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ORIGINAL ARTICLE

Influence of chemical permeation enhancers on transdermal permeation of alfuzosin: an investigation using response surface modeling

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Abstract

Context: A nonoral alternative such as transdermal system is desired to improve bioavailability and to maintain a constant and prolonged drug level with reduced frequency of dosing. *Objective*: The objective of the investigation is to develop a transdermal therapeutic system for alfuzosin hydrochloride and to study the influence of chemical permeation enhancers (CPEs) on the percutaneous permeation pattern. *Material and methods*: A D-optimal mixture design was used to study the influence of CPE with oleic acid (OA), lauric acid, and propylene glycol (PG) as mixture components. The influence of chemical enhancers on skin permeation was compared using one-way analysis of variance followed by multiple comparison analysis. Criterion of desirability was used to optimize the therapeutic system. Preclinical studies in rabbits were also carried out to establish an ex vivo–in vivo correlation (EVIVC). *Results*: The drug permeation pattern suggested Higuchian diffusion as predominant mode followed by case II to super case II transport as drug transport mechanism. The optimized formulation was obtained using 5% (w/w) CPE consisting of a blend of 62.41% OA and 37.59% PG. About twofold increase in alfuzosin permeation was achieved with the optimized transdermal patch. An approximate linear EVIVC was established (R^2 = 0.971). *Discussion*: The optimized blend of enhancers could improve skin permeation parameters. A higher extent of in vivo skin permeation compared with cadaver skin permeation may be due to more permeable nature of rabbit skin. *Conclusion*: The investigations suggest an effective alternative delivery strategy such as transdermal systems for alfuzosin hydrochloride.

Key words: D-optimal mixture design, permeation enhancers, polymeric system, preclinical studies, transdermal delivery

Introduction

The permeation through stratum corneum (SC) is the rate-limiting step for delivery of most of the drugs and this has led to considerable research toward different percutaneous permeation enhancement technologies[1]. Chemical permeation enhancement has been studied most extensively and is expected to play a leading role in the introduction of more transdermal products. Over the past three decades, much research has concentrated on studying the enhancing ability of a wide range of substances and their mechanisms of action. Chemical agents have been shown to enhance the permeation of drugs through the skin by either increasing the solubility of the drug in SC or disrupting the lipid matrix of SC or interacting with the intracellular protein[2].

Alfuzosin hydrochloride is an alpha-adrenergic receptor blocker approved by the FDA for the symptomatic treatment of benign prostatic hyperplasia (BPH). Currently, it is available as an immediate-release standard form (administered 2.5 mg thrice a day) and a 10-mg sustained-release once-daily dosage form[3]. The absolute bioavailability of alfuzosin is about 49% under fed condition, whereas the corresponding value under fasting condition is around 25%[3,4]. This shows that food has a significant impact on the oral absorption of alfuzosin. Moreover, because the therapy is palliative and is indicated for symptomatic management of BPH, alfuzosin is

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prescribed, usually, for a long period of time. This originates the need of an alternative nonoral route of administration for chronic therapy, which can bypass the hepatic first-pass metabolism. Transdermal route is an alternative choice of route of administration for such drugs. Transdermal patches offer added advantages such as maintenance of constant and prolonged drug level, reduced frequency of dosing, self-administration, and easy termination of medication, leading to patient compliance.

From our laboratory, we have already reported the optimum polymeric system and drug loading levels for passive transdermal delivery of alfuzosin hydrochloride[5]. In this study, we have analyzed the influence of chemical permeation enhancers (CPEs), oleic acid (OA; monounsaturated fatty acid, C_{18} with one double bond), lauric acid (LA; C_{12} saturated fatty acid), and propylene glycol (PG) on the permeation of alfuzosin hydrochloride through the human cadaver skin ex vivo. The percutaneous permeation enhancement of alfuzosin hydrochloride was compared with published passive permeation data[5]. Further, in vivo skin permeation profile of the developed optimized transdermal system was investigated in rabbits.

Materials

Alfuzosin hydrochloride (MW 425.9 and log *P*: 1.51) 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) was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Polyvinylalcohol (PVA, MW 125,000), OA, LA, and PG were purchased from SD Fine-Chem. Ltd., Boisar, India. Di-*n*-butylphthalate was purchased from Central Drug House (Pvt.) Ltd., Mumbai, India. All the chemicals were used as received without any further purification.

Methods

Design of experiment to study influence of CPE

A D-optimal mixture design was used to study the influence of CPEs on permeation pattern of alfuzosin hydrochloride. In a mixture experiment, which is suitable for pharmaceutical formulations, the independent factors are the components of a mixture and the response is dependent on the relative proportions of each ingredient[6]. The method involves changing mixture composition and exploring how such changes will affect the properties of the mixture[7]. Unlike standard and classical design of experiments (DOEs) such as factorials and fractional factorials, D-optimal design matrices are usually not orthogonal and effect estimates may be correlated. These types of designs are always an option regardless of the type of model the researcher wishes to fit. In this investigation, fraction of OA (X_1), fraction of LA (X_2), and fraction of PG (X_3) were selected as mixture components. The cumulative amount of alfuzosin hydrochloride permeated per square centimeter of human cadaver skin at 24 hours (P_{24}) and permeation flux (J) were chosen as dependent response variables. In a mixture design, the level of a single mixture component cannot be changed independently[8] and the sum of the mixture components has to be equal to 100%[9]. The restrictions imposed on the mixture component proportions are as follows:

$$0\% \le X_1 \le 100\%; 0\% \le X_2 \le 100\%; 0\% \le X_3 \le 100\%$$
$$\text{and} \quad X_1 + X_2 + X_3 = 100\%.$$

Design-Expert® software (version 7.1.4, Stat-Ease Inc., Minneapolis, MN, USA) was used for the generation and evaluation of the statistical experimental design.

Fabrication of matrix-type transdermal patches

We have already reported transdermal systems with the optimum levels of polymeric system and drug loading dose (ratio of EC : PVP = 10 : 90 with 50% drug loading) and fabrication method of the same[5]. In brief, experimental transdermal films were prepared at all possible combinations incorporating 5% (w/w) CPEs (Table 1). Films composed of EC- and PVP-containing alfuzosin hydrochloride (1.31 mg/cm^2 film) were prepared by solvent evaporation technique. Di-*n*-butylphthalate was incorporated as a plasticizer at a concentration of 30% (w/w) of dry weight of polymers. Alfuzosin hydrochloride was dissolved in chloroform followed by the addition of polymers, plasticizer, and permeation enhancers with constant stirring. The matrix was prepared by pouring the homogeneous dispersed solution on 4% PVA backing membrane in a flat-bottomed Petri dish, covered with perforated aluminum foil, and dried at 40°C for 24 hours. The dry films with drug dissolved in the matrix were removed and kept in desiccators until use.

Preparation of human cadaver skin for permeation studies

Human cadaver skin samples from abdominal region of male young adults (about 30 years) were obtained from the Forensic Medicine and Toxicology Department of MKCG Medical College, Berhampur, India. The skin samples were collected in formalin solution and used immediately without storing for a prolonged period (skin samples were used within 24 hours of procurement).

Subcutaneous fat was carefully removed from the skin sample using forceps and a scalpel. Following removal of subcutaneous fat, individual portions of skin were immersed in water at 60°C for 3 minutes[10]. The skin was then pinned, dermis side down, on a wax board and the epidermis (comprising SC and epidermal layer) gently removed from the underlying dermis. The latter was discarded and the epidermal membrane floated onto the surface of water and was taken up on a Whatman filter

Table 1. Formulation of the transdermal patches.

Run	EC : PVP	ALF[a] (%)	DBP[b] (%)	Enhancer blend composition OA : LA : PG	Formulation code
1	10 : 90	50	30	0 : 0 : 1	$FO_0L_0G_1$
2	10 : 90	50	30	0 : 1 : 1	$FO_0L_1G_1$
3	10 : 90	50	30	0 : 1 : 0	$FO_0L_1G_0$
4	10 : 90	50	30	1 : 1 : 4	$FO_1L_1G_4$
5	10 : 90	50	30	1 : 4 : 1	$FO_1L_4G_1$
6	10 : 90	50	30	1 : 1 : 1	$FO_1L_1G_1$
7	10 : 90	50	30	1 : 1 : 0.4	$FO_1L_1G_{0.4}$
8	10 : 90	50	30	1 : 1 : 0	$FO_1L_1G_0$
9	10 : 90	50	30	1 : 0 : 1	$FO_1L_0G_1$
10	10 : 90	50	30	4 : 1 : 1	$FO_4L_1G_1$
11	10 : 90	50	30	1 : 0 : 0	$FO_1L_0G_0$
Control	10 : 90	50	30	0 : 0 : 0	F

[a]Percentage of total polymer (EC/PVP) weight. [b]Percentage of total polymer (EC/PVP) weight.

paper (Whatman International Ltd., Maidstone, England). The resultant epidermal sheets were thoroughly dried and stored flat in aluminum foil at 4–8°C until use[11]. The mean thickness of the prepared epidermal sheets was 81.40 ± 2.37 μm. Utmost care was exercised during fat tissue removal not to damage the skin samples. The integrity of the skin samples, after removal of fat tissue, was assessed carefully under a microscope[12]. The skin samples, prior to permeation studies, were placed in contact with normal saline (receptor media) for a period of 24 hours to allow any water-soluble UV-absorbing materials to leach from the skin[13]. At least two changes of the receptor phase were made during this period.

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 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 32 ± 0.5°C. Owing to higher aqueous solubility of alfuzosin HCl, normal saline has been chosen as the receptor fluid[5]. The diffusional area (cross-section area) of the diffusion cell was 1.766 cm^2. The receptor fluid was constantly agitated at 100 rpm by a teflon-coated magnetic bead. The film 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 this study to minimize variability in results. The whole of the receptor fluid was collected from the sampling port at predetermined time intervals 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 patch control system to avoid the influence of inherent extracts from the skin or leaching of any material from the film without drug on absorbance at 242 nm, at which the sample aliquots were analyzed spectrophotometrically. The amount of drug permeated per square centimeter at each time interval was estimated and subjected to further data analysis.

Permeation data analysis and statistics

The concentration of the permeant in the receptor solution was corrected for previous sample removal. The cumulative amount of permeant, P (μg/cm^2), which permeated the skin per unit surface area, was plotted against time. The slope of linear portion of the plot was taken as being the steady-state flux (J) (μg/cm^2/h).

Permeation kinetic model fitting

Several mathematical models[14] can be used to describe the kinetic behavior of the drug release mechanism from matrix systems, the most suitable being the one that best fits the experimental results. The choice of a specific model for a particular dataset depends on the shape of the plot obtained, as well as on the underlying mechanism. The kinetics of alfuzosin release from transdermal matrix systems was determined by finding the best fitting of the data to distinct models such as zero order, first order, and Higuchi as follows:

$$Q_t = Q_0 + k_0 t \quad (1)$$

where Q_t is the amount of drug released at time t, Q_0 is the amount of drug in the solution at $t = 0$ (usually, $Q_0 = 0$), and k_0 is the zero-order release rate constant.

$$Q_t = Q_\infty (1 - e^{k_1 t}) \quad (2)$$

Q_∞ being the total amount of drug in the matrix and k_1 is the first-order kinetic constant.

$$Q_t = k_H t^{1/2} \quad (3)$$

where k_H represents the Higuchi rate constant.

Moreover, to better characterize the drug release behavior for the polymeric systems, under study, and particularly to gain some insight into the corresponding mechanism, the Korsmeyer–Peppas semiempirical model was applied:

$$\frac{Q_t}{Q_\infty} = kt^n \tag{4}$$

where Q_t/Q_∞ is the fraction of drug released at time t, k is a constant comprising the structural and geometric characteristics of the matrix, and n, the release exponent, is a parameter that depends on and is used to characterize the release mechanism. For the case of a slab, in particular, $n = 0.5$ corresponds to a Fickian diffusion release (case I diffusional), $0.5 < n < 1$ to an anomalous (non-Fickian) transport, $n = 1$ to a zero-order (case II) release kinetics, and $n > 1$ to a super case II transport.

The best-fit model was determined statistically employing comparison of coefficient of determination (R^2). The preparation of graphs and statistical calculations were carried out with the help of Microsoft Excel® software.

In vivo percutaneous absorption studies in rabbits

The mass balance technique[15] was employed for indirect estimation of in vivo alfuzosin permeation from the transdermal patches in rabbits. The Institutional Animal Ethics Committee of College of Pharmaceutical Sciences, Berhampur, India (Regd. No. 1170/ac/08/CPCSEA), approved all experiments with animals. Male albino rabbits of either sex weighing 1.5–2.5 kg were used for the experiments. Animals were allowed to be acclimatized for a period of 1 week in our laboratory environment prior to this study and maintained under controlled conditions of temperature as well as humidity and had free access to water and food. The instructions given by our institutional animal ethical committee were followed throughout the experiment to ensure good laboratory practice.

Four healthy rabbits were selected for this study. One day before the experiment, hair on the abdominal area was clipped by a scissor and washed with distilled water. In the first treatment period, the transdermal films (5.0 cm^2) were applied on hair-free abdominal skin of each rabbit and occluded with Leucoplast®[16]. The patches were removed after 3 hours and the drug content in the patches was estimated. A cotton swab was used to extract any adhering drug particles on the application site. Drug content was determined by dissolving the films and shaking the cotton swab in chloroform and filtering with Whatman filter paper (0.45 μm). The filtrate was evaporated and the drug residue was dissolved in normal saline. The drug content was analyzed at 242 nm using a UV spectrophotometer (Shimadzu, Kyoto, Japan). After a washout period of 1 week, the second treatment was carried out. The same procedure in the same animals, as in first treatment period, was followed in the second treatment period but the patches were applied for 6 hours. The drug content was analyzed at the end of the treatment period. Similarly, in subsequent treatments the patches were applied for 8, 10, 12, and 24 hours with a washout period of 1 week between treatment periods. Thus, the amount of drug remaining unabsorbed at the end of the treatment period was estimated. The amount of drug permeated into the skin was calculated by subtracting the estimated unabsorbed amount from the initial known amount of drug in the patch. The calculated amount of drug permeated was plotted against time.

Ex vivo–in vivo correlation

Drug permeated in vivo at various time points were plotted against cumulative amounts of drug permeated, ex vivo at same time points. A correlation was established between the ex vivo permeation profile of the optimized formulation and the in vivo permeation pattern.

Results and discussion

In this investigation, a model mono-unsaturated fatty acid (OA), a saturated fatty acid (LA), and a glycol (PG) were employed as permeation enhancers. Effects of the mixture components (CPEs) on the ex vivo drug permeation from the transdermal patches were studied by statistical experimental design. The experimental design has been widely used in pharmaceutical field to study the effect of formulation variables and their interactions on response variables[5,17–19]. The purpose of response surface methodology is to obtain a model allowing to understand as fully as possible the effects of the factors and their levels, over the whole of the experimental domain, and also to predict the response inside this domain. Moreover, it can be used for optimizing a process (i.e., maximizing one or more of the responses, keeping the remainder within a satisfactory range), carrying out simulations with the model equation and plotting the responses[20].

Influence of CPE

A D-optimal mixture design (Table 2) was used with fraction of OA (X_1), fraction of LA (X_2), and fraction of PG (X_3) as mixture components to study the influence of CPE. 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 3). Cubic model has terms that are aliased and hence that model was not selected. Based on statistical analysis such as adjusted multiple correlation coefficient (adjusted R^2) and predicted residual sum of squares (PRESS), a special cubic model was fitted to the data ($p < 0.05$) for interpreting data results for the permeation responses, P_{24} and J (Table 4). Analysis of variance (ANOVA) was applied to estimate the significance of the model at the

Table 2. Composition and observed responses from runs in D-optimal mixture design.

	Mixture components			Observed responses[a]		
Run	X_1 (% OA)	X_2 (% LA)	X_3 (% PG)	P_{24} (μg/cm^2)	J (μg/cm^2/h)	ER_{flux}
1	0.000	0.000	100.000	172.33 ± 2.13	8.04 ± 0.88	1.05 ± 0.08
2	0.000	50.000	50.000	245.71 ± 2.64	10.67 ± 0.79	1.39 ± 0.06
3	0.000	100.000	0.000	237.75 ± 1.58	10.30 ± 0.67	0.35 ± 0.05
4	16.667	16.667	66.667	192.67 ± 2.08	8.75 ± 0.82	1.14 ± 0.07
5	16.667	66.667	16.667	244.13 ± 1.77	10.62 ± 0.66	1.39 ± 0.05
6	33.333	33.333	33.333	206.61 ± 1.49	9.14 ± 0.64	1.19 ± 0.05
7	41.667	41.667	16.667	267.52 ± 3.16	11.58 ± 0.92	1.51 ± 0.08
8	50.000	50.000	0.000	258.42 ± 2.61	11.12 ± 0.43	1.45 ± 0.02
9	50.000	0.000	50.000	332.11 ± 3.68	14.42 ± 0.74	1.89 ± 0.05
10	66.667	16.667	16.667	307.91 ± 2.51	13.18 ± 0.66	1.73 ± 0.04
11	100.000	0.000	0.000	296.43 ± 1.79	12.65 ± 0.83	1.66 ± 0.06
Control	–	–	–	167.21 ± 2.05	7.62 ± 0.19	1.00

[a]Data shown are mean ± standard error of the mean (n = 4).

Table 3. Model analysis by sequential model sum of squares.

	P_{24}		J	
Source	Sum of squares	p-Value	Sum of squares	p-Value
Mean versus total	1.023E + 006	–	1949.02	–
Linear versus mean	20,427.93	0.0080	30.15	0.0123
Quadratic versus linear	10,110.95	0.0418	17.47	0.0354
Special cubic versus quadratic	4869.86	0.0068	7.88	0.0069
Cubic[a] versus special cubic	2722.13	0.0070	4.34	0.0086
Residual	870.41	–	1.50	–
Total	1.062E + 006	–	2010.35	–

[a]Cubic model is aliased and hence not to be selected.

Table 4. D-optimal mixture model summary statistics.

	P_{24}		J	
Source	Adjusted R^2	PRESS	Adjusted R^2	PRESS
Linear	0.4505	26,275.65	0.4133	44.16
Quadratic	0.6745	16,351.70	0.6645	26.44
Special cubic	0.8465	33,510.06	0.8414	55.09
Cubic[a]	0.9522	21,391.62	0.9476	36.87

[a]Aliased model not to be selected.

5% significance level. The mathematical model generated by the design for the responses are as follows.

Special cubic model (for P_{24} and J)

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{123}X_1X_2X_3$$

where Y is the dependent variable, b_i (b_1, b_2, b_{12}, b_{23}, and b_{13}) is the estimated coefficient for the corresponding component X_i (X_1, X_2, X_3, X_1X_2, X_2X_3, X_1X_3, and $X_1X_2X_3$). The interaction terms (X_1X_2, X_2X_3, X_1X_3, and $X_1X_2X_3$) show how the response changes when the components are changed simultaneously.

Final equation in terms of real components

$$P_{24} = +300.68079\,X_1 + 239.68779\,X_2 + 166.40310\,X_3 + 35.74632\,X_1X_2 + 384.23831\,X_1X_3 + 151.37231\,X_2X_3 - 914.77645\,X_1X_2X_3$$

$$J = +12.82174\,X_1 + 10.38504\,X_2 + 7.80602\,X_3 + 1.42567\,X_1X_2 + 16.00264\,X_1X_3 + 5.55724\,X_2X_3 - 77.01272\,X_1X_2X_3$$

The coefficient estimate and standardized main effects (SMEs) for the responses are listed in Table 5. SME values were calculated by dividing the main effects by the standard error of the main effects. In addition, three-dimensional response surface plots were presented to estimate the effects of the mixture components on each response. Results of multiple regression analysis and SMEs revealed that CPEs (X_1, X_2, and X_3) had statistically significant influence on all dependent variables ($p < 0.05$, Table 5).

The influence of mixture components on P_{24} and J is evident from the three-dimensional response surface plots (Figures 1 and 2). It appeared that the amount of drug permeated at 24 hours (P_{24}) and steady-state permeation flux were affected more by the fraction of OA, which was cleared from the response surface plots (Figures 1 and 2). The higher SME value (21.67 and 22.89, respectively, for P_{24} and J) of X_1 indicated that the effect of fraction of OA was found to be the main influential factor on the measured responses followed by LA and PG having lower SME values. From our laboratory we have already reported encouraging percutaneous enhancement of ondansetron hydrochloride using fatty acids including OA at levels up to 5% (w/w) and the formulation was found dermatologically safe[19]. OA is an unsaturated fatty acid with a *cis* configuration. The bent

Table 5. Standardized main effects of the factors on the measured responses.

Coefficient of regression parameter	Measured responses					
	P_{24}			J		
	Coefficient estimate	95% confidence interval	SME[a]	Coefficient estimate	95% confidence interval	SME[a]
b_1	300.68	269.30 to 332.06	21.67	12.82	11.56 to 14.09	22.89
b_2	239.69	208.31 to 271.07	17.28	10.39	9.12 to 11.65	18.55
b_3	166.40	135.02 to 197.79	11.99	7.81	6.54 to 9.07	13.94
b_1b_2	35.75	–158.52 to 230.01	0.41	1.43	–6.41 to 9.26	0.41
b_1b_3	384.24	228.42 to 540.06	5.57	16.00	9.72 to 22.28	5.75
b_2b_3	151.37	–4.45 to 307.19	2.19	5.56	–0.72 to 11.84	2.00
$b_1b_2b_3$	–1914.78	–3154.89 to –674.66	–3.49	–77.01	–127.00 to –27.02	–3.48

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

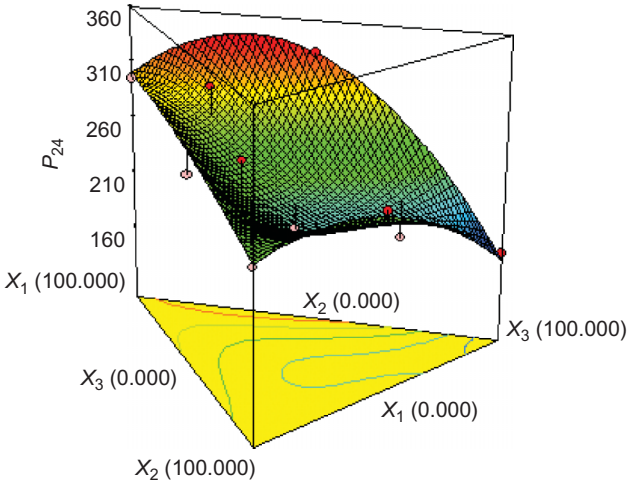

Figure 1. Three-dimensional response surface plot for cumulative amount drug released at 24 hours (P_{24}) indicating the effect of the mixture components.

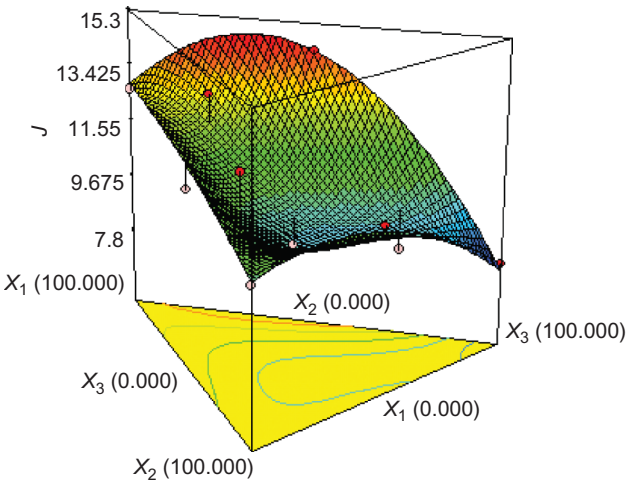

Figure 2. Three-dimensional response surface plot for steady-state permeation flux (J) indicating the effect of the mixture components.

cis configuration of OA is expected to disturb intercellular lipid packing more so than the saturated straight chain LA or PG[21]. This explains the higher P_{24} and J values with OA compared with the other two CPEs. It was found that both P_{24} and J values increased with increase in fraction of mixture components. Among interactions of mixture components, it was observed that both the dependent variables were affected more by interaction of X_1 and X_3 (i.e., fraction of OA and PG), which was indicated by the higher SME value of 5.57 and 5.75 for P_{24} and J, respectively. The highest amount of drug permeated at 24 hours (332.11 ± 3.68 μg/cm^2) and steady-state permeation flux (14.42 ± 0.74 μg/cm^2/h) was observed with a blend of the mixture components consisting of 50% each of OA and PG (run 9). PG is known to have relatively low skin cell toxicity[22,23] and has been widely used for formulation of transdermal delivery systems. It was suggested that the probable permeation enhancing mechanism of PG is by solvating alpha-keratin and occupying hydrogen-bonding sites, thus reducing drug/tissue binding[24]. On the contrary, fatty acids are known to be enhancers with lipophilic properties, and many studies have shown that the skin permeability enhancing effects of fatty acids are greatest along with PG[25–27]. The enhancing effect by the addition of fatty acids to PG has been widely studied, and the binary system was considered to disorganize the multilaminate hydrophilic–lipophilic layers located intercellularly in the SC, consequently promoting percutaneous absorption of drugs[28].

To examine the influence of CPEs on permeation patterns of alfuzosin hydrochloride, patches without incorporating CPE (control) were fabricated and the permeation pattern[5] was compared with patches containing permeation enhancers (Figure 3). Transdermal flux values obtained for all conditions were statistically compared performing a one-way ANOVA, followed by a multiple comparison test such as Holm–Sidak test at an overall significance level of 0.05 using Sigma Stat software (Sigma Stat 3.5, SPSS Inc., Chicago, IL, USA). For this purpose, the permeation enhancing activities, expressed as enhancement ratio of flux (ER_{flux}), were calculated as the ratio between the flux value obtained with the CPE and that observed with the control. The results are shown in Figure 4. One-way ANOVA followed by

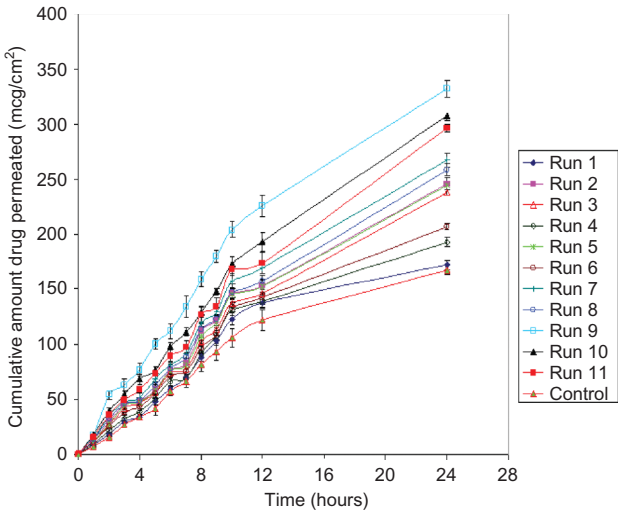

Figure 3. Ex vivo permeation profile of transdermal patches demonstrating the influence of chemical permeation enhancers. Error bars indicate standard error of the mean.

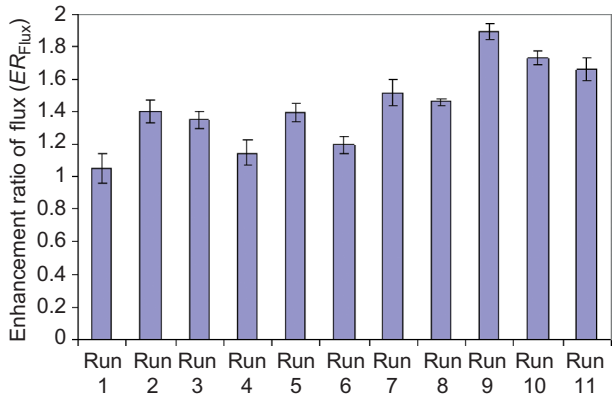

Figure 4. Enhancement ratio of flux (ER_{flux}) for the different runs. Error bars indicate standard error of the mean.

multiple comparisons versus control (Holm–Sidak method) detected statistically significant difference ($p < 0.05$) in ER_{flux} except run 1 when all the runs were compared with control run.

Maximum increase in steady-state permeability flux (J) was observed with run 9 (patches containing chemical enhancer blend consisting of 50% each of mixture components OA and PG). It was also observed that the efficacy of blend of permeation enhancers is higher than either of the permeation enhancer investigated in this study. A statistically significant difference in the permeation parameters of alfuzosin hydrochloride in different formulations was observed when compared by using one-way ANOVA followed by all pair-wise Holm–Sidak test ($p < 0.05$).

The drug permeation data were fitted to various kinetic models such as zero order, first order, Higuchi, and Korsmeyer–Peppas. The corresponding coefficients of determination (R^2) values are presented in Table 6. R^2

Table 6. Permeation kinetic model fitting.

Models / Formulation	Zero order R^2	First order R^2	Higuchi R^2	Korsmeyer-Peppas R^2	Korsmeyer-Peppas n
$FO_0L_0G_1$	0.886	0.894	0.943	0.971	1.032
$FO_0L_1G_1$	0.961	0.971	0.972	0.984	0.948
$FO_0L_1G_0$	0.968	0.976	0.974	0.988	0.962
$FO_1L_1G_4$	0.917	0.926	0.959	0.977	1.014
$FO_1L_4G_1$	0.962	0.971	0.972	0.985	0.959
$FO_1L_1G_1$	0.938	0.948	0.971	0.983	0.951
$FO_1L_1G_{0.4}$	0.964	0.974	0.973	0.984	0.929
$FO_1L_1G_0$	0.971	0.980	0.974	0.986	0.936
$FO_1L_0G_1$	0.944	0.962	0.983	0.973	0.909
$FO_4L_1G_1$	0.973	0.984	0.980	0.992	0.910
$FO_1L_0G_0$	0.976	0.985	0.973	0.989	0.936
F[a]	0.924	0.933	0.970	0.979	1.065

[a]Formulation without enhancer as control.

is a statistic that will give information about the goodness of fit of a model. In regression, the R^2 (coefficient of determination) is a statistical measure of how well the regression line approximates the real data points. An R^2 of 1.0 indicates that the regression line perfectly fits the data. In this study, R^2 statistics is utilized to determine the goodness of fit of the model. The R^2 statistics clearly indicated that the data best fit to Higuchian diffusion model (Table 6). Alfuzosin hydrochloride is freely soluble in the receptor media. Thus, both diffusion and erosion could contribute to the drug release process from the patches. In fact, it is well known that in polymeric hydrophilic matrices similar to the ones considered, water-soluble drugs are released mainly by diffusion across the gel layer. The mechanism of release from polymeric matrix systems is complex and is not completely understood. Even if some processes could be characterized as either purely diffusional or purely erosion controlled, several others could only be rationalized as being due to a coupling of both. The use of the Korsmeyer–Peppas equation, and particularly the interpretation of the release exponent values (n), allows getting insight into the balance between these mechanisms.

The diffusion exponent values obtained after fitting the permeation data to Peppas kinetic model were found to range from 0.909 to 1.032 indicating case II to super case II transport as the mechanism of drug release from the matrix-type transdermal systems possibly owing to chain disentanglement and swelling of polymeric system.

Optimization of formulation

The aim of the optimization was to obtain the defined targets for the responses simultaneously with respect to the predefined constraints. The optimum values of the mixture components were obtained by numerical analyses using the Design-Expert® software and based on the criterion of desirability[29]. It optimizes any combination of one or more goals and the goals may apply to either factors or responses. The goals are combined into an

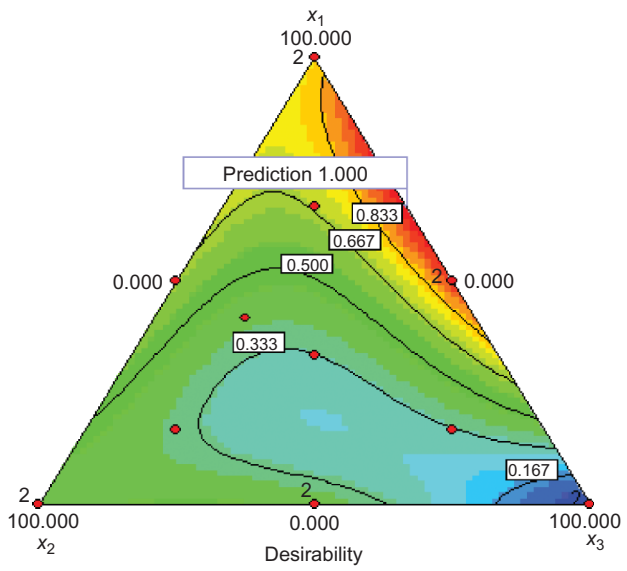

Figure 5. Desirability plot showing the predicted region of optimum goal response.

overall desirability function. The program seeks to maximize this function. The goal seeking begins at a random starting point and proceeds up the steepest slope to a maximum. There may be two or more maximums because of curvature in the response surfaces and their combination into the desirability function (Figure 5). By starting from several points in the design space, chances improve for finding the 'best' local maximum. In our study, a desired goal was established to maximize the dependent permeation responses P_{24} and J. At this stage, the defined desirable areas of three responses were superimposed and the region of interest was generated. The software suggested 14 solutions with high desirability (0.456–1.0) and the optimized formulation predicted was with 62.41% OA and 37.59% PG. Transdermal patches with the predicted optimum levels of formulation variables were fabricated and analyzed to validate the optimization procedure. Comparative values of predicted and observed responses along with the formulation components are reported in Table 7, which demonstrated that the observed values of a new batch were mostly similar with predicted values within 5% of predicted error.

In vivo percutaneous absorption studies in rabbits

Mass balance technique is an alternative noninvasive method for assessment of in vivo drug permeation. The amount of drug permeated into the animal skin was calculated by subtracting the unabsorbed amount of drug outside the skin surface (amount in patch and amount on skin surface) from the known initial amount of drug in the applied patch. A comparison of permeation profiles obtained in the ex vivo and in vivo permeation studies is presented in Figure 6. It has been observed that the in vivo permeation values (396.24 ± 14.37 μg/cm^2 at 24 hours) are higher than the ex vivo permeation values (344.82 ± 3.24 μg/cm^2 at 24 hours) at corresponding time points. It has been reported elsewhere that rabbit skin is more permeable to permeants and yields permeation values much greater than those seen in humans[15]. Though the permeability of drugs through rabbit skin is higher than human skin, preclinical studies in rabbits serve as a screening tool for further clinical studies in healthy human subjects.

A key goal in development of pharmaceutical dosage forms is a good understanding of the in vitro/ex vivo and in vivo performance of the dosage forms. One of the challenges of biopharmaceutics research is correlating in vitro/ex vivo drug release information of various drug formulations to the in vivo drug profiles (EVIVC). Thus, the need for a tool to reliably correlate in vitro and in vivo drug release data has exceedingly increased. Such a tool shortens the drug development period, economizes the resources, and leads to improved product quality[30]. The main objective of an in vitro–in vivo correlation is to serve as a surrogate for in vivo bioavailability and to support biowaivers.

In this work, in vivo drug permeation profile was attempted to correlate to ex vivo cadaver skin permeation

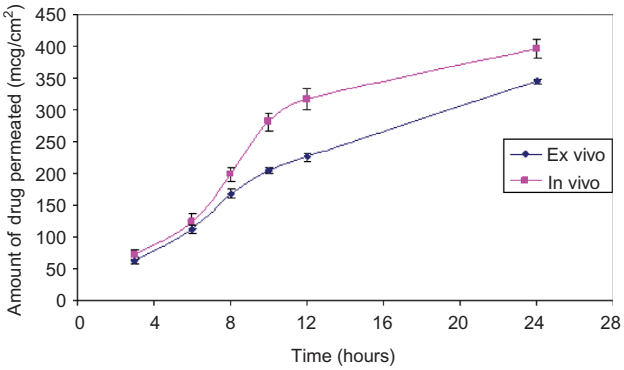

Figure 6. Ex vivo and in vivo permeation profiles of alfuzosin hydrochloride. Error bars indicate standard error of the mean.

Table 7. Optimized levels for formulation variables and comparative values of predicted and observed responses for numerically optimized formulation.

	Mixture components			Goal response					
				P_{24} (μg/cm^2)			J (μg/cm^2/h)		
Formulation	X_1 (%)	X_2 (%)	X_3 (%)	Observed[a]	Predicted	Predicted error[b] (%)	Observed[a]	Predicted	Predicted error[b] (%)
Optimized	62.41	0	37.59	344.82 ± 3.24	340.34	1.31	14.87 ± 0.83	14.69	1.22

[a]Data shown are mean ± standard error of the mean (n = 4). [b]Predicted error (%) = (observed value–predicted value)/predicted value × 100.

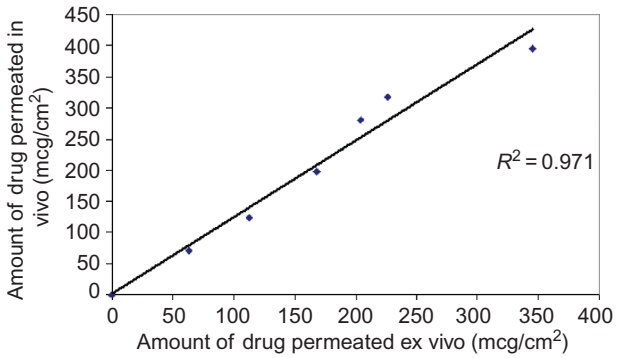

Figure 7. Correlation between ex vivo and in vivo performance.

pattern. A relationship (Figure 7) was established between the ex vivo and the in vivo data using Sigma Stat® software. The equation that best explained (R^2 = 0.971) the relation (approximately linear) is as follows:

$$Y = 1.2294X + 2.0188.$$

Conclusion

The influence of CPEs on ex vivo transdermal permeation of alfuzosin hydrochloride was investigated employing statistical DOE. The optimized formulation was obtained using 5% (w/w) CPE consisting of a blend of 62.41% OA and 37.59% PG using a D-optimal mixture design and the observed responses are found close to the predicted responses. Experimentally observed cumulative amount of alfuzosin permeated at 24 hours (P_{24}) and steady-state permeation flux (J) were found to be 344.82 ± 3.24 μg/cm^2 and 14.87 ± 0.83 μg/cm^2/h, respectively. Hence, about twofold increase in alfuzosin permeation was achieved with the optimized transdermal patch. A relationship was established between the ex vivo and the in vivo data. Though the in vivo method adopted in this study is not a superior method to direct assessment of plasma drug concentration, this will spare the animals from painful invasive procedures. At the same time, the method reported here may be utilized at resource-poor setup in the absence of bioanalytical facilities.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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