Response Surface Methodology to Investigate the Iontophoretic Delivery of Tacrine Hydrochloride

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Purpose. The objective of this work was to apply response surface approach to investigate the main and interaction effects of delivery parameters for iontophoretic delivery of tacrine HCl *in vitro*.

Methods. Iontophoresis was used to deliver tacrine HCl across rat skin. Experiments were performed according to Box-Behnken design to evaluate effects of drug concentration (X_1), current density (X_2), and donor buffer molarity (X_3) on cumulative drug delivered in 24 h (Y_1), 6 h (Y_2), iontophoretic flux (Y_3), and post-iontophoretic flux (Y_4).

Results. Mathematical model for Y_1 was $Y_1 = 0.653 + 0.163 * X_1 + 0.456 * X_2 - 0.156 * X_3 + 0.190 * X_1X_2 + 0.139* X_3X_3$. Response surface plot indicated that at low level of X_2 (0.1mA/cm^2), X_1 had little effect on Y_1. However, at high level of X_2 (0.5 mA/cm^2), Y_1 significantly increased from 0.75 mg/cm^2 to 1.46 mg/cm^2 when X_1 increased from 1% to 9%. Regression equations predicted responses for Y_1 to Y_4, for optimal formulation, which were in reasonably good agreement with experimental values.

Conclusions. Experimental design methodology revealed an interaction between drug concentration and current density, which would have been difficult to predict from one factor at a time classic experimental approach.

KEY WORDS: iontophoresis; response surface; tacrine hydrochloride; transdermal delivery.

INTRODUCTION

Tacrine (1,2,3,4-tetrahydro-5 aminoacridine) is a potent, centrally active, reversible cholinesterase inhibitor (1) used to treat the symptoms of mild to moderate dementia of Alzheimer disease (2). It is one of the five drugs currently approved by the FDA for the treatment of Alzheimer disease. However, its oral administration has been associated with extensive first-pass metabolism and rapid clearance from the systemic circulation. A low peroral bioavailability and large interindividual differences have been reported in man (3,4).

A potential alternative route for tacrine delivery could be the transdermal route. Advantages with transdermal delivery of tacrine are reduced first-pass hepatic metabolism, improved therapy by providing fairly constant blood levels for extended period of time unlike conventional oral dosage forms, and potential minimization of the occurrence of gastrointestinal side effects and hepatotoxicity, which is associated with peroral administration. However, passive delivery of tacrine through human epidermis was not able to deliver sufficient amounts to be in the therapeutic range (5). In the current work, iontophoresis was used to assist the delivery of tacrine into and through the skin. Iontophoresis enhances the delivery of drug through the skin by applying an external electric field across the skin. It uses an electrode of the same polarity as the drug to drive the drug into the skin by electrorepulsion and electroosmosis. Electroosmosis is a current-induced convective solvent flow. When the skin is buffered at a physiologic pH of 7.4, it acquires a net negative charge, causing electro-osmotic flow to occur from anode to cathode during iontophoresis. This aids in enhancing the anodic delivery of positively charged and neutral solutes (6). Iontophoretic drug transport occurs mainly through existing pathways such as sweat ducts and hair follicles. However, it can also cause increased skin permeability through the creation of new pathways, but this is seen as a secondary mechanism of delivery (7).

Iontophoresis is a complex process governed by a multitude of factors. These factors mainly include physicochemical factors like drug concentration, molecular size, and strength of the donor buffer and electrical factors like current density and mode of current used. Each of these factors has its own influence on the amount of drug delivered across the skin (8). These factors might also interact, influencing the amount of drug delivered. The relative importance of each factor needs to be assessed.

In the current work, the influence of various factors, namely drug concentration, current density, and donor buffer molarity, on the iontophoretic delivery of tacrine HCl were investigated using response surface methodology. Iontophoresis was performed *in vitro* across full-thickness rat skin. An experimental design approach was used in the study, in contrast to a one-factor-at-a-time classic experimental approach. The advantage of using this experimental design method included reduction in the number of experiments, identification of interaction between factors, detection of the optimal response within the experimental region, and empirical modeling of the data. The optimal iontophoretic drug delivery parameters, providing the maximal transdermal delivery, were then tested across human dermatomed skin to determine the feasibility of optimized formulation conditions to achieve clinically significant levels of tacrine.

MATERIALS AND METHODS

Experimental Design

Experiments were performed according to a three-factor, three-level Box-Behnken design. This design is suitable for exploring quadratic response surfaces and permits the development of a polynomial model. In this design, the experimental region is assumed to be a cube, and experiments are performed at points corresponding to midpoint of each edge and replicated experiments at the center of this multidimensional cube. The second-order polynomial model generated by the Box-Behnken design is of the form: $Y = B_o + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_1X_2 + B_5X_2X_3 + B_6X_1X_3 + B_7X_1^2 + B_8X_2^2 + B_9X_3^2$, in which Y is the measured response associated with each factor-level combination; X_1, X_2, and X_3 are the factors studied; B_o is an intercept; B_1 to B_9 are the regression coefficients (9).

The independent factors and the dependent responses used in the study are listed in Table I. The matrix of the

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Table I. Variables in Box-Behnken Design

Factor	Independent variable		
	Low level (–1)	Middle level (0)	High level (+1)
X_1: drug concentration	1%	5%	9%
X_2: current density	0.1 mA/cm^2	0.3 mA/cm^2	0.5 mA/cm^2
X_3: donor buffer molarity	25 mM	100 mM	175 mM
	Dependent variable		
	Response		
	Y_1 = cumulative drug delivered in 24 h		
	Y_2 = cumulative drug delivered in 8 h		
	Y_3 = iontophoretic flux		
	Y_4 = post-iontophoretic flux		

Box-Behnken design is depicted in Table II. Each row in the matrix identifies an experiment, and each experiment provides a result (response). The levels of the factors studied were chosen so that their relative difference was adequate to have a measurable effect on the response, along with the information that the selected levels are within practical use. The constant and regression coefficients were calculated using commercial software (Statgraphics Plus, Version 5, Manugistics, Rockville, MD, USA). The polynomial equations from this optimization technique were used to predict the iontophoretic delivery of tacrine HCl in the experimental region. Comparison of the predicted values for Y_1, Y_2, Y_3, and Y_4 with the experimental data was also used to test the validity of the response surface models.

Materials

Tacrine HCl was obtained from Sigma (St. Louis, MO, USA). Monobasic sodium phosphate, dibasic sodium phosphate, sodium chloride, triethylamine, and High Performance Liquid Chromatography (HPLC) grade acetonitrile and water were purchased from Fisher Scientific (Pittsburgh, PA, USA). All the chemicals were used as received.

In Vitro Permeation Studies

Full-thickness abdominal skin was freshly excised from hairless rats and mounted on the Franz diffusion cells. This research adhered to the "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, revised 1985). Iontophoretic experiments (n = 3) were performed according to the Box-Behnken design, varying the levels of factors, that is, drug concentration (1%, 5%, and 9%), current density (0.1, 0.3, and 0.5 mA/cm^2), and strength of donor buffer (25, 100, and 175 mM) as shown in Table II. The studies were carried out at 37°C. The donor compartment contained the drug solution in phosphate buffer, pH 7.4. The receptor compartment was filled with plain phosphate buffer. Tacrine is a basic drug with a pKa of 10 and has a positive charge at a pH of 7.4 (10). It was thus delivered under the positively charged anode. The anode (silver wire) was placed in the donor solution and the cathode (silver/silver chloride matrix) in the receptor. Current was applied for a period of 6 h. However, sampling was continued for 24 h to see if enhanced delivery would stop once the current is stopped. Sodium chloride, 50 mM, was added to drive the electrochemistry of the silver/silver chloride electrodes. Current was applied using silver/silver chloride electrodes as these do not cause electrolysis of water

Table II. Matrix of Box-Behnken Design and Results for Each Experimental Run

Exp. no.	X_1	X_2	X_3	Cumulative release in mg/cm^2 (±SE)		Flux in mg/cm^2 h (±SE)	
				24 h (Y_1)	6 h (Y_2)	Iontophoretic (Y_3)	Post-iontophoretic (Y_4)
1	1	1	0	1.43 (0.122)	0.515 (0.216)	0.119 (0.027)	0.041 (0.003)
2	0	1	1	1.211 (0.112)	0.466 (0.73)	0.090 (0.015)	0.028 (0.002)
3	0	0	0	0.712 (0.048)	0.192 (0.061)	0.035 (0.007)	0.022 (0.002)
4	0	0	0	0.679 (0.049)	0.121 (0.040)	0.027 90.004)	0.029 (0.002)
5	0	1	–1	1.434 (0.097)	0.426 (0.057)	0.089 (0.020)	0.038 (0.006)
6	–1	0	–1	0.939 (0.096)	0.298 (0.163)	0.060 (0.020)	0.032 (0.002)
7	1	–1	0	0.253 (0.023)	0.054 (0.017)	0.011 (0.002)	0.010 (0.001)
8	–1	1	0	0.6 (0.135)	0.158 (0.079)	0.035 (0.010)	0.023 (0.003)
9	1	0	1	0.716 (0.105)	0.216 (0.132)	0.049 (0.017)	0.026 (0.003)
10	–1	0	1	0.411 (0.065)	0.1 (0.055)	0.022 (0.006)	0.017 (0.001)
11	0	0	0	0.715 (0.008)	0.189 (0.004)	0.044 (0.001)	0.027 (0.001)
12	0	–1	1	0.206 (0.004)	0.050 (0.005)	0.033 (0.021)	0.008 (0)
13	0	–1	–1	0.388 (0.035)	0.092 (0.034)	0.020 (0.004)	0.016 (0.002)
14	–1	–1	0	0.181 (0.03)	0.037 (0.013)	0.007 (0.001)	0.008 (0.001)
15	1	0	–1	1.03 (0.163)	0.358 (0.167)	0.074 (0.027)	0.029 (0.002)

(unlike platinum electrodes) and thus avoid pH changes in solutions as a result of iontophoresis. Samples, 0.5 ml, were withdrawn at specified time intervals from the sampling arm of the receptor compartment and replaced with 0.5 ml of plain phosphate buffer. The samples were later analyzed by HPLC, and the dilution of the receptor resulting from replacement of samples was taken into account in the calculation spreadsheet.

Passive delivery of a 5% solution of tacrine HCl in 50 mM phosphate buffer, pH 7.4, was also studied to compare the passive profile of the drug to that obtained by iontophoresis.

HPLC Analysis

An HPLC method modified from the literature (5) was used to analyze tacrine HCl. The column used was Microsorb-MV 100 C18 (150 mm × 4.6 mm; 5 μm) at ambient temperature. The mobile phase consisted of acetonitrile, triethylamine, and water at pH 6.5 (22:1:77). The mobile phase was vacuum filtered through an 0.45-μm filter and degassed before use. The flow rate was adjusted to 1 ml/min. Samples were injected into the chromatographic system (Hewlett Packard Series 1100, Wilmington, DE), and the effluent was monitored at 320 nm. The retention time for tacrine was approximately 9 min. The assay was found to be linear over a range of 0.5–500 μg/ml, with a correlation coefficient of 0.999. Percent Y intercept was less than 1%.

RESULTS AND DISCUSSION

The passive delivery of tacrine HCl across full-thickness rat skin was found to be negligible. Delivery was significantly enhanced by iontophoreis (Fig. 1). The magnitude of enhancement was, however, determined by the operational experimental conditions. As shown in Table II, 15 experiments were performed. After performing *in vitro* permeation experiments, the amount of tacrine HCl permeating per unit area of the full-thickness rat skin was measured up to 24 h. The cumulative release profiles for the 15 iontophoresis experiments are illustrated in Figs. 1 and 2.

The lag time for permeation of tacrine HCl by iontophoresis was approximately 2 h; hence, iontophoretic flux (mg/cm^2 h) was calculated by linear regression of the permeation

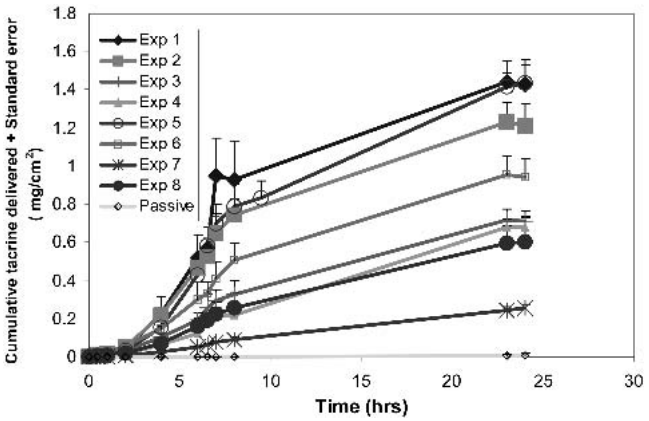

Fig. 1. Cumulative release plots for the passive delivery and iontophoretic delivery (Exp. 1–8) of tacrine HCl. Iontophoresis (n = 3) was applied for 6 h followed by passive delivery up to 24 h.

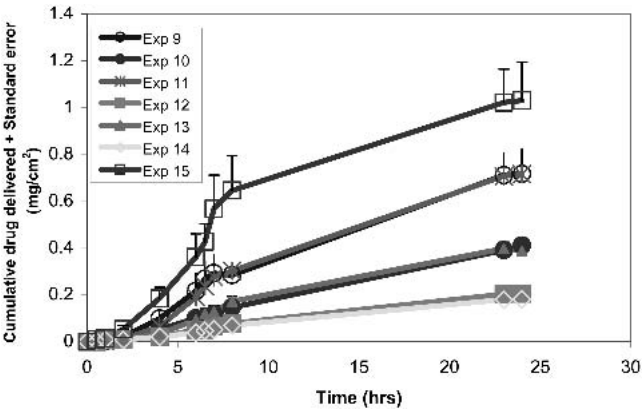

Fig. 2. Cumulative release plots for the iontophoretic delivery (Exp. 9–15) of tacrine HCl. Iontophoresis (n = 3) was applied for 6 h followed by passive delivery up to 24 h.

profiles from 2 to 6 h. The post-iontophoretic flux (mg/cm^2 h) was calculated from the slope of the linear portion of the plot from 6 h to 24 h. It was seen that enhanced tacrine delivery did not stop once the current was discontinued at 6 h, however, the post-iontophoretic flux was lower than the iontophoretic flux in most of the experiments. Enhanced tacrine delivery even after stopping iontophoresis has been attributed to formation of a tacrine depot in the skin (11). Tacrine is a lipophilic cation with a log P (octanol/water partition coefficient) of 3.3 (5), which might be getting associated with the lipophilic negatively charged sites in the skin and released slowly from there into the receptor compartment. Such an association of lipophilic positively charged drugs with skin has previously been reported for propranolol and nafarelin (6).

Table II summarizes the values for responses: Y_1, cumulative amount of drug delivered in 24 h; Y_2, cumulative amount of drug delivered in 6 h; Y_3, iontophoretic flux; and Y_4, post-iontophoretic flux. This data was analyzed using a statistical package (Statgraphics Plus, version 5) in order to generate mathematical models for each of the responses. The model for Y_3 (iontophoretic flux) was obtained after Box-Cox transformation (lambda = 0.111), which minimized the mean squared error for the fitted model. The results of analysis for each response variable were as follows:

$$Y_1 = 0.653 + 0.163 * X_1 + 0.456 * X_2 - 0.156 * X_3 + 0.190 * X_1X_2 + 0.139^* X_3^2 \quad (1)$$

$$Y_2 = 0.181^* + 0.06 * X_1 + 0.166 * X_2 - 0.043\, X_3 + 0.085\, X_1X_2 + 0.07^* X_3^2 \quad (2)$$

$$Y_3 = 0.673 + 0.024 * X_1 + 0.063 * X_2 - 0.011 * X_3 + 0.0322^* X_3^2 \quad (3)$$

$$Y_4 = 0.026 + 0.003 * X_1 + 0.011 * X_2 - 0.004 * X_3 + 0.003 * X_1X_2 + 0.003^* X_1X_3 - 0.004\, X_2^2 \quad (4)$$

The above equations indicate the quantitative effect of process variables (X_1, X_2, and X_3) and their interactions on the responses Y_1, Y_2, Y_3, and Y_4. The values of the coefficients X_1 to X_3 are associated with the effect of these variables on the response. Coefficients with more than one factor represent an interaction effect, whereas those with higher order terms denote quadratic relationships. A positive sign signifies a synergistic effect, whereas a negative sign stands for an

antagonistic effect. Only the coefficients that were statistically significant ($p < 0.05$) were retained in the equations except for the coefficient of X_3 in the model for iontophoretic flux (Y_3). X_3 was retained in the equation, as X_3^2 was statistically significant. The confidence with which the regression equations predicted responses for Y_1, Y_2, Y_3, and Y_4 were 87%, 71%, 65%, and 86%, respectively. Thus, it is seen that the mathematical model for Y_3 had a poor predictive capacity than other models. Lack of fit test (p value greater than 0.05 for all the models) indicated that models as fitted adequately represent the observed data at 95% confidence level. The standard error of estimate for Y_1 to Y_4 were 0.146, 0.096, 0.038, and 0.004, respectively.

From all the regression equations (1 to 4), it is seen that the regression coefficient of X_2 is larger than any other regression coefficients, and hence current density (X_2) was the main factor having a positive impact on the iontophoretic delivery of tacrine HCl. Current provides driving force for the movement of ionic species across the skin, and hence the iontophoretic flux of a 9% solution of tacrine in 100 mM phosphate buffer increased approximately 10-fold from 11 $\mu g/cm^2$ h to 119 $\mu g/cm^2$ h, when current density was increased from 0.1 to 0.5 mA/cm^2. The equations also indicate that tacrine permeation enhanced by an increase in drug concentration (X_1), as contribution to iontophoretic flux by electrorepulsion and electro-osmosis increases with an increase in drug concentration (6). However, permeation was decreased on increasing the molarity of donor buffer (X_3).

The relationship between the dependent and independent variables is further illustrated using response surface plots. Response surface plot (Fig. 3) eliciting the effect of drug concentration (X_1) and current density (X_2) and their interaction on cumulative drug delivered in 24 h (Y_1) indicated that at low level of X_2 (0.1 mA/cm^2), X_1 had a little effect on Y_1. However, at high levels of X_2 (0.5 mA/cm^2), Y_1 significantly increased from 0.75 mg/cm^2 to 1.46 mg/cm^2, when X_1 was increased from 1% to 9%.

At a low current density of 0.1 mA/cm^2, there was no significant change in permeation when drug concentration was increased from 1% to 9%. This seems to support findings of Al-Khalili *et al.* (12), who recorded no increase in the iontophoretic flux of buspirone hydrochloride on increasing drug concentration at 0.1 mA/cm^2. Iontophoretic drug delivery occurs through charged pores (i.e., skin appendages), and the ion flow through these pores may be diffusion limited. As the pores become saturated with drug, the membrane (pore) conductivity reaches a limiting value at high drug concentrations, which may partially explain the above observation. Tacrine being a positively charged lipophilic molecule might be getting anchored to the negatively charged skin sites. As the donor drug concentration increases, sufficient tacrine might be able to enter the pores and cause significant neutralization of the negative charges of the membrane, thereby attenuating electro-osmotic flow and cation permselectivity. Such a concentration-dependent inhibition of conventional electroosmosis has been reported for cationic lipophilic β blockers (13). Thus, at 0.1 mA/cm^2, a negligible change in permeation with increasing drug concentration may be a result of a counterbalance between two effects i) decrease in tacrine transport due to attenuation of electrosmosis and ii) an increased drug transport with concentration by electrorepulsion.

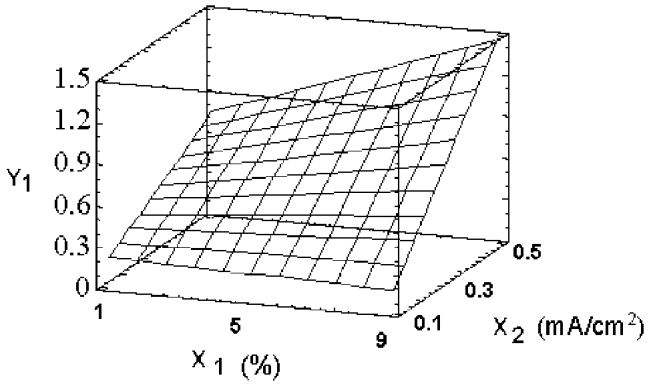

Fig. 3. Response surface plot eliciting the effect of X_1 (drug concentration) and X_2 (current density) on Y_1 (cumulative tacrine delivered in 24 h).

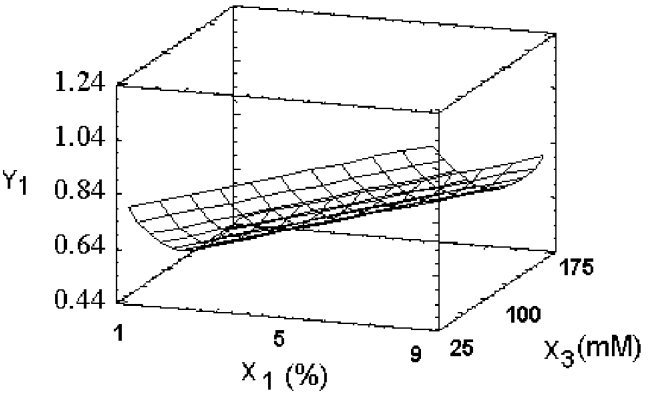

Fig. 4. Response surface plot showing the effect of X_1 (drug concentration) and X_3 (donor buffer molarity) on Y_1 (cumulative tacrine delivered in 24 h).

However, at 0.5 mA/cm^2, the delivery increased with an increase in drug concentration. In order to explain the interaction term between X_1X_2 (drug concentration and current density), a drop in skin resistance as a function of current density was studied. At a low current of 0.1 mA/cm^2, the resistance of the skin dropped only 4.4% after 6 h of ionto-

Table III. Observed, Predicted, and Residual Values for Response Y_1

Exp. no.	Predicted (mg/cm^2)	Observed (mg/cm^2)	Residual (mg/cm^2)
1	1.461	1.43	0.031
2	1.092	1.211	–0.119
3	0.653	0.712	–0.059
4	0.653	0.679	–0.026
5	1.404	1.434	–0.030
6	0.785	0.939	–0.154
7	0.171	0.253	–0.082
8	0.757	0.6	0.157
9	0.799	0.716	0.083
10	0.473	0.411	0.062
11	0.653	0.715	–0.062
12	0.18	0.206	–0.026
13	0.492	0.388	0.104
14	0.223	0.181	0.042
15	1.11	1.03	0.08

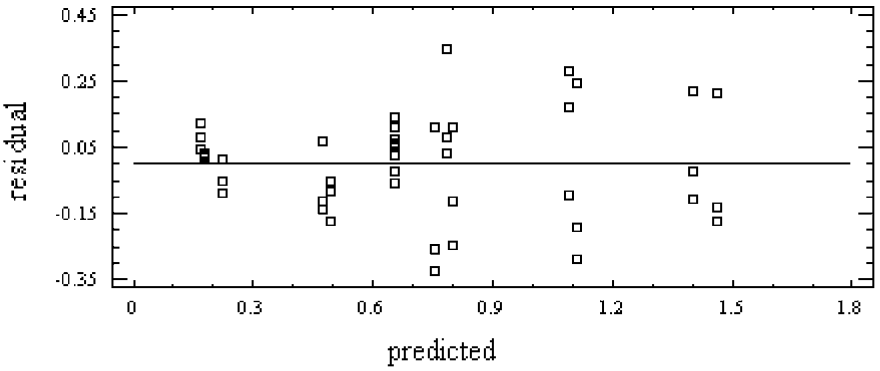

Fig. 5. Residual plot for response Y_1.

phoresis. Also at 0.3 mA/cm^2, the drop in skin resistance was only 7.5%. However the resistance dropped 35.4% at 0.5 mA/cm^2. At low current densities, iontophoresis disorganizes the stratum corneum only locally. However, at high current densities, a general disruption of the stratum corneum lipid structure observed by freeze fracture electron microscopy has been reported (7). Jadoul *et al.* have also observed a disordering of the lamellar intercellular organization in the stratum corneum above 0.33 mA/cm^2 by small-angle X-ray scattering (14). At 0.5 mA/cm^2, the layer structure of two main families of lipids, namely glycerides and ceramides, are highly perturbed, which might be responsible for the significant drop in skin resistance. The authors have concluded that loss of coupling between intercellular lipid bilayers might be favoring interlamellar diffusion of molecules. In a study performed by Pechtold *et al.,* it was seen that ion and water accessibility within the stratum corneum lipid lamellae increased with an increase in iontophoretic current density from 0.1 to 1 mA/cm^2 (15). Hence, as the intercellular regions in the stratum corneum become more accessible to the drug and the number of transport pathways increase, more drug would be required for neutralization of negative charges in the membrane and attenuation of electro-osmotic flow. Thus, it is probable that a progressive reduction of electro-osmotic contribution to transport would be slower at 0.5 mA/cm^2 than at 0.1 mA/cm^2. Also, a higher current density of 0.5 mA/cm^2 provides more driving force for the drug, and hence drug is transported by electromigration. Marro *et al.* (16) studied the contributions of electromigration and electro-osmosis to the iontophoretic delivery of lipophilic cations, quinine and propranolol, at 0.5 mA/cm^2 and concluded that the primary mechanism of the iontophoretic transport of these lipophilic cations was electrorepulsion rather than electro-osmosis. Hence, a possible explanation for our results is that, at 0.5 mA/cm^2 as the effect of electrorepulsion predominates and also due to secondary contribution to tacrine delivery by electro-osmosis, tacrine permeation increased with an increase in drug concentration.

The molarity of donor phosphate buffer (X_3), composed of monobasic and dibasic sodium phosphate, had a negative effect on drug permeation. A 25 mM donor buffer yielded greater drug permeation than did a donor buffer of 100 mM (Fig. 4). However, on further increasing the buffer molarity to 175 mM, tacrine permeation was not further retarded. Wearley *et al.* reported similar results for the iontophoretic transport of positively charged verapamil ions (17). When the concentration of competing sodium ions in the donor solution was increased from 0 to 1 M, verapamil flux initially decreased and then showed a slight increase. Also, the iontophoretic flux of sodium benzoate displayed a sharp initial decrease with addition of 86 mM sodium chloride followed by

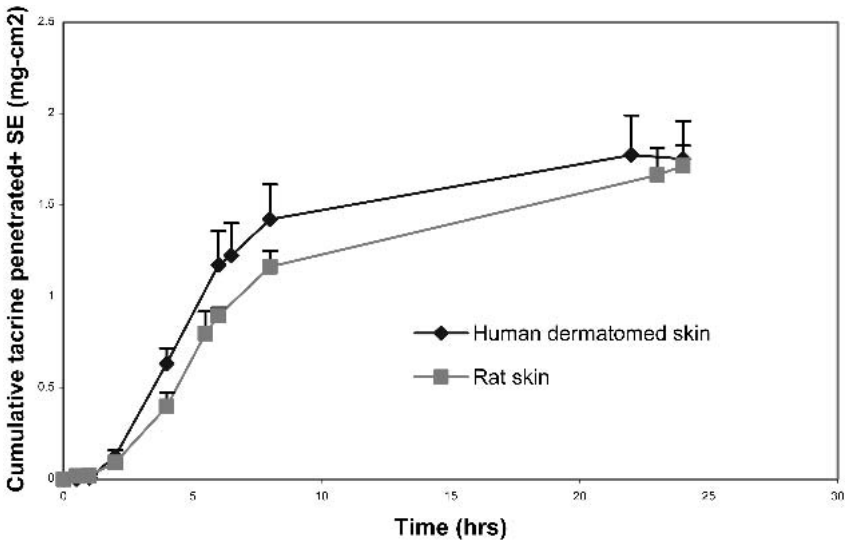

Fig. 6. Comparison of iontophoretic delivery of tacrine across rat and human dermatomed skin.

only a slight decrease upon further addition of sodium chloride (18). A convective force of sodium ions, which act to drag along drug cations, had been invoked as an explanation for the increase in flux of verapamil. The authors also commented on these convective forces acting at lower concentrations of sodium ions, which tend to restrain the initial decrease. An analogous situation is seen in case of iontophoretic delivery of tacrine cation. Delivery of a 5% solution of tacrine in 25 mM buffer yielded a delivery of 1 mg/cm^2. Increasing the buffer strength to 100 mM sharply decreased tacrine delivery to 0.72 mg/cm^2. As the molarity of the donor buffer increases, the number of buffer sodium co-ions competing with drug ions to be delivered across the skin increase. Sodium ions are estimated to carry a substantial amount of the total current, thereby diminishing the rate of drug permeation. Upon further increasing the buffer strength to 175 mM, tacrine permeation did not decrease. It is probable that at a high buffer concentration, convective force of sodium ions sweep along tacrine ions in their path and hence the observed response.

The values of X_1 to X_3 were substituted in the response surface model for Y_1, to obtain the theoretical values of Y_1 for the 15 experiments. The theoretical (predicted) values and the experimental (observed) values were in close agreement as seen from Table III. The residual plot for response Y_1 is shown as Fig. 5.

The models predicted levels of X_1 = 9%, X_2 = 0.5 mA/cm^2, and X_3 = 25 mM, for the optimal formulation having a maximal transdermal delivery rate, within the experimental region. An experiment was performed based on these optimal levels. The regression equations predicted responses of Y_1 = 1.756 mg/cm^2, Y_2 = 0.613 mg/cm^2, Y_3 = 0.141 mg/cm^2 h, and Y_4 = 0.041 mg/cm^2 h for the optimal formulation. These predicted values were in reasonably good agreement with the observed values of 1.713 ± 0.248 (SD) mg/cm^2, 0.894 ± 0.044 mg/cm^2, 0.202 ± 0.018 mg/cm^2 h, and 0.034 ± 0.015 mg/cm^2 h, respectively, for Y_1 to Y_4. Best correlation between the experimental data and predicted values was seen for responses Y_1 and Y_4. The model developed for cumulative drug delivered in 8 h (equation not reported) also showed good correlation between the observed (1.162 ± 0.119 mg/cm^2) and predicted response (1.096 mg/cm^2 h). So, it is seen that predictive capacity of the models is better in later stages of delivery. This might be due to formation of tacrine reservoir in the skin and hence poor correlation in the earlier stages. The release of tacrine from the skin into the receptor compartment at later stages might be improving the correlation between the observed and predicted responses.

Extrapolation of *in Vitro* Data to Predict Plasma Levels

The optimal drug delivery conditions were then tested across human dermatomed skin and compared to that across rat skin (Fig. 6). Single-factor ANOVA (performed on iontophoretic flux, post-iontophoretic flux, cumulative tacrine delivered in 6, 8, and 24 h) indicated that there was no significant difference ($p > 0.05$) in the iontophoretic delivery of tacrine across human dermatomed skin and rat skin.

The iontophoretic flux through human skin was 242 μg/cm^2 h. Steady-state plasma levels after transdermal drug permeation can be predicted by the following equation (19): $C_{ss} = J_{ss}$ A/CL, where A is surface area for drug absorption, J_{ss} is the steady-state flux (μg/cm^2 h), and CL is the clearance of the drug from the body. The CL for tacrine is reported to be about 150 L/h (20). Using this relationship, iontophoretic flux extrapolated to 25 cm^2 would result in plasma levels of 40.33 ng/ml. Thus, the optimized iontophoretic delivery is well within the therapeutic range of 5–70 ng/ml (21), suggesting that clinically significant doses can be delivered.

CONCLUSIONS

Box-Behnken experimental design was used to investigate transdermal iontophoretic delivery of tacrine HCl. It revealed an interaction between drug concentration and current density, which would have been difficult to predict from a one-factor-at-a-time classic experimental approach. At low current density, drug concentration had a negligible effect on drug permeation; however, at a high current density, tacrine delivery was significantly enhanced on increasing drug concentration. Response surface method is a powerful, competent method that can estimate main and interaction effects between drug delivery parameters for a complex system like transdermal iontophoresis. It aided in determining the optimal formulation conditions within the experimental region. Thus, the feasibility of using an optimization model to predict the iontophoretic delivery of tacrine hydrochloride was established.

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