

EFFECT OF PENETRATION ENHANCERS ON SKIN PERMEATION OF TRAZODONE HYDROCHLORIDE FROM MATRIX TYPE TRANSDERMAL FORMULATION THROUGH MOUSE AND HUMAN CADAVER EPIDERMIS

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Abstract: A transdermal dosage form of trazodone hydrochloride (TZN) may be useful in the treatment of moderate to severe depression in schizophrenic patients by providing prolonged duration of action. It will also improve patient compliance and bioavailability. Controlled input of TZN would attenuate fluctuating plasma level of TZN resulting from oral therapy. The aim of the current investigation was to evaluate its flux and the effects of various penetration enhancers, viz., isopropyl myristate (IPM), isopropyl palmitate (IPP), butanol and octanol on transdermal permeation from matrix-based formulations through the skin. The enhancing effect on the permeation of TZN was determined using the mouse and human cadaver epidermis. *In vitro* permeation data were collected at 37°C using Keshary-Chien diffusion cells. The skin permeation was then evaluated by measuring the steady state permeation flux of TZN, enhancement ratio and the diffusion parameter. The highest enhancing effect was obtained with IPM followed by butanol, octanol and IPP. In general, higher fluxes were observed through mouse epidermis as compared with the human cadaver epidermis. The skin retention of TZN for both the species in the presence of different enhancers was nearly 3 times higher than for the control formulation. Based on the observed results, a transdermal patch of about 70 cm² consisting of 10 % IPM should be able to attain and maintain therapeutic plasma concentration of TZN at 0.75 mg/mL over a period of 24 h.

Keywords: trazodone hydrochloride, matrix-based transdermal formulations, mouse epidermis, human cadaver epidermis, penetration enhancers, isopropyl myristate

Trazodone hydrochloride (TZN), a serotonin uptake inhibitor, is used in the treatment of moderate to severe depression in schizophrenic patients. The undesirable side effects of TZN administered orally can be offset by using the transdermal route, which attenuates the fluctuating TZN levels (52 – 81%) resulting from oral therapy. The plasma half-life of TZN is about 6 hours, which requires frequent dosing necessary to maintain the therapeutic blood level (0.75 mg/mL) for a long-term treatment (1-3). For the treatment of depression, the dose of TZN for adults ranges from 150 to 600 mg administered as one to four tablets a day. The dose and frequency may cause enhanced drug related side effects and may pose compliance problems. Therefore, TZN is an ideal drug candidate for transdermal drug delivery.

It has been proposed (4) that the major permeability barrier in stratum corneum results from an ordered intracellular lipid matrix and has a low water content. It has been reported that among the proteins in the stratum corneum, approximately 90% of the protein is water insoluble (5). The water insoluble protein in the stratum corneum also plays a role in the

barrier function of the skin. There have been many attempts to increase the percutaneous absorption of drugs. It has been reported that penetration enhancers may partition into, and interact with skin constituents to induce a temporary and reversible increase in skin permeability (6). Thus, the use of penetration enhancers could possibly increase the percutaneous penetration of TZN so that a therapeutically effective dose can be delivered from a patch of reasonable size. The objective of the present study was to investigate the effect of various enhancers belonging to aliphatic acid esters [isopropyl myristate (IPM) and isopropyl palmitate (IPP)] and alkanols (butanol and octanol) in the matrix-based transdermal formulation on the *in vitro* transport of TZN across mouse and human cadaver epidermis. The skin retention of TZN after permeation study was also measured.

EXPERIMENTAL

Materials

Trazodone hydrochloride (ICN Pharmaceuticals, Inc., Germany), Eudragit RL100 and RS 100

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(gift samples from Rohm Pharma, Darmstadt, Germany), isopropyl myristate (Loba Chemie, India), isopropyl palmitate (Biolab, India), butanol (Ranbaxy Laboratory, New Delhi), octanol (E. Merck, India), isopropanol (Ranbaxy Laboratory, New Delhi), triethylcitrate (E. Merck, Germany) were used.

METHODS

Determination of target flux

The flux was determined by the following equation (19) at steady state: $I.R. = C_{SS} \times Cl$, where I.R. is the input rate into the body through the skin, C_{SS} is the steady state concentration (0.75 mg/mL) (1,3) and Cl is the clearance of the drug from the body (2.1 mL/min/kg or 147 mL/min for a normal healthy person of 70 kg) (2).

Fabrication of transdermal devices

At first, the backing membrane was prepared using Eudragit NE 30D. The method of backing membrane preparation was based on that developed by Panigrahi and Ghosal (8). The films were prepared using Eudragit RL 100 and RS 100 polymers. The polymeric solution (10% w/v) was prepared by dissolving Eudragit RL 100 and RS 100 at 1 : 3 ratio, along with 10% w/w of TZN, 13% w/w of triethylcitrate as plasticizer and 10% w/w of penetration enhancers in a binary mixture of isopropanol and acetone (3 : 2, v/v). TZN was dispersed in the polymeric solution being kept under magnetic stirring for uniform dispersion. The enhancers were added to the mixture after dissolving the polymers. The control film was fabricated with no enhancers. The mixture was cast over backing membrane in aluminum Petri dish (13 cm²) and dried under controlled conditions for 24 h. The solvent got completely evaporated in 24 h whereas triethylcitrate and penetration enhancers remained in the drug-polymer matrix.

Preparation of epidermis with intact stratum corneum (SCE)

The method of SCE preparation was based on that developed by Kligman and Christophers (9). The mouse skin was freshly excised from the abdomen of male mouse, 6-7 weeks of age (obtained from M/S. Ghosh Enterprises, Kolkata, India). The human cadaver skin was obtained from Forensic Science Department, Assam Medical College, Dibrugarh. The skin was excised from the abdomen of human cadaver of either sex (age 27-62 years) within 48 h of post-mortem. The SCE membrane from mouse and

human cadaver were removed after soaking the whole skin in distilled water at 60°C for 1 min. These were then rinsed thoroughly with distilled water, dried in a desiccator at approximately 25% RH, wrapped in aluminum foil and stored at $4 \pm 1^\circ\text{C}$ (10). The SCE membrane was immersed in the saline phosphate buffer of pH 7.4 in a closed vessel for 1 h in order to allow complete hydration at room temperature (10,11). Some workers reported 12 h (12) or more than 12 h (13) for complete skin hydration before permeation study. This is because they hydrated the skin in contact with the receptor phase in receptor compartment of the diffusion cell. The skin was gently blotted dry with a tissue paper. The integrity of the skin was tested microscopically before being used in Keshary-Chien diffusion cell, to detect any histological change. No significant histological changes were observed.

In vitro permeation studies

The *in vitro* permeation study was performed using Keshary-Chien glass diffusion cell (14). The film sample was fixed on the skin sample (mouse or human cadaver epidermis) previously fixed in between the donor and receptor compartment of Keshary-Chien diffusion cell. Both the effective skin area and the area of the film sample placed on the skin were 2.84 cm². The stratum corneum side of the skin was kept in intimate contact with the release surface of the transdermal films. The receiver phase was 18 mL of saline phosphate buffer of pH 7.4 stirred on a magnetic stirrer. The cell was thermostated at 37°C producing a skin surface temperature of 32°C. The amount of drug permeated was determined spectrophotometrically at 246 nm by removing 1 mL aliquot through a hypodermic syringe fitted with a 0.22 μm membrane filter, at designated time intervals, for 32 h period. The volume was replenished with the same volume of pre-warmed receiver solution. Blanks were run for each set as described above without using films and calculated accordingly.

TZN was estimated by UV spectrophotometric assay using Hitachi U-2001 UV-VIS spectrophotometer at 246 nm. For this purpose, a standard calibration curve of TZN powder (reference standard) in saline phosphate buffer of pH 7.4 was constructed, which was found to be linear between 4 – 32 μg/mL. The equation of the resulting line was “absorbance = - 0.004 + 0.0264 × concentration” [r = 0.999]. The amount of TZN released into the receptor phase from transdermal films was then calculated by determining the UV absorbance of samples removed at set intervals at 246 nm against blank.

Determination of TZN retained in the skin (15)

At the end of permeation experiment, the skin was removed from the diffusion cell and washed using several milliliters of distilled water and blotted dry with a tissue paper. The exposed skin area was weighed, cut into small pieces and placed in 5 mL of saline phosphate buffer of pH 7.4 with occasional stirring for 24 h. The desorbing solution was then filtered through membrane filter (pore size 0.22 μm) and the amount of TZN in the filtrate was determined spectrophotometrically at 246 nm.

Data and statistical analysis

The TZN concentration was corrected for sampling effects according to the equation described by Hayton and Chen (16). The cumulative amount of TZN permeated per unit skin surface area (Q , mg/cm^2) was plotted against time (h) and the slope of the linear portion of the plot was estimated as steady-state flux (J_{ss} , $\text{mg}/\text{cm}^2/\text{h}$). The lag time, T_L , was determined from the x-intercept of the slope at the steady state. The apparent diffusion parameter (D/h^2 , h^{-1}) was calculated from the following equation (17): $D/h^2 = 1/6T_L$, where h (cm) is the thickness of the skin and D is diffusion coefficient within the skin (cm^2/h). The penetration enhancing effect of the permeation enhancer was calculated in terms of enhancement ratio (ER) using the following equation: $ER = J_{ss} \text{ with enhancer} / J_{ss} \text{ without enhancer}$.

Data were expressed as mean \pm SEM ($n = 4$). Statistical comparisons were made using the Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Target flux

The input rate required based on the steady state equation was 6615 $\mu\text{g}/\text{h}$ or 6.615 mg/h to achieve a therapeutic plasma concentration of TZN (0.75 $\mu\text{g}/\text{mL}$). Therefore, the target flux (transdermal absorption rate) to attain therapeutic concentration of TZN was calculated at 66.15 $\mu\text{g}/\text{cm}^2/\text{h}$ or 0.066 $\text{mg}/\text{cm}^2/\text{h}$ from a patch size of maximum 100 cm^2 for a 70 kg person.

As mentioned previously, the present study was carried out to investigate the effect and the mode of action of IPM, IPP, butanol and octanol as penetration enhancers on the permeability of TZN across mouse and human cadaver epidermis. For this purpose, all experiments were done using matrix formulation of TZN (10% w/w) containing penetration enhancers at 10% w/w level. The con-

centrations of the enhancers were selected from the previous reports in the literatures (18-20).

The permeation through mouse epidermis

The *in vitro* permeation of TZN across mouse epidermis is summarized in Figure 1. A control transdermal formulation without enhancer was placed in contact with the stratum corneum of mouse epidermis. The permeation of TZN reached steady state after 6 h (Figure 1).

When various enhancers were incorporated into the transdermal formulation, the permeation of TZN increased significantly ($p < 0.05$) against the control (Table 1). The permeation of TZN in the presence of 10% w/w of IPM, IPP, butanol and octanol was 3.79, 2.00, 3.32 and 2.40 times greater, respectively, than that in absence of these enhancers. The lag time was significantly ($p < 0.05$) decreased in the presence of these enhancers (Table 1)

The amount of TZN retained in the skin at the conclusion of the permeation studies for IPM, IPP, butanol and octanol was nearly 3 times higher than

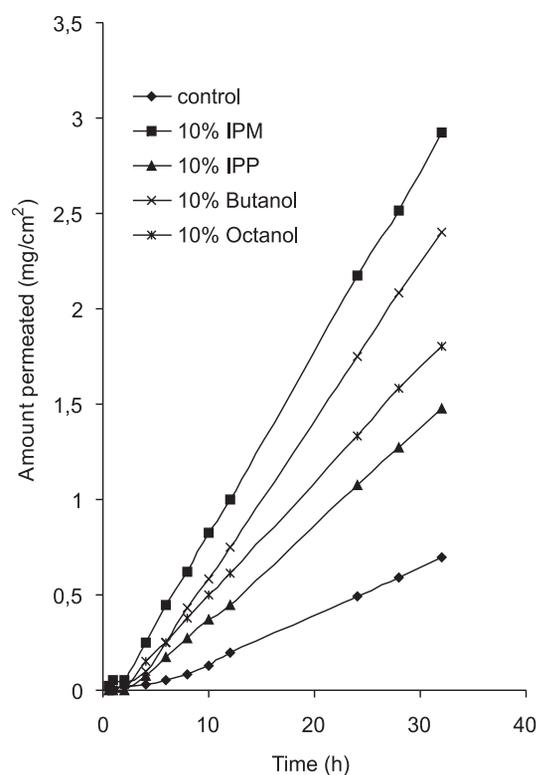


Figure 1. Permeation profiles of TZN from matrix-based transdermal formulations containing different penetration enhancers through mouse epidermis. Each data point represents the mean of 4 determinations.

Table 1. The penetration data of TZN delivered from matrix-based transdermal formulations containing different penetration enhancers through mouse epidermis¹.

Enhancers (% w/w)	Jss ² (mg/cm ² /h)	T _L ³ (h)	ER ⁵	D/h ² ⁴ (×100 h ⁻¹)	Best fit of equation for permeation plot	R ⁶	Skin retention (µg/mg)
Control	0.025 ± 0.005	6 ± 0.2	1.00	2.78 ± 0.5	Q = 0.0252 t - 0.1123	0.9994	2.74 ± 0.67
10% IPM	0.095 ± 0.02	1.5 ± 0.3	3.79	11.11 ± 1.25	Q = 0.0952 t - 0.1308	0.9998	7.84 ± 1.31
10% IPP	0.050 ± 0.012	3 ± 0.4	2.00	5.56 ± 2.12	Q = 0.0501 t - 0.1298	0.9997	7.29 ± 1.21
10% Butanol	0.083 ± 0.015	2 ± 0.1	3.32	8.33 ± 2.05	Q = 0.0826 t - 0.238	0.9999	7.79 ± 1.64
10% Octanol	0.060 ± 0.021	4 ± 0.6	2.40	4.17 ± 1.05	Q = 0.0595 t - 0.0975	0.9999	7.37 ± 0.98

¹ Values are the mean ± SEM (n = 4)

² Steady state flux

³ Lag time

⁴ Diffusion parameter

⁵ Enhancement ratio

⁶ Coefficient of correlation

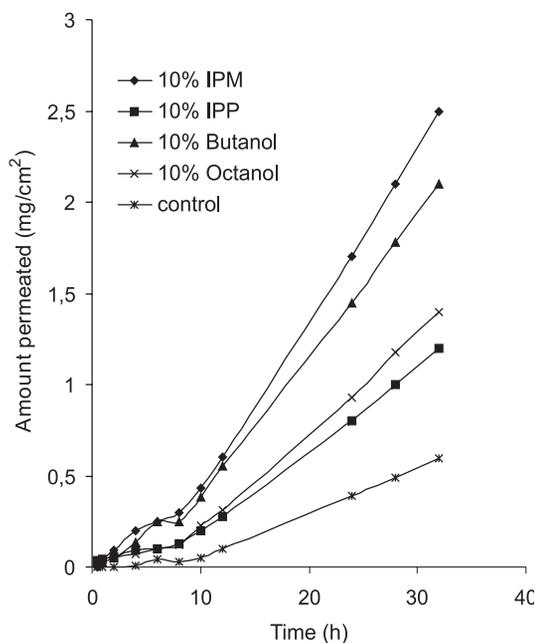


Figure 2. Permeation profiles of TZN from matrix-based transdermal formulations containing different penetration enhancers across human cadaver epidermis. Each data point represents the mean of 4 determinations.

for the control formulation (Table 1), indicating that tissue concentration could be related to the flux across the skin.

The permeation through human cadaver epidermis

In a separate experiment using human cadaver epidermis, the enhancing effects of the above penetration enhancers were examined. The *in vitro* per-

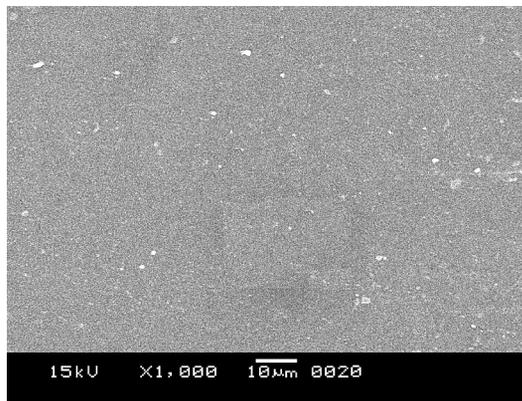


Figure 3. SEM photomicrograph of one formulation.

meation of TZN from the control formulation reached steady state after 8 h (Figure 2). The incorporation of penetration enhancers at 10% w/w level into the formulation increased *in vitro* permeation of TZN significantly ($p < 0.05$). The transport of TZN in the presence of IPM, IPP, butanol and octanol was 3.83, 1.87, 3.20 and 2.20 times greater, respectively, than the control formulation. The lag time was significantly reduced in the presence of these enhancers (Table 2).

The results in Table 2 show that the amount of TZN found in the skin for various enhancers was nearly 3 times higher than for the control formulation.

DISCUSSION

All the transdermal formulations studied here were of matrix-dispersion type as indicated by the

Table 2. The penetration data of TZN delivered from matrix-based transdermal formulation containing different penetration enhancers through human cadaver epidermis¹.

Enhancers (% w/w)	Jss ² (mg/cm ² /h)	T _L ³ (h)	ER ⁵	D/h ² ⁴ (×100 h ⁻¹)	Best fit of equation for permeation plot	R ⁶	Skin retention (µg/mg)
Control	0.024 ± 0.02	8 ± 1.8	1.00	2.08 ± 0.52	Q = 0.0241 t - 0.1827	0.9994	3.42 ± 1.23
10% IPM	0.092 ± 0.03	3 ± 0.7	3.83	5.56 ± 1.22	Q = 0.0923 t - 0.4013	0.9995	9.89 ± 2.87
10% IPP	0.045 ± 0.05	5 ± 1.2	1.87	3.33 ± 0.78	Q = 0.0447 t - 0.2409	0.9994	8.24 ± 1.97
10% Butanol	0.077 ± 0.07	4 ± 0.9	3.20	4.16 ± 0.55	Q = 0.0771 t - 0.3797	0.9999	9.73 ± 2.54
10% Octanol	0.053 ± 0.004	6 ± 0.5	2.20	2.77 ± 0.42	Q = 0.0531 t - 0.3146	0.9995	8.96 ± 1.50

¹ Values are the mean ± SEM (n = 4)² Steady state flux³ Lag time⁴ Diffusion parameter⁵ Enhancement ratio⁶ Coefficient of correlation

Table 3. Physicochemical parameters of various enhancers.

Enhancers	Molecular weight (Da)	Boiling point (°C)	Log P	*δ (cal/cm ³) ^{1/2}
IPM	270	167	1.96	8.5
IPP	298	342	2.09	7.5
Butanol	74	117	0.89	11.18
Octanol	130	195	3.15	10.3

*Solubility parameter

Table 4. Student's *t*-test results of the species difference for the skin retention.

Skin Sample	Penetration enhancers				
	Control	10% IPM	10%IPP	10% Butanol	10% Octanol
Mouse epidermis	2.74 ± 0.67	7.84 ± 1.31	7.29 ± 1.21	7.79 ± 1.64	7.37 ± 0.98
Human cadaver epidermis	3.42 ± 1.23	9.89 ± 2.87	8.24 ± 1.97	9.73 ± 2.54	8.96 ± 1.50
Student's <i>t</i> -test	<i>t</i> = 1.05 p = 0.20	<i>t</i> = 1.27 p = 0.17	<i>t</i> = 1.08 p = 0.19	<i>t</i> = 1.87 p = 0.10	<i>t</i> = 2.00 p = 0.07

microscopic study using JEOL, JSM-6360, Scanning Electron Microscope (SEM). A microphotograph of one formulation is shown in Figure 3. Then, as the skin was supposed to provide the only barrier to drug penetration, flux changes in the presence of different enhancers were considered indicative of alteration of skin permeability caused by a direct action of these enhancers on the skin.

Effect of aliphatic acid esters

As observed from Table 1 and 2, Jss, D/h² and amount of TZN retained in the skin increased significantly (p < 0.05) with aliphatic acid esters suggesting that these enhancers increased the partition-

ing of TZN into the skin. Thus, the major effect of these esters is on the partition coefficient of TZN, that is, they affect the skin lipids and alter its solubility in the skin. This is in agreement with the results of Sato et al. (19), who concluded that aliphatic esters mainly act on the lipids of the skin, which is a main barrier against the drug permeation, and increase the diffusivity in the skin and/or partition coefficient between the skin and the delivery system. Solutes can be transported across the skin by at least two pathways: a polar pathway associated with the protein component of the skin and a non-polar pathway associated with the lipid component (21). Varying amount of TZN retained in the skin

with IPM and IPP indicate the variable partitioning of TZN into the skin, which in turn implies that the solubility of the TZN in the epidermis had altered. Therefore, it may be concluded that the aliphatic esters used act through the nonpolar route to increase the flux across the epidermis. It is assumed that these enhancers being the lipophilic in nature may penetrate into the lipid bilayers of the skin and due to their chain structure, disrupt the lipid, which increase the fluidity of lipids (6). As shown in Table 1 and 2, there was a statistically significant difference ($p < 0.05$) between the flux values obtained with IPM and IPP. It may be ascribed to their different physicochemical properties (Table 3). It has been reported that the flux of the solutes through the membrane is enhanced when the solubility parameter of the vehicle is close to that of the skin (22). The solubility parameter of IPM (Table 3) is very near to the solubility parameter of human skin [$10 \text{ (cal/cm}^3)^{1/2}$] (22) and this helps in higher flux of TZN with short lag time. As aliphatic esters used in the present study did not differ significantly in their log P values (Table 3), differences in their permeation activities cannot be ascribed to their lipophilicity. On the other hand, they significantly differed in boiling points. A low boiling point of IPM is an indication of weak cohesiveness or self-association of the molecules, and therefore, it may more easily associate or interact with lipid components of the skin and thereby alter the barrier property. The enhancement effect of IPM can also be manifested by virtue of its intermediate polar nature; it may be partitioned into both the lipid (nonpolar) and protein (polar) phase of the skin.

Effect of alkanols

As described in the Results, butanol and octanol exhibited significant TZN permeation enhancing effects across the skins of both species. The higher values of D/h^2 parameter observed in the Table 1 and Table 2 indicate that these penetration enhancers reduced the barrier properties of the skin to the diffusion of drug resulting in an increase of the flux values. In effect, these alkanols may interact with lipid components of the skin by swelling the skin lipids or causing them to become a more fluid-like material (23). This may improve the permeability characteristics of the skin, and thus reducing its resistance to the diffusion of TZN. Among the two, the more pronounced enhancing effect was obtained with butanol regarding the permeation flux, and diffusion parameter. The possibility may be ascribed to its physicochemical properties (Table 3). Butanol, a polar solvent, may partition into both the polar and

nonpolar phases of the skin. In polar pathway, it interacts with keratin fragment of the skin and this interaction and/or association is more pronounced due to its low boiling point (weak cohesiveness or self-association of the molecules) and similar solubility parameter to that of keratin fragments of the skin ($11.8 \text{ (cal/cm}^3)^{1/2}$). The keratin fragments become fluidized by loosening of its packing structure and also the formation of water 'pools' around polar head groups resulting in a facilitated polar pathway or channel through the lipid bilayers (22). TZN with a pK_a value of 6.7 would be cationic at the skin surface pH (varying between 4 – 5) and anionic within the viable tissue (pH 7.4). The ionized TZN may permeate through polar channel when butanol is used as enhancer. In contrast, octanol, a nonpolar liquid, acts only through lipid pathway. The larger molecular size (Table 3) of octanol decreased its mobility into the skin and its interaction and/or association with the lipid is less pronounced due to its high boiling point (strong cohesiveness of the molecules – Table 3) resulting in less permeable membrane. Further, the percutaneous absorption involves drug partitioning from the vehicle into the skin, drug diffusion through the skin, drug partitioning from skin to aqueous viable tissue and drug diffusion through the viable tissue to the dermal microcirculation. The last process is generally regarded as the fastest step. A penetration enhancer, therefore, is likely to be effective when it acts on one or more of the first three processes (24). In the present study, the low flux value of TZN observed with octanol may be due to its lower impact on the skin diffusion process.

Skin retention

As observed from Table 1 and 2, the amount of TZN retained in the skin for both the species significantly increased ($p < 0.05$) against the control in the presence of different enhancers. However, the difference between the skin retention for both the species in the presence of various enhancers is not significant statistically ($p > 0.05$). This may be due to the gradual increase of skin retention up to the saturation level with increasing the flux value across the skin.

The retention of TZN in the human cadaver epidermis was slightly higher than the retention in the mouse epidermis. However, this difference is insignificant statistically ($p > 0.05$ – Table 4).

CONCLUSIONS

The required input rate to attain therapeutic concentration of TZN was set at 6.615 mg/h. The

passive diffusion of TZN through the human cadaver skin was small due to its ionic behavior at the site of permeation. The presence of various enhancers at 10% w/w level increased the flux of TZN significantly. The maximum flux attained with IPM was 0.092 ± 0.03 mg/cm²/h followed by butanol (0.077 ± 0.07 mg/cm²/h), octanol (0.053 ± 0.004 mg/cm²/h) and IPP (0.045 ± 0.05 mg/cm²/h). With the flux of 0.092 mg/cm²/h obtained from a formulation consisting of 10% IPM, it can be estimated (input rate) that a transdermal patch of about 70 cm² consisting of 10% IPM should be able to attain and maintain 0.75 µg/mL of TZN over a period of 24 h.

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