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Original Research Article

Temperature influencing permeation pattern of alfuzosin: An investigation using DoE

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ARTICLE INFO

Article history:
Received 3 October 2014
Received in revised form
11 July 2015
Accepted 15 July 2015
Available online 30 July 2015

Keywords:
Alfuzosin hydrochloride
Transdermal delivery
Temperature
Permeation enhancer
Ex vivo

ABSTRACT

Background and objective: There has been relatively little investigation of the effect of temperature on skin permeation compared to other methods of penetration enhancement. A principal physicochemical factor which controls the passive diffusion of a solute from a vehicle into the skin arises from the skin temperature. The aim of this ex vivo study was to probe into the effect of heat on transdermal absorption of alfuzosin hydrochloride from ethyl cellulose-polyvinyl pyrrolidone (EC-PVP) based transdermal systems.

Materials and methods: Principles of design of experiment (DoE) were used to systematically study the influence of temperature on transdermal permeation of alfuzosin. Ex vivo transdermal permeation studies were carried out at varied donor compartment temperatures. Permeation data analysis was carried out and activation energy for transdermal permeation was estimated.

Results: Temperature found to enhance ex vivo permeation parameters of alfuzosin hydrochloride from its transdermal systems. It was also noted that chemical permeation enhancers potentiate permeation enhancing effect of temperature. The permeation flux values approximately doubled after exposure to 45 °C. The activation energy for transdermal permeation was found lower for the runs with chemical permeation enhancers indicating existence of a lower energy barrier in the presence of chemical permeation enhancers.

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Peer review under the responsibility of the Lithuanian University of Health Sciences.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.medici.2015.07.002

Conclusion: The method reported here is a simple and useful tool for studying the effect of heat on percutaneous absorption. Such temperature dependent enhancement of flux can be more pronounced at skin surface temperatures >45 °C.

1. Introduction

The use of heat as a means of enhancing percutaneous absorption has been documented historically [1], but it has never been fully exploited as a means of aiding drug delivery across the skin. Thus, there has been relatively little investigation of the effect of temperature on skin permeation compared to other methods of penetration enhancement. It is essential that the effect of temperature on penetrant and also the cellular components (keratin, ceramides, etc.) and functions of the skin be well defined in order to understand the mechanism of enhancement. In addition, the FDA has produced an article describing the possibility of toxicity due to the percutaneous absorption of topically applied material as a result of the increased temperature and exposure to UV radiation associated with the sun [2]. A principal physicochemical factor which controls the passive diffusion of a solute from a vehicle into the skin arises from the skin temperature. It is an established fact that skin temperature rise increases penetration of solute [3,4]. Additionally, temperature enhanced permeabilities of the solutes were associated with the gel to liquid-crystalline transition of lipid hydrocarbon chains [5] and increasing temperatures of the stratum corneum (SC) resulted in increased fluidity (rotational disorder) of the intercellular lipids [6,7]. Phase behavior studies of SC have shown that arrangement and state of lipid bilayer is changing with temperature. Lipid bilayer of SC can exist in crystalline gel, liquid-/crystalline state or mesomorphic form depending upon the temperature.

In a recent study reported by Petersen et al. [8], controlled heat application (43 °C) caused significant cutaneous hyperaemia (up to ninefold increase in skin perfusion) with an increase in nicotine uptake (up to 13-folds). In another study, the transdermal permeation was found to increase exponentially when the donor environment temperature was varied from 2 to 47 °C [9].

Use of heat energy to enhance transdermal delivery of drugs has been adopted by few pharmaceutical companies. The controlled heat-aided drug delivery patch (CHADD) developed by Zars Inc. (Salt Lake City, Utah) consists of a patch containing a series of holes at the top surface regulating the entry of oxygen into the patch. This works on generation of heat chemically in a powder filled pouch by an oxidative process regulated by the rate of flow of oxygen through the holes into the patch. The CHADD technology was used in the delivery of a local anesthetic system (lidocaine and tetracaine) from a patch (S-Caine®) and found to enhance the depth and duration of the anesthetic action in human volunteers when the results obtained in active and placebo groups were compared [10]. Zars Inc. together with Johnson and Johnson, recently developed Titragesia™ (a combination of CHADD disks and Duragesic Patches, the latter contains fentanyl for treatment of acute pain).

Consequently, increasing the temperature of the skin and its environment may well provide the potential for overcoming the barrier properties of the SC, and thus, warrants a systematic investigation.

The absolute bioavailability of alfuzosin is about 49% under fed conditions, while the corresponding value under fasting conditions is approximately 25% [11]. This shows that food has a significant impact on the oral absorption of alfuzosin. This originates the need for an alternative route of administration, which can bypass the hepatic first-pass metabolism. Transdermal route is an alternative choice of route of administration for such drugs. Various physicochemical parameters like molecular weight, log *P* value and aqueous solubility of alfuzosin hydrochloride are 425.92, 1.51 at a pH of 7.4 and >10%, respectively [12]. These favorable parameters make it an ideal drug candidate for transdermal delivery.

The aim of this ex vivo study was to probe into the effect of heat on transdermal absorption of alfuzosin hydrochloride from EC-PVP based transdermal systems. Temperatures used in this study were in the range 30–50 °C range so as to minimize the thermal damage to the skin [9,13].

2. Materials and methods

2.1. Materials

Alfuzosin hydrochloride was obtained as a gift sample from Cipla Ltd. (Mumbai, India). Ethyl cellulose (EC; ethoxy content 47.5%–49%, viscosity 14 cps in 5% (w/w) solution in 80:20 toluene/ethanol at 25 °C) was purchased from BDH Chemicals Ltd., Poole, England. Polyvinylpyrrolidone (PVP; *K* value: 26–35) and polyvinylalcohol (PVA) were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India and S.D. Fine-Chem. Ltd., Boisar, India, respectively. Di-n-butylphthalate was purchased from Central Drug House (P) Ltd., Mumbai, India.

2.2. Experimental design

A D-optimal response surface design was used to study the influence of temperature on ex vivo human cadaver skin permeation of alfuzosin hydrochloride. One numeric factor (X_1) and one categoric factor (X_2) were evaluated. The categoric factor was evaluated at two levels and the numeric factor was evaluated at five levels. The levels of categoric factor indicate either absence (Level 1) or presence (Level 2) of chemical permeation enhancers. Temperature was taken as the numeric factor and was studied at 30, 35, 40, 45 and 50 °C.

The cumulative amount of alfuzosin hydrochloride permeated per cm^2 of human cadaver skin at 24 h (P_{24}), permeation flux (J) and steady state permeability coefficient (P_{ss}) were chosen as dependent variables. Design-Expert software (Version 7.1.4, Stat-Ease Inc., Minneapolis, USA) was used for the generation and evaluation of the statistical experimental design.

2.3. Preparation of transdermal films

From our laboratory, we have already fabricated alfuzosin transdermal systems and reported the influence of chemical permeation enhancers on transdermal permeation of alfuzosin [12]. In brief, the films were composed of EC and PVP at a ratio of 10:90 with 50% (w/w) drug loading and 5% (w/w) chemical permeation enhancer (a blend of 62.4% oleic acid and 37.6% propylene glycol). Di-n-butylphthalate was incorporated as a plasticizer at a concentration of 30% (w/w) of dry weight of polymers. Transdermal patches were fabricated using solvent evaporation technique.

2.4. Ex vivo human cadaver skin permeation studies

The extent and rate of skin permeation of alfuzosin hydrochloride through the human cadaver skin were carried out using modified Franz diffusion cells (Keshary–Chien diffusion cell). The receptor compartment was filled with 20 ml normal saline (0.9% (w/v) of NaCl) and its temperature was maintained at 30, 35, 40, 45 or 50 °C during the experiment. To control the temperature during the permeation studies, distilled water from a precision water bath maintained at desired temperature was allowed to circulate through the jackets of the diffusion cells. Owing to higher aqueous solubility of alfuzosin HCl, normal saline has been chosen as the receptor fluid [14]. The diffusional area (cross section area) of the diffusion cell was 1.766 cm^2. The receptor fluid is constantly agitated at 100 rpm by a Teflon coated magnetic bead. The film (about 1.8 cm^2) was applied under occlusion (using Leucoplast® tape) on the epidermal surface of the human cadaver skin fitted between the donor and receptor compartments of the diffusion cell. Abdominal sections of skin from the same donor were used throughout the study to minimize variability in results. The whole of the receptor fluid was collected from the sampling port at predetermined time interval and replaced immediately with fresh normal saline. A similar set was run simultaneously using the film (without drug) at the donor compartment as a skin film control system to avoid the influence of inherent extracts from the skin or leaching of any material from the film without drug on the absorbance at 242 nm, at which the sample aliquots were analyzed spectrophotometrically. The amount of drug permeated per square cm at each time interval was estimated and subjected to further data analysis

2.5. Estimation of activation energy

Activation energy for permeation provides insight to mechanisms of transmembrane mobility of drug molecules [15,16]. Activation energy for permeability of alfuzosin hydrochloride from its transdermal system across human cadaver skin was estimated by measuring the permeability of drug at various temperatures like 30, 35, 40, 45 and 50 °C using the Arrhenius equation:

$$k = Ae^{-Ea/RT}$$

where k is the specific reaction rate, A is a constant commonly referred to as the frequency factor, R is the gas constant, and T is temperature. The slope of the plot of $\log P_{ss}$ vs $1/T$ can be related to E_a as follows:

$$\text{Slope} = \frac{-E_a}{2.303R}$$

2.6. Permeation data analysis and statistics

The flux (μg/cm^2 h) of alfuzosin hydrochloride was calculated from the slope of the plot of the cumulative amount of alfuzosin hydrochloride permeated per cm^2 of human cadaver skin at steady state against the time using linear regression analysis. The steady state permeability coefficient (P_{SS}) of the drug through human cadaver skin was calculated by using the following equation:

$$P_{SS} = \frac{J}{C}$$

where J is the flux and C is the initial concentration of alfuzosin hydrochloride in the patch. The observed difference in the permeation parameters of alfuzosin hydrochloride in different formulations were compared by using one way analysis of variance (ANOVA) followed by all pair wise multiple comparison procedure such as Holm–Sidak test at overall significance level of 0.05 using SigmaStat software (SigmaStat 3.5, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Ex vivo permeation of alfuzosin

Effects of temperature on the ex vivo drug permeation from the transdermal patches were studied by statistical design of experimental (DoE). In this study, a D-optimal response surface design (Table 1) was used with temperature as numeric factor and presence or absence of chemical permeation enhancers as categoric factor. A suitable statistical model was selected using Design Expert® software. Sequential model sum of squares select the highest order polynomial where the additional terms are significant and the model is not aliased (Table 2). Based on statistical analysis like adjusted multiple correlation coefficient (adjusted R^2) and predicted residual sum of squares (PRESS), a cubic model was fitted to the data ($p < 0.05$) for interpreting data results for the permeation response; P_{24}, and a reduced cubic model (automatically done by backward elimination method) for J and P_{ss} (Table 3). Analysis of variance (ANOVA) was applied to estimate the significance of the model at the 5% significance level.

The mathematical model generated by the design for the responses are as follows:

$$\text{Cubic model (for } P_{24}): \quad Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{112}X_1^2X_2 + b_{111}X_1^3$$

Table 1 – Composition and observed responses from randomized runs in D-optimal response surface design.

Run	Factors		Observed responses[c]			ER_{flux}
	X_1[a] (temperature) (°C)	X_2[b] (CPE)	P_{24} (μg/cm²)	J (μg/cm²/h)	$P_{ss} \times 10^3$ (cm/h)	
1	30	Level 1	107.44 ± 2.17	5.2 ± 0.58	3.95 ± 0.37	0.68
2	30	Level 2	273.37 ± 4.26	12.06 ± 0.43	9.17 ± 0.68	0.81
3	35	Level 1	166.53 ± 2.33	7.57 ± 0.66	5.75 ± 0.44	0.99
4	35	Level 2	308.34 ± 4.16	13.45 ± 0.51	10.23 ± 0.51	0.90
5	40	Level 1	209.54 ± 3.83	9.77 ± 0.62	7.43 ± 0.71	1.28
6	40	Level 2	467.81 ± 4.87	20.62 ± 0.26	15.68 ± 0.53	1.39
7	45	Level 1	317.77 ± 4.08	14.56 ± 0.46	11.07 ± 0.38	1.91
8	45	Level 2	662.33 ± 5.37	29.4 ± 0.37	22.36 ± 0.42	1.98
9	50	Level 1	375.28 ± 4.66	17.2 ± 0.93	13.08 ± 0.44	2.25
10	50	Level 2	796.37 ± 5.89	34.07 ± 0.87	25.91 ± 0.79	2.29
Control-1	–	–	167.21 ± 2.05	7.62 ± 0.19	5.80 ± 0.35	–
Control-2	–	–	344.824 ± 3.24	14.87 ± 0.83	11.31 ± 0.31	–

[a] The numeric factor was varied from 30 to 50 °C.
[b] The levels of categoric factor indicate either absence (Level 1) or presence (Level 2) of chemical permeation enhancers.
[c] Data shown are mean ± standard error of the mean ($n = 4$).

Table 2 – Model analysis by sequential model sum of squares.

Source	P_{24}		J		P_{ss}	
	Sum of squares	p-Value	Sum of squares	p-Value	Sum of squares	p-Value
Mean vs total	2.427E+006	–	4734.82	–	2.738E−003	–
Linear vs mean	7.979E+005	<0.0001	1425.85	<0.0001	8.246E−004	<0.0001
2FI vs linear	46,728.78	<0.0001	73.77	<0.0001	4.266E−005	<0.0001
Quadratic vs 2FI	4342.21	0.0233	6.12	0.0598	3.538E−006	0.0598
Cubic vs quadratic	4238.53	0.0083	9.22	0.0106	5.331E−006	0.0106
Residual	1832.04	–	4.36	–	2.523E−006	–
Total	3.282E+006	–	6254.14	–	3.617E−003	–

Table 3 – Model summary statistics.

Source	P_{24}		J		P_{ss}	
	Adjusted R-squared	PRESS	Adjusted R-squared	PRESS	Adjusted R-squared	PRESS
Linear	0.9220	91,665.9	0.9282	149.07	0.9282	8.621E−005
2FI	0.9845	17,857.76	0.9835	33.10	0.9835	1.914E−005
Quadratic	0.9901	12,347.13	0.9875	26.31	0.9875	1.522E−005
Cubic	0.9963	8276.452	0.9950	19.91	0.9950	1.152E−005

Reduced cubic model (for J and P_{ss}): $Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{111}X_1^3$

where Y is the dependent variable, b_0 is the arithmetic mean response of the runs and b_i (b_1, b_2, b_{12}, b_{11}, b_{112} and b_{111}) is the estimated coefficient for the corresponding factor X_i (X_1, X_2, X_1X_2, X_1^2, $X_1^2X_2$ and X_1^3) which represents the average result of changing one factor at a time from its low to high value. The interaction terms show how the response changes when the factors are changed simultaneously. The polynomial terms are included to investigate nonlinearity.

Final equation in terms of coaded factors

$$P_{24} = +347.58 + 271.04X_1 + 120.89X_2 + 67.65X_1X_2 + 40.92X_1^2 + 24.83X_1^2X_2 - 73.68X_1^3$$

$$J = +15.59 + 12.46X_1 + 5.65X_2 + 2.72X_1X_2 + 1.58X_1^2 - 3.95X_1^3$$

$$P_{ss} = +0.012 + 9.47E-003X_1 + 4.29E-003X_2 + 2.07E-003X_1X_2 + 1.20E-003X_1^2 - 3.00E-003X_1^3$$

Final equation in terms of actual factors

CPE Level-1 of X_2

$$P_{24} = +4386.30824 - 346.20917 \times \text{Temperature} + 9.00271 \times \text{Temperature}^2 - 0.073681 \times \text{Temperature}^3$$

$$J = +249.25988 - 19.26340 \times \text{Temperature} + 0.49011 \times \text{Temperature}^2 - 3.95259E-003 \times \text{Temperature}^3$$

$$P_{ss} = +0.18955 - 0.014649 \times \text{Temperature} + 3.727E-004 \times \text{Temperature}^2 - 3.005E-006 \times \text{Temperature}^3$$

CPE Level-2 of X_2

Table 4 – Standardized main effects of the factors on the measured responses.

Coefficient of regression parameter	Measured responses								
	P_{24}			J			P_{ss}		
	Coefficient estimate	95% confidence interval	SME[a]	Coefficient estimate	95% confidence interval	SME[a]	Coefficient estimate	95% confidence interval	SME[a]
b_0	347.58	330.52 to 364.63	46.97	15.59	14.71 to 16.47	39.97	0.012	0.011 to 0.013	40.11
b_1	271.04	224.34 to 317.73	13.38	12.46	10.05 to 14.87	11.75	9.474E−003	7.643E−003 to 0.011	11.71
b_2	120.89	103.83 to 137.94	16.34	5.65	5.18 to 6.12	26.90	4.294E−003	3.938E−003 to 4.651E−003	27.25
b_{12}	67.65	56.46 to 78.84	13.95	2.72	2.15 to 3.30	10.88	2.072E−003	1.634E−003 to 2.510E−003	10.70
b_{11}	40.92	19.09 to 62.76	4.32	1.58	0.46 to 2.71	3.16	1.202E−003	3.461E−004 to 2.057E−003	3.18
b_{112}	24.83	2.99 to 46.66	2.62	–	–	–	–	–	–
b_{111}	−73.68	−122.80 to −24.56	−3.46	−3.95	−6.48 to −1.42	−3.53	−3.006E−003	−4.931E−003 to −1.080E−003	−3.53

[a] Standardized main effects (SME) were calculated by dividing the main effect by the standard error of the main effect.

$$P_{24} = +4881.31716 - 372.40103 \times \text{Temperature} + 9.49924 \times \text{Temperature}^2 - 0.073681 \times \text{Temperature}^3$$

$$J = +238.75385 - 18.71841 \times \text{Temperature} + 0.49011 \times \text{Temperature}^2 - 3.95259\text{E} - 003 \times \text{Temperature}^3$$

$$P_{ss} = +0.18156 - 0.0142 \times \text{Temperature} + 3.727\text{E} - 004 \times \text{Temperature}^2 - 3.0057\text{E} - 006 \times \text{Temperature}^3$$

The coefficient estimate and standardized main effects (SME) for the responses are listed in Table 4. SME values were calculated by dividing the main effects by the standard error of the main effects. Results of multiple regression analysis and standardized main effects (SME) revealed that both the factors (X_1 and X_2) had statistically significant influence on all dependent variables ($p < 0.05$, Table 4).

The permeation profile of different runs is presented in Fig. 1. The influence of temperature and chemical permeation enhancer on the studied permeation parameters is evident from Figs. 2–4. SME values were found higher for the categoric factor, i.e. chemical permeation enhancer (16.34, 26.90 and 27.25 for P_{24}, J and P_{ss}, respectively) compared to the numeric factor (13.38, 11.75 and 11.71 for P_{24}, J and P_{ss}, respectively).

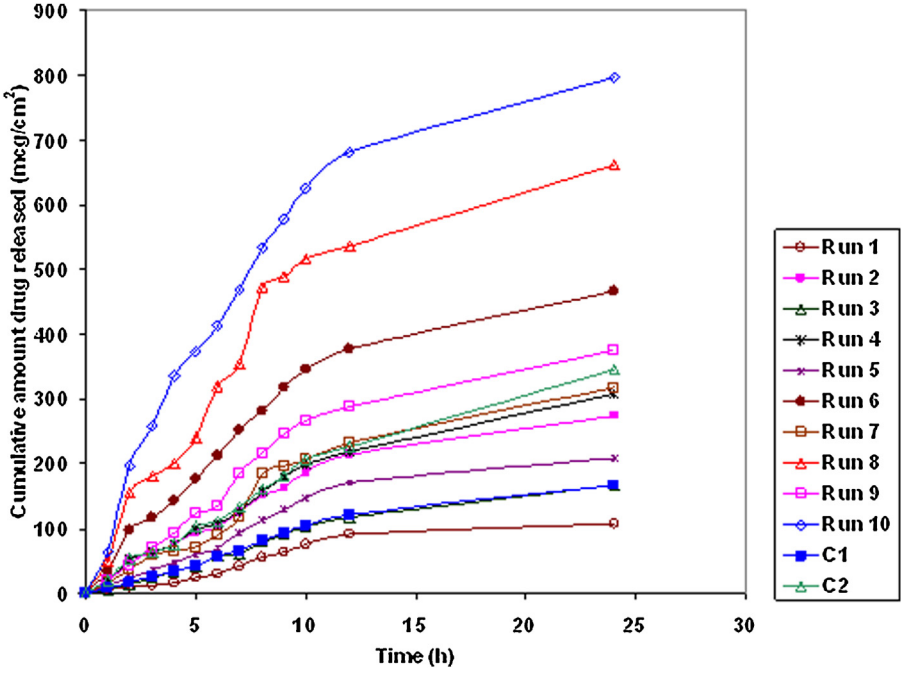

Fig. 1 – Ex vivo permeation profile of alfuzosin hydrochloride from transdermal patches ($n = 6$).

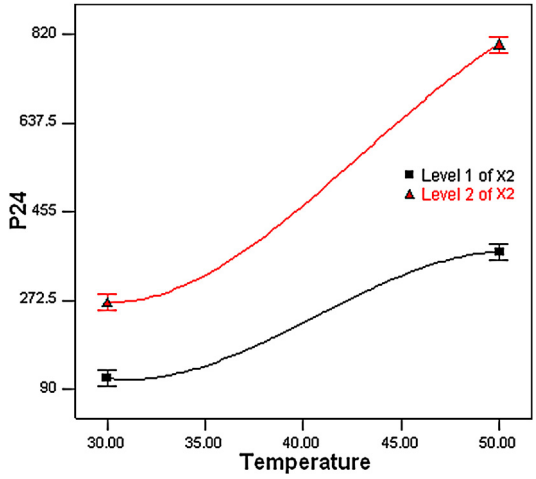

Fig. 2 – Plot showing influence of temperature (X_1) and chemical permeation enhancer (X_2) on P_{24} ($n = 6$).

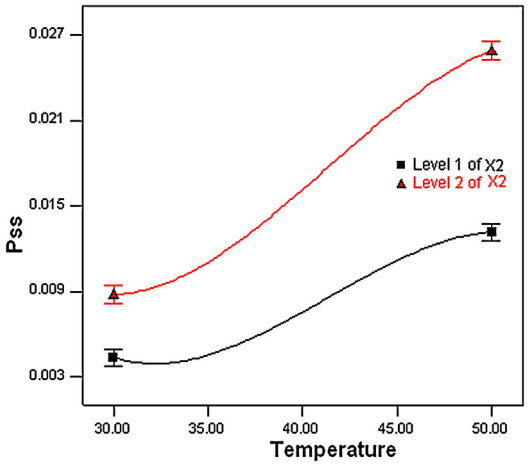

Fig. 4 – Plot showing influence of temperature and chemical permeation enhancer (X_2) on P_{ss} ($n = 6$).

The permeation parameters improved with increasing temperature from 30 to 50 °C and the effect was found more prominent in the presence of chemical permeation enhancer indicating a synergistic action. In the absence of chemical permeation enhancer, the cumulative amount of drug permeated at 24 h (P_{24}) found to increase from 107.44 ± 2.17 to 375.28 ± 4.66 μg/cm^2 when the temperature was varied from 30 to 50 °C. Similar increments in J (5.2 ± 0.58 to 17.2 ± 0.93 μg/cm^2/h) and P_{ss} (3.95 ± 0.37 × 10^{-3} to 13.08 ± 0.44 × 10^{-3} cm/h) were also observed. In the presence of chemical permeation enhancer, the cumulative amount of drug permeated at 24 h (P_{24}) found to increase from 273.37 ± 4.26 to 796.37± 5.89 μg/cm^2 when the temperature was varied from 30 to 50 °C. Similar increments in J (12.06 ± 0.43 to 34.07 ± 0.87 μg/cm^2/h) and P_{ss} (9.17 ± 0.68 × 10^{-3} to 25.91 ± 0.79 × 10^{-3} cm/h) were also observed.

The permeation responses were subjected to one way ANOVA followed by all pair wise multiple comparisons and comparisons vs control. When the permeation flux values of different runs with categoric factor at Level 1 were compared with that of Control 1, no statistically significant differences ($p < 0.05$) were observed except runs 7 and 9. Similarly, when the flux values of runs with categoric factor at Level 2 were compared with that of Control 2, statistically significant differences ($p < 0.05$) were observed except run 4.

Further, the permeation enhancing activities, expressed as enhancement ratio of flux (ER_{flux}), were calculated as the ratio between the flux value obtained with the change in temperature and that observed with the control. For the patches with categoric factor at Level-1, Control-1 (fabricated without incorporating chemical permeation enhancers; Pattnaik et al. [11]) was used for estimation of ER_{flux} and Control-2 (fabricated incorporating chemical permeation enhancers, Pattnaik et al. [11]) was used while estimating the same for the patches with categoric factor at Level-2. The results are shown in Fig. 5. One way ANOVA followed by all pair wise multiple comparisons (Holm–Sidak method) using Sigma Stat software (Sigma Stat 3.5, SPSS Inc., Chicago, IL, USA) detected

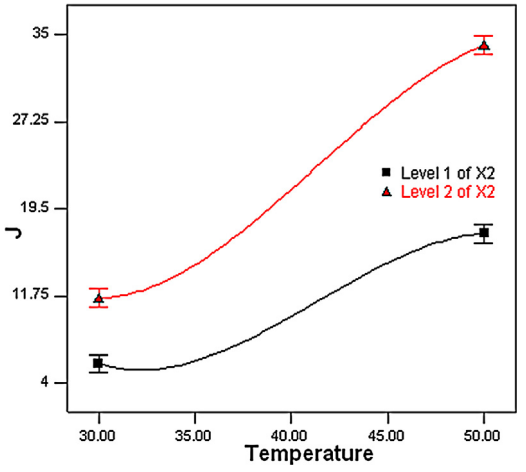

Fig. 3 – Plot showing influence of temperature and chemical permeation enhancer (X_2) on J ($n = 6$).

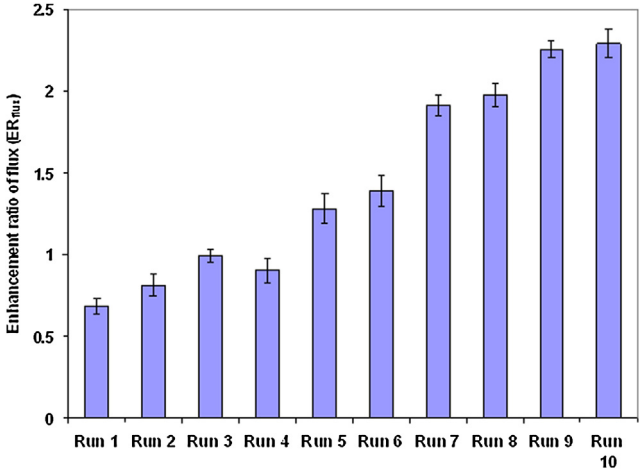

Fig. 5 – Enhancement ratio of flux for the experimental runs. Error bars indicate standard error of the mean.

statistically significant difference ($p < 0.05$) in ER_{flux} among all the runs with categoric factor at Level-1 and all the runs with categoric factor at Level-2 except between run 2 vs run 4 and run 8 vs run 10.

Maximum increase in steady state permeability flux (J) was observed with run 9 (patches without chemical permeation enhancers and studied at 50 °C) with ER_{flux} value of 2.25 and run 10 (patches containing chemical permeation enhancer blend and studied at 50 °C) with ER_{flux} value of 2.29.

3.2. *Activation energy for transdermal permeability*

Good linearity ($R^2 = 0.9887$ and 0.966, respectively, for Levels 1 and 2 of the categoric factor) was observed in the Arrhenius plot (Fig. 6). The activation energy for transdermal permeation was estimated to be 49.72 and 46.61 kJ/mol in the absence and presence of chemical permeation enhancers, respectively indicating lowering of energy barrier using chemical permeation enhancers.

4. Discussion

DoE has been successfully employed in pharmaceutical field to study the effect of independent variables and their interactions on response variables [12,17,18]. The present investigation is also a successful application of DoE to understand the influence of temperature on transdermal permeation of alfuzosin. It is confirmed from the study that the dependent responses are much more influenced by the presence or absence of chemical permeation enhancer than by the changes in numeric factor, i.e. temperature. The chemical permeation enhancer used in the patches consists of a blend of 62.4% oleic acid (OA) and 37.6% propylene glycol (PG). Oleic acid is an unsaturated fatty acid with a *cis* configuration. The bent *cis* configuration of oleic acid is expected to disturb intercellular lipid packing more so than the saturated straight chain counterparts [19]. Propylene glycol is known to have relatively low skin cell toxicity [20,21] and has been widely used for formulation of transdermal delivery systems. The enhancing effect by the addition of fatty acids to propylene glycol has been widely studied, and the binary system was considered to disorganize the multilaminate hydrophilic–lipophilic layers

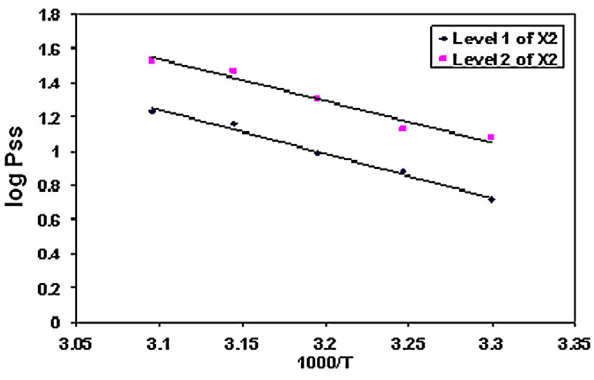

Fig. 6 – Arrhenius plot for runs with Level 1 and Level 2 of categoric factor.

located intercellularly in the stratum corneum, consequently promoting percutaneous absorption of drugs [22].

About twofold increase in the permeation responses were found after exposure at 45 °C. The enhancement in percutaneous absorption with temperature observed in this study is consistent to that reported by other authors [10,23–25]. These authors attributed this enhancement to an increase in the fluidity of stratum corneum lipids, which might also lead to an associated increase (expansion) in intercellular space with this leading to the increase in epidermal permeability. At low temperatures, the lipids are much more rigid and close packed and this hinders the diffusion of the penetrant molecules across the SC. With the aid of differential scanning calorimetry (DSC), thermal transitions of stratum corneum lipids has been shown to occur near 40, 65–70 and 80–85 °C [10,26]. Since SC lipid transitions occur at skin surface temperatures >40 °C, the enhanced permeation of drug at the studied higher temperatures may be attributed to the thermal transition of SC lipids resulting in a reduced barrier property.

The increase in enhancement of transdermal flux due to exposure at higher temperature in the presence of chemical permeation enhancers clearly indicated the existence of synergism of chemical permeation enhancers and temperature for transdermal permeation of alfuzosin hydrochloride. Similar potentiating activity in permeation at higher temperature was reported for hydrophilic and lipophilic drugs in the presence of oleic acid [23].

The limitations of using the procedure employed in this study to simulate increases in skin surface temperatures may not reflect physiological conditions. Homeostasis ensures that the blood circulation is maintained at 37 °C. Therefore, at diffusion cell receptor temperatures below and above 37 °C, the clearance of the permeant from the underlying layer of skin in contact with receptor fluid may be slower and faster, respectively. This temperature difference will affect the in vitro measured fluxes and might not parallel in vivo transdermal flux. This method, however, is a simple and useful tool for studying the effect of heat on percutaneous absorption. Such temperature dependent enhancement of flux can be more pronounced at skin surface temperatures >45 °C. However, the use of such high temperatures (>45 °C) for a long period of time as employed in this study may cause patient discomfort. This calls for further investigation into the effect of periodic increases in physiologically acceptable temperature on percutaneous absorption.

The slope of the Arrhenius plot which is proportional to the activation energy (E_a) gives an indication of the energy level necessary for penetrants to break restraining bonds and diffuse, i.e. it provides a measure of the resistance to diffusion of the penetrant to cross the epidermis. In general, the value of the activation energy is a function of both the diffusing molecule and the diffusion pathway [10]. Due to the lipophilic nature of the skin, polar compounds diffusing through the intercellular pathway of normal, intact epidermis might be expected to encounter greater energy barriers and therefore have higher activation energies compared to non-polar molecules. The latter would permeate more easily via the lipoidal pathway due to their higher stratum corneum/water partition coefficients. The activation energy, therefore takes into account the processes involved in the diffusion of a

penetrant across the epidermis (including diffusion of penetrant through the vehicle, partitioning from the vehicle into SC, diffusion in the SC, the partitioning of the compound from SC into epidermal layer, diffusion through the epidermal layer and finally partitioning into the receptor solution). The activation energy for transdermal permeation was estimated to be 49.72 kJ/mol and 46.61 kJ/mol in the absence and presence of chemical permeation enhancers, respectively indicating lowering of energy barrier using chemical permeation enhancers.

5. Conclusions

Temperature found to enhance ex vivo permeation parameters of alfuzosin hydrochloride from its transdermal systems. It was also noted that chemical permeation enhancers potentiate permeation enhancing effect of temperature. The permeation flux values approximately doubled after exposure to 45 °C for both the categoric levels (i.e. in the presence and absence of chemical permeation enhancer) but the enhancement ratio was found to be higher for runs with chemical permeation enhancers. The activation energy for transdermal permeation was also found lower for the runs with chemical permeation enhancers indicating existence of a lower energy barrier in the presence of chemical permeation enhancers. The method reported here is a simple and useful tool for studying the effect of heat on percutaneous absorption. Such temperature dependent enhancement of flux can be more pronounced at skin surface temperatures >45 °C. However, the use of such higher temperatures (>45 °C) for a long period of time, as employed in this study, may cause patient discomfort. This calls for further investigation into the effect of periodic increases in physiologically acceptable temperature on percutaneous absorption.

Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES

[1] Blank IH, Scheuplein RJ, Macfarlane DJ. Mechanism of percutaneous absorption III. The effect of temperature on the transport of non-electrolytes across the skin. J Invest Dermatol 1967;49:582–9.

[2] Lewis C. Sunning for science: the effects of common substances on sun-exposed skin. FDA Consum 2002;36:26–9.

[3] Fritsch WC, Stoughton RB. The effect of temperature and humidity on the penetration of acetylsalicylic acid-^{14}C in excised human skin. J Invest Dermatol 1963;41:307–12.

[4] Barry BW, editor. Effect of temperature. Dermatological formulations: percutaneous absorption. New York: Marcel Dekker; 1983. p. 158–60.

[5] Knutson K, Krill SL, Lambert WJ, Higuchi WI. Physicochemical aspects of transdermal permeation. J Controlled Release 1987;6:59–74.

[6] Golden GM, Donald BS, Guzek DB, Harris RR, Mc Kie JE, Potts RO. Lipid thermotropic transitions in human stratum corneum. J Invest Dermatol 1986;86:255–9.

[7] Ogiso T, Ogiso H, Paku T, Iwaki M. Phase transition of rat stratum corneum lipids by an electron spin resonance study and relationship of phase states to drug penetration. Biochim Biophys Acta 1996;1301:97–104.

[8] Petersen KK, Rousing ML, Jensen C, Nielsen LA, Gazerani P. Effect of local controlled heat on transdermal delivery of nicotine. Int J Physiol Pathophysiol Pharmacol 2011;3 (3):236–42.

[9] Tominaga K, Tojo K. Effect of environmental temperature on transdermal drug penetration. Biol Pharm Bull 2010;33 (12):1983–7.

[10] Shomaker TS, Zhang J, Love G, Basta S, Ashburn MA. Evaluating skin anaesthesia after administration of a local anaesthetic system consisting of an SCaine™ patch and a controlled heat-aided drug delivery (CHADD™) patch in volunteers. Clin J Pain 2000;16:200–4.

[11] Liu Q, Fassihi R. Zero-order delivery of a highly soluble, low dose drug alfuzosin hydrochloride via gastro-retentive system. Int J Pharm 2008;348:27–34.

[12] Pattnaik S, Swain K, Bindhani A, Mallick S. Influence of chemical permeation enhancers on transdermal permeation of alfuzosin: an investigation using response surface modeling. Drug Dev Ind Pharm 2011;37 (4):465–74.

[13] Akomeah F, Nazir T, Martin GP, Brown MB. Effect of heat on the percutaneous absorption and skin retention of three model penetrants. Eur J Pharm Sci 2004;21: 337–45.

[14] Nicoli S, Colombo P, Santi P. Release and permeation kinetics of caffeine from bioadhesive transdermal films. AAPS J 2005;7 [Article 20].

[15] Grass GM, Robinson JR. Mechanisms of corneal drug penetration. 1: In vivo and in vitro kinetics. J Pharm Sci 1988;77:3–14.

[16] Kawaju K, Midori Y, Shiono H, Ota A. Charaterization of the carrier mediated transport of levofloxacin, a fluoroquinolone antimicrobial agent, in rabbit cornea. J Pharm Pharmacol 1999;51:797–801.

[17] Pattnaik S, Swain K, Mallick S. Influence of polymeric system and loading dose on drug release from alfuzosin hydrochloride transdermal films. Lat Am J Pharm 2009;28:62–9.

[18] Swain K, Pattnaik S, Mallick S, Chowdary KA. Influence of hydroxypropyl methylcellulose on drug release pattern of a gastroretentive floating drug delivery system using a 3^2 full factorial design. Pharm Dev Technol 2009;14:193–8.

[19] Choi A, Gang H, Chun I, Gwak H. The effects of fatty acids in propylene glycol on the percutaneous absorption of alendronate across the excised hairless mouse skin. Int J Pharm 2008;357:126–31.

[20] Ponec M, Haverkort M, Soei YL, Kempenaar J, Brussee H, Bodde HE. Toxicity screening of n-alkylazacycloheptan-2-one derivatives in cultured human skin cells: structure–toxicity relationships. J Pharm Sci 1989;78:738–41.

[21] Kadir R, Ponec M, Tiemessen HLGM, Junginger HE, Boddé HE. Oleyl surfactants as skin penetration enhancers: effects on human stratum corneum permeability and in vitro toxicity to cultured human skin cells. In: Walters KA, Hadgraft J, editors. Pharmaceutical skin penetration enhancement, vol. 59. New York: Marcel Dekker, Inc.; 1993. p. 215–28.

[22] Nomura H, Fusao K, Sugimoto Y, Miyashita Y, Dohi M, Kato Y. Percutaneous absorption of indomethacin from mixtures of fatty alcohol and propylene glycol (FAPG base) through rat skin: effects of oleic acid added to FAPG base. Chem Pharm Bull 1990;38:1421–4.

[23] Ohara N, Takayama K, Nagai T. Influence of temperature on the percutaneous absorption for lipophilic and hydrophilic

drugs across the rat skin pretreated with oleic acid. Int J Pharm 1995;123:281–4.
[24] Ogiso T, Hirota T, Masahiro I, Hino T, Tadatoshi T. Effect of temperature on percutaneous absorption of terodiline and relationship between penetration and fluidity of stratum corneum lipids. Int J Pharm 1998;176:63–72.
[25] Clarys P, Alewaeters K, Jadoul A, Barel A, Manadas OR, Preat V. In vitro percutaneous penetration through hairless rat skin: influence of temperature, vehicle and penetration enhancers. Eur J Pharm Biopharm 1998;46:279–83.
[26] Tanojo H, Bouwstra JA. Subzero thermal analysis of human stratum corneum. Pharm Res 1994;11(11):1610–6.