and was reproducible. A relatively higher weight is attributed to the presence of the backing layer (Shabbir *et al*., 2017).

Thickness
The thickness of the patches ranged from 0.62 ± 0.02 to 0.75 ± 0.02 cm (Table III), and the low SDs signify that the process employed was capable of reproducing films with minimal variability (Shabbir *et al*., 2017; Ali *et al*., 2016).

TABLE III - Results for weight variation, thickness, swelling index, percentage weight increase due to swelling and percentage erosion of transdermal patches of TZD HCl

Formulations	Weight ± S.D. (mg)	Thickness ± S.D. (cm)	Swelling index	Percentage weight increase	Percentage Erosion
S01-A	1.63 ± 0.068	0.75 ± 0.02	0.701	70.69	2.25
S02-A	1.63 ± 0.014	0.74 ± 0.01	0.83	82.99	4.12
S03-A	1.68 ± 0.003	0.71 ± 0.02	0.98	97.78	4.92
S04-A	1.64 ± 0.020	0.71 ± 0.01	1.29	129.08	6.53
S05-A	1.62 ± 0.048	0.64 ± 0.01	1.39	138.61	4.36
S06-A	1.68 ± 0.054	0.73 ± 0.02	1.47	147.19	4.23
S07-A	1.69 ± 0.015	0.64 ± 0.02	1.49	148.94	7.73
S08-A	1.67 ± 0.001	0.72 ± 0.02	1.69	169.42	6.03
S09-A	1.63 ± 0.009	0.62 ± 0.03	2.26	225.74	6.48
S10-A	1.68 ± 0.067	0.67 ± 0.02	Disintegrated after 10 min		
S11-A	1.60 ± 0.031	0.62 ± 0.02	Disintegrated after 10 min		

Page 6 / 12

Braz. J. Pharm. Sci. 2018;54(4):e00130

Swelling index and percentage weight change due to swelling

As shown in Table III, the swelling index and percentage weight increase were minimum in S01-A (0.71 and 70.69%, respectively) and maximum in S09-A (2.26 and 225.74% respectively). The films S10-A and S11-A disintegrated after 10 minutes since they had maximum amount of hydrophilic polymer (HPMC). The swelling index depicts the hydration of film when it is immersed in water. Increased hydration of polymer in a film leads to the formation of empty spaces which may affect sustained release profile of matrix patch. In addition, the patch becomes less resistant to mechanical stress (Perioli *et al.*, 2009).

The percentage erosion of the formulated patches was calculated to estimate the weight loss or disintegration after they had been immersed in water for 60 minutes. As the amount of EL-100 was increased, the rate of disintegration or erosion decreased because of the hydrophobic nature of polymer. Erosion was least in S01-A (2.25%) (Table III).

Drug content and content uniformity

The percentage drug content is shown in Table IV. The low value of SD is an indication of uniformity of drug content in different parts of the film. This ensures that the rheological properties of the casting solution are suitable for homogeneous distribution of drug in the film. For sustained release formulations, the drug should be distributed throughout the patch homogeneously and uniformly (Ali *et al.*, 2016).

Percentage moisture content

The percentage moisture content varied from 2.88 to 9.87% (Table IV). Formulation S01-A showed the lowest moisture content, whereas S11-A showed the highest content of moisture. It was observed that as the concentration of HPMC increased, the amount of moisture content also increased. This is due to the fact that HPMC is hydrophilic in nature, and so absorbs moisture from the atmosphere (Ammar *et al.*, 2009). The acceptable amount of moisture in a patch is higher than 2% but less than 10%. This prevents the patch from becoming brittle and bulky, and reduces its susceptibility to microbial contamination (Ali *et al.*, 2016).

Percentage moisture uptake

Formulation S01-A showed the least moisture uptake, while S11-A had maximum moisture uptake capacity (Table IV). As the concentration of HPMC increased the moisture uptake capacity of the film also increased due to the hydrophilic nature of HPMC. On the other hand, EL-100 is hydrophobic but still possesses some hydrophilicity due to the presence of quaternary ammonium groups in its structure. Thus, formulations containing higher concentration of EL-100 are able to absorb moisture from the atmosphere at higher humidity. The presence of PEG 400, a hydrophilic substance, further increases the moisture uptake capacity of the films (Eva, Milan, 2012). It makes the network of polymers less dense because of increase in the mobility of polymeric chains

TABLE IV - Results for moisture content, moisture uptake, water vapor transmission rate (WVTR) and water vapor permeability (WVP) of transdermal patches of TZD HCl

Formulation	Moisture content (%)	Moisture uptake (%)	WVTR (g/m².h)	WVP (mg.Pa^{-1}.cm^{-1}.h^{-1})	Drug content (%)
S01-A	2.88	4.49	4.34×10^{-6}	8.89×10^{-7}	100.12 ± 0.01
S02-A	2.89	5.12	4.35×10^{-6}	9.15×10^{-7}	99.22 ± 0.05
S03-A	2.93	5.50	4.41×10^{-6}	9.47×10^{-7}	100.06 ± 0.02
S04-A	4.89	8.67	4.35×10^{-6}	9.02×10^{-7}	99.08 ± 0.02
S05-A	5.85	9.06	4.54×10^{-6}	9.21×10^{-7}	97.30 ± 0.02
S06-A	5.53	9.54	4.44×10^{-6}	9.70×10^{-7}	98.20 ± 0.08
S07-A	5.84	9.22	4.44×10^{-6}	9.84×10^{-7}	97.90 ± 0.05
S08-A	5.73	11.03	4.38×10^{-6}	9.91×10^{-7}	99.78 ± 0.04
S09-A	5.81	11.99	4.50×10^{-6}	9.61×10^{-7}	100.28 ± 0.10
S10-A	7.61	11.72	4.43×10^{-6}	9.73×10^{-7}	97.89 ± 0.02
S11-A	9.87	16.91	4.63×10^{-6}	9.92×10^{-7}	99.04 ± 0.10

WVTR: Water vapor transmission rate; WVP: Water vapor permeability

Braz. J. Pharm. Sci. 2018;54(4):e00130

Page 7 / 12

which increases the free volume between the matrix systems. This pore formation increases the absorption of water molecules into the film, resulting in increased porosity (El-Gendy *et al.*, 2008). Moisture uptake studies are used to estimate the maximum amount of moisture a patch can hold when it is exposed to high humid conditions. For transdermal patches, moisture uptake up to 15% w/w is thought to prevent film bulkiness, thus avoiding discomfort (Ubaidulla *et al.*, 2007).

Water vapor transmission rate (WVTR)

Maximum WVTR was seen in S11-A (4.63×10^{-6} g/m^2. h), while WVTR was least in S01-A (4.34×10^{-6} g/m^2.h, Table IV). This parameter (WVTR) serves to estimate the passage of vapors through a patch per unit area per unit time, so to ensure its integrity during storage (Xiangrong *et al.*, 2007).

Water vapor permeability (WVP)

The lowest WVP was observed in S01-A (8.89×10^{-7} mg.Pa^{-1}.cm^{-1}.h^{-1}), while the highest value was seen in S11-A (9.92×10^{-7} mg.Pa^{-1}.cm^{-1}.h^{-1}, Table IV). The high permeability of water through S11-A is due to the high level of HPMC in the formulation. The WVP is a parameter that determines the onset of drug release and drug release rate during dissolution (Xiangrong *et al.*, 2007).

In vitro drug release studies

In vitro drug release from formulations containing single polymer

The percentage drug release was estimated with reference to the calibration curve ($R^2 = 0.9995$). Drug release from formulations containing EL-100 (S01-A) and HPMC (S11-A) in maximum ratio is shown in Figure 2. It is evident from the *in vitro* release studies that 94.4% drug was released in 12 h from S01-A, whereas 99.0% of drug was released from SII-A within the third hour of dissolution. The relative hydrophobic nature of EL-100 decreased its affinity for water, resulting in decreased thermodynamic activity of the drug in film, and decreased drug release. Due to its higher hydrophilic nature, HPMC absorbs water. Dissolution of an aqueous soluble fraction of polymer matrix leads to the swelling of the polymer, resulting in a faster release of drug from the pores of film because of adequate porosity and diffusivity. The formation of such pores decreases the mean diffusion path length of the drug molecules into the dissolution medium (Ansari, Singhai, Saraogi, 2011).
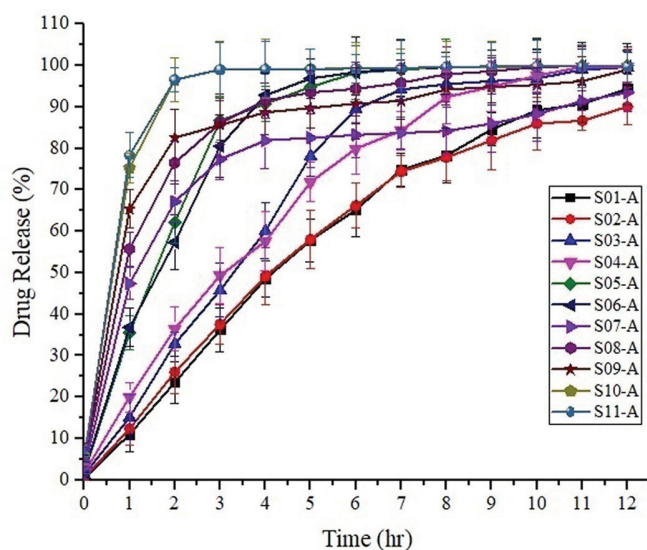

FIGURE 2 - *In vitro* drug release (%) from TZD HCl transdermal patch containing EL100 and HPMC at different ratios. S01-A and S11-A contained EL100: HPMC at a ratio of 10:0 and 0:10 respectively. The results are expressed as mean ± S.D, n=3.

It appears that EL-100 is a better release retardant at higher concentrations than HPMC. The addition of hydrophilic component to a patch enhances its release rate because HPMC is more permeable to water. It has been shown that release rate and permeation rate can be controlled for a long period of time by using a combination of lipophilic polymer and hydrophilic polymer (Ansari, Singhai, Saraogi, 2011). The *in vitro* drug release profile of S01-A ($R^2 = 0.9728$) best fitted in Higuchi model. The drug release mechanism was found to be anomalous (non-Fickian diffusion) i.e. combination of diffusion and erosion.

Formulations containing combination of HPMC and EL 100 in different ratios

The *in vitro* drug release profile of formulations S02-A to S10-A is depicted in Figure 2. Initially, there was a rapid release of drug from the transdermal patches containing higher proportion of HPMC. This rapid drug release (burst effect) is attributed to the hydrophilic nature of HPMC (Arora, Mukherjee, 2002). The addition of hydrophilic components to a formulation tends to enhance its release-rate constants, resulting in the leaching of hydrophilic polymeric matrix. This leads to the formation of pores in the matrix system (Darwhekar, Jain, Patidar, 2011). Burst effect was lowest in formulation S04-A, relative to the other formulations because of the higher concentration of EL-100 and lower HPMC concentration. No burst effect was evident in S02-A and S03-A because of the presence of high proportions of hydrophobic polymer. By decreasing the hydrophilic polymer, and increasing the hydrophobic portion of the

polymer matrix, the rapid drug release effect was gradually reduced, and sustained effect gradually increased. A 3-D surface plot (Figure 3) of formulations at t_{10} showed that as the concentration of HPMC increased, drug release from the matrix patch also increased, whereas a decrease in release rate was observed with an increase in EL-100 concentration (Kusum *et al.*, 2003). The values obtained from formulations S02-A, S03-A, S04-A, S05-A, S06-A and S08-A (with correlation constants of R^2 of 0.9694, 0.9662, 0.9831, 0.9896, 0.9748 and 0.9065, respectively) best fitted the Higuchi model. This signifies that the main drug release mechanism was diffusion, because proportionality between cumulative percentage drug release and square root of time is usually regarded as an indicator of diffusion-controlled drug release (Siepmann, Peppas, 2012). All other formulations did not fit into any kinetic model because their R^2 values were too small. The values 'n' of Korsmeyer-Peppas equation depicted that drug release mechanism from transdermal patches S01-A to S04-A (with 'n' values of 0.78, 0.85, 0.78 and 0.64 respectively) occurred via anomalous diffusion because the value of 'n' was greater than 0.5 but less than 1. Formulations S05-A to S11-A (with 'n' values 0.44, 0.39, 0.14, 0.28, 0.21, 0.10 and 0.07, respectively) showed Fickian diffusion drug release mechanism (Siepmann, Peppas, 2012). *Ex vivo* permeation studies were performed for the optimized formulations S03-A and S04-A which were selected according to the *in vitro* drug release profile data and physicochemical evaluation.
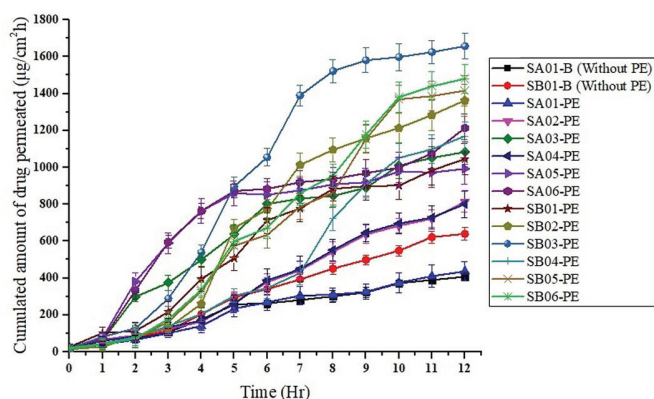

FIGURE 3 - Ex *vivo* cumulative drug permeated (µg/cm² h) from TZD HCl patches containing permeation enhancers. SA01-B and SB01-B represents the control patches (without permeation enhancers). The results are expressed as mean ± SD, n=3.

Ex vivo permeation studies

Ex vivo permeation of transdermal patches without penetration enhancer (control patches)

The cumulative amount of drug permeated from SA$_{01}$-B and SB$_{01}$-B is given in Figure 4. Although patch SA$_{01}$-B (R^2 = 0.9772) followed Higuchi model, only 20.3% of the drug permeated from the matrix system to the skin after 12 h. When compared to SA$_{01}$-B, permeation increased by a factor of 1.58 in SB$_{01}$-B. Patch SB$_{01}$-B with R^2 value of 0.9897 followed zero order kinetic model. According to Korsmeyer-Peppas equation, the patch showed anomalous drug release and permeation, favoring both swelling and diffusion (Costa, Sousa, 2001). Flux rate also increased from 23.20 µg/cm²h in SA$_{01}$-B, to 39.40 µg/cm²h in SB$_{01}$-B (Table V). The increase in permeation rate was due to the increase in concentration of hydrophilic polymer HPMC. Although there was an increase in cumulative drug permeation, the two patches failed to achieve the desired target flux over a period of 12 hours. Therefore, a penetration enhancer was required to improve the drug flux.
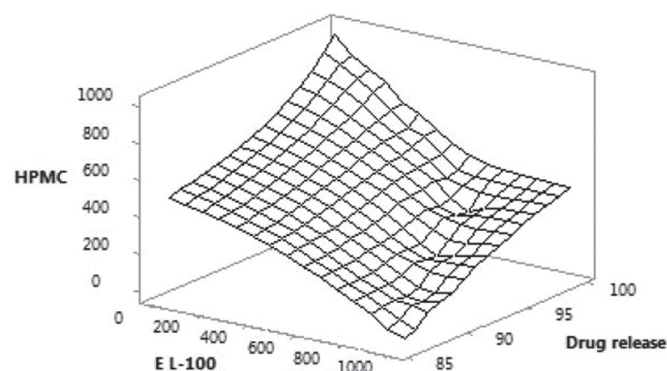

FIGURE 4 - 3-D plot of *in vitro* drug release study of formulations at t_{10}.

As the concentration of Span 20 increased from 5 to 15% w/w in a formulation containing 8 : 2 (EL 100 : HPMC) drug polymer matrix, the cumulative amount of drug permeated also increased (Figure 4). Patches SA$_{01}$-PE and SA$_{03}$-PE with R^2 values of 0.9749 and 0.9833 respectively, followed Higuchi model which signified a diffusion-controlled drug release and permeation. On the other hand, SA$_{02}$-PE (R^2 = 0.9705) fitted better in zero order kinetics. This permeation profile indicated that the drug-polymer matrix ensured constant concentration and was independent of the initial concentration. According to the 'n' value of Korsmeyer-Peppas equation, SA$_{01}$-PE, SA$_{02}$-PE and SA$_{03}$-PE (n = 1.03, 1.12, and 1.03, respectively) had super case II transport mechanism in which polymer relaxation on imbibition of water is the rate controlling step (Siepmann, Peppas, 2012). Increases in flux were also observed with increases in permeation enhancer concentration (Table V).

Braz. J. Pharm. Sci. 2018;54(4):e00130

Page 9 / 12

TABLE V - Slope, flux and permeability coefficient of formulations containing permeation enhancer

Formulation	Slope	Flux (μg/cm^2.h)	Permeability coefficient (cm/h)
SA$_{01}$-B	27.84	23.20	0.0136
SB$_{01}$-B	47.28	39.40	0.0232
SA$_{01}$-PE	30.82	25.69	0.0151
SA$_{02}$-PE	61.46	51.22	0.0301
SA$_{03}$-PE	73.06	60.89	0.0354
SA$_{04}$-PE	61.81	51.50	0.0303
SA$_{05}$-PE	65.47	54.56	0.0327
SA$_{06}$-PE	68.61	57.18	0.0336
SB$_{01}$-PE	95.52	79.60	0.0468
SB$_{02}$-PE	118.29	98.57	0.0580
SB$_{03}$-PE	122.52	102.10	0.0606
SB$_{04}$-PE	77.92	64.93	0.0382
SB$_{05}$-PE	113.88	94.90	0.0558
SB$_{06}$-PE	138.15	115.13	0.0677

The cumulative amount of drug permeated from formulations containing EL 100 and HPMC at a ratio of 7:3, with Span 20 is shown in Figure 4. It is clear that as the concentration of Span 20 increased from 5 to 15% (w/w), the percentage permeation of drug also increased from 363.44 to 902.94 μg/cm^2. When fitted in various models SB$_{01}$-PE, SB$_{02}$-PE and SB$_{03}$-PE (R^2 = 0.9905, 0.9794, and 0.9863, respectively) followed zero order kinetics. The *'n'* value of Korsmeyer-Peppas equation was greater than 1, indicating super case II transport drug release and permeation mechanism, possibly due to swelling and chain disentanglement of hydrophilic polymer. The flux of patches containing 15% (w/w) Span 20 was greater than formulations containing 5 and 10% (w/w) Span 20 (Table V).

The cumulative amount of drug permeated from the matrix patch increased with increase in the concentration of HPMC and Span 20. The hydrophilic property of HPMC contributed to increases in the penetration of solvent molecules into the polymeric matrix. This disturbed the compactness of the system, leading to the formation of pores and faster release and permeation of drug (Patel *et al*., 2009). The presence of PEG 400 further increased the permeation rate and made the network less dense due to increased mobility of the polymeric chain which caused the polymer to relax. Span 20 is a non-ionic surfactant that is considered to be the safest enhancer in relation to anionic, cationic and zwitterionic surfactants (Williams, Barry, 2012). It acts as a penetration enhancer by increasing the fluidity of the lipid components of *stratum corneum* through solubilization. It also interacts and binds with keratin filaments which cause disruption of corneocytes. Statistical analysis using one-way ANOVA and Tukey's multiple comparison tests revealed a significant difference in drug permeation rate at t$_{10}$ and flux ($p < 0.001$) at different Span 20 concentrations (Shabbir *et al*., 2016).

An increase in cumulative drug penetration was seen as DMSO concentration was increased in the 8 : 2 (EL 100 : HPMC) polymer matrix system, with drug permeation values of 667.52, 826.92, and 1010.84 μg/cm^2 at 5, 10 and 15% (w/w) DMSO, respectively (Figure 4). Formulation SA$_{04}$-PE (R^2=0.9876) followed zero order kinetics whereas SA$_{05}$-PE and SA$_{06}$-PE (R^2 = 0.9839 and 0.9119, respectively) appeared to follow Higuchi model. The values of *'n'* for SA$_{04}$-PE and SA$_{05}$-PE in the Korsmeyer-Peppas equation were 1.15 and 1.07, respectively, which implies super case II transport mechanism of drug permeation and erosion. On the other hand, SA$_{06}$-PE (*n* = 0.97) had an anomalous transport mechanism favoring both diffusion and swelling (Siepmann, Peppas, 2012). An increase in flux was obtained by increasing DMSO concentration as shown in Table V.

The cumulative drug permeation for 7 : 3 (EL 100 : HPMC) polymeric system containing DMSO as permeation enhancer is shown in Figure 4. Drug permeation increased with increase in DMSO concentration, and the patches followed Higuchi model. The *'n'* value of Korsmeyer-Peppas equation indicated that SB$_{04}$-PE, SB$_{05}$-PE and SB$_{06}$-PE had super case II transport mechanism with values greater than 1. The flux increased as the amount of DMSO concentration increased (Ali *et al*., 2016).

An increase in cumulative drug permeation was observed when the concentrations of HPMC and DMSO were increased. DMSO has been shown to change the intercellular keratin confirmation from α helical structure to β sheets, thereby denaturing proteins. It also interacts with the head group of some bilayer lipids to distort the packing geometry within the intercellular domain of human *stratum corneum* (Williams, Barry, 2012). Statistical results using the one-way ANOVA and Tukey multiple comparison test showed a significant difference in drug permeation rate at t$_{10}$ and flux at different DMSO concentrations ($p < 0.001$, Shabbir *et al*., 2016).

CONCLUSION

Based on the results of the present study, it can be reasonably concluded that 7 : 3 ratio of EL-100 and

Page 10 / 12

Braz. J. Pharm. Sci. 2018;54(4):e00130

HPMC, with 15% Span 20, can be successfully prepared for the sustained release of TZD HCl. The drug release increases commensurately with increases in HPMC concentration, while EL-100 acts a retardant at high polymer concentration. The transdermal flux increases at higher Span 20 and DMSO concentrations. The highest permeation enhancement can be obtained with formulations containing 15% (w/w) Span 20. Maximum cumulative drug release with higher flux through skin can be achieved by SB_{03}-PE at 95% CI. The formulation followed zero order kinetic model with Super case II transport mechanism of drug permeation.

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Braz. J. Pharm. Sci. 2018;54(4):e00130

Page 11 / 12

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