

Response surface method as an approach to optimization of iontophoretic transdermal delivery of pilocarpine

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Abstract

Optimization via experimentation in iontophoresis was investigated. A response surface method was applied in optimizing the operating conditions in iontophoretic transdermal delivery of pilocarpine. With an objective for a transitory application of pilocarpine, i.e. in diagnosis of cystic fibrosis or in treatment of hyperhidrosis, the best operating condition was achieved via a response surface method by adjusting the pH of the buffer, ionic strength, current amplitude and frequency of pulsed current or active temporal ratio based on unknown structural models with stationary or slowly varying optima in the region of interest. This method was easy to approach and efficient in seeking optimal experimental conditions for multiple variables and an unknown structural system. Satisfactory results in optimizing the operating conditions in iontophoretic transdermal delivery of pilocarpine were thereby achieved.

Keywords: Optimization; Iontophoretic transdermal delivery; Pilocarpine; Response surface method

1. Introduction

Iontophoresis is a process or technique that causes an increased penetration of ionic or charged molecules into tissue using electrical potential or current, in either continuous (Bagniefski and Burnette, 1990) or pulsed mode (Chien, 1993). It is used to enhance the transdermal flux of many compounds (Tyle, 1986; Banga and Chien, 1988) and at present receives considerable attention

(Guy, 1992). In dermatology, diverse medications with an iontophoretic system of drug delivery, ranging from steroids and antibiotics to local anesthetics, and the treatment of hyperhidrosis or diagnosis of cystic fibrosis, are used (Sloan and Soltani, 1986). In more recent work, transdermal iontophoresis is also investigated as a system for delivery of peptide and protein drugs in systemic medication (Liu et al., 1988; Srinivasan et al., 1989; Green et al., 1992). Transdermal iontophoresis can avoid hepatic first-pass effects, can provide a controlled and large rate of delivery, and can be employed for varying periods.

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In the application of iontophoresis, there are many factors that influence delivery of drugs, including physicochemical and electric properties of the system. Physicochemical factors include the charges that drugs possess, the conductivity of the system, the pH of the donor solution, the ionic strength of the buffer and the concentration of the drug. The electric factors involved in the rate of delivering a drug include the electrical potential applied, the type of electrical potential (constant voltage or constant current), the mode used (continuous mode or pulsed mode), the electrode, the current amplitude, the frequency of pulsed current and the active temporal ratio. Each factor has its individual influence. Some effects would be counterbalanced by others. The significance of the influence of these parameters also varies from one drug to another.

Pilocarpine, a cholinergic agent that was chosen as a model drug, is a widely used drug for diagnosis of cystic fibrosis. Cystic fibrosis is an inherited systemic disorder of mucus-producing exocrine glands affecting the pancreas as well as the bronchi, intestine and liver. There is a diagnostically abnormal content of sodium and chloride in the perspiration. A physician invariably analyzes the patient's perspiration to diagnose the syndrome of cystic fibrosis. The major effect of pilocarpine on the body is to produce marked sweating. In order to improve control of perspiration and to decrease the total water lost, transdermal iontophoresis of pilocarpine is the best choice (Gibson and Cooke, 1959). Rapid diagnosis and quick action are needed. Transitory application and immediate pharmacodynamic response are the main objectives of this application. In this work, optimization based on a response surface method via experiments in iontophoresis with periodic pulsed current was investigated.

2. Optimizing method

The response surface method is a useful and efficient tool to obtain an appropriate model with minimum experiments. It is used in pharmaceutical processes in optimization of granulation (Wehrle et al., 1993; Vojnovic et al., 1994), cap-

sule and tablet formulation (Iskandarani et al., 1993), or oral solution (Senderak et al., 1993). The structure of this method is outlined as follows (Fig. 1). Before experiments are designed, a definite purpose of the experiments and a quantitative objective function of the system should be clearly defined. In iontophoretic transdermal delivery of pilocarpine for marked sweating, for example, it can be defined as the maximum rate of permeation of pilocarpine in the first hour. Then we need to select the most important variables that affect the system response and to predetermine the range of each input variable. Systematic experiments proceed according to a two-level factorial design.

2.1. Two-level factorial design

In the case of transdermal iontophoresis of pilocarpine under a periodically pulsed current device, the most important factors affecting the rate of permeation of pilocarpine include pH and ionic strength of the donor solution, the frequency of pulsed current, the magnitude of the current and the duty cycle. In these tests, the variables of pH (x_1), ionic strength (x_2), frequency (x_3), and duty cycle (x_4) were chosen as key parameters. Based on previous tests in the literature and our preliminary experiments of iontophoretic delivery of pilocarpine, the condition $x^0 = (4, 0.012, 2000, 50)$ was chosen as a starting point and the spacing $s = (\pm 1, \pm 0.008, \pm 1000, \pm 10)$ was used. To

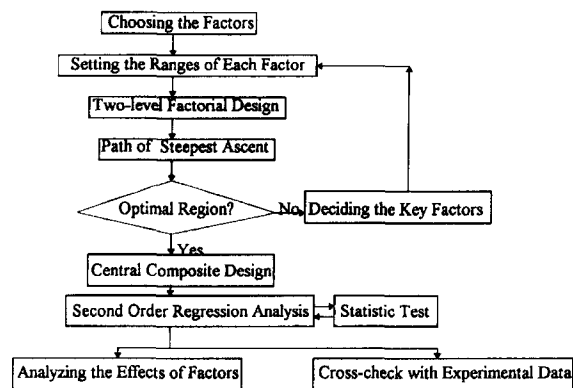


Fig. 1. Outline of the response surface method.

simplify the calculation, all variables are normalized in coded level, -1 (low) $\sim +1$ (high); 2^{4-1} experiments were conducted first to find the path of steepest ascent.

2.2. First-order response surface methods and path of steepest ascent

A first-order response surface can be constructed based on these experimental results by the least squares method. The gradient direction $\nabla g(x^k)$ for the next step can be estimated subsequently. Experiments are then conducted at points along this direction to a new point x^{k+1} that represents the best solution obtained along the direction $\nabla g(x^k)$. If the optimal objective function is in the region of the corresponding current point x^k , a detailed and complete experimental design (central composite design) should be performed to improve understanding of the response surface by using a response surface model of high order. If the optimal objective function is outside the region of the current point x^k , another starting point near this optimal point and spacing should be chosen. The corresponding two-level coded factorial experiments should be conducted again to obtain a new path of steepest ascent, proceeding repeatedly as above until a response surface model of higher order is established and a maximum of the objective function is attained.

2.3. Central composite design

If an 'optimum' region is once decided according to the method of the path of steepest ascent, it is necessary to conduct extra experiments to achieve a more detailed description of the response surface, i.e. a response surface model of greater order. We generally add design points near the center to complete a second-order response surface to test this optimum solution. Such a model with more than three variables is expressed mathematically as

$$Y = a_0 + \sum_{i=1}^n a_i x_i + \sum_{i=1}^n \sum_{j=i+1}^n a_{ij} x_i x_j + \varepsilon \quad (1)$$

in which Y is the value of the response, the dependent variable, a_0 is equivalent to the re-

sponse at the central point, a_i shows the degree of effect of x_i on Y , a_{ij} shows the interaction of x_i and x_j , and ε is the vector of errors.

If several successive experiments are performed at scattered points about the known experimental region without achieving an improved solution, the search can be terminated and the best available solution can be adopted.

2.4. Effect analysis of factors

From the final surface response of high order, the degree of effect of x_i on \hat{Y} , and the interaction of x_i and x_j can be determined from the coefficients of a_i and a_{ij} . Student's t -test was used to verify the significance of these coefficients and the validity of this model.

3. Materials and methods

Pilocarpine hydrochloride (reagent grade) was obtained from Nacalai Tesque (Japan). All other chemicals used in the experiments and analysis were also reagent grade (Sigma Chemical Company, St. Louis, MO, USA or Wako Chemicals, Japan).

3.1. Iontophoretic flux determination in vitro

Iontophoretic diffusion experiments were carried out in vitro in the Valia-Chien side-by-side diffusion cell (model VSC-1, Crown Glass, Somerville, NJ, USA), with a controlled rate of stirring (600 rev./min) in both half cells. An isothermal condition $37 \pm 0.2^\circ\text{C}$ was maintained in both half cells by circulating temperature controlled water through the water jacket cells. An orifice (0.69 cm^2) connected the two diffusion cells. The volume of each cell was 3.5 ml. Platinum wires were used as electrodes. The electrodes were connected to a universal power source, (Hewlett Packard, HP 3245A, Palo Alto, CA, USA), that produces a constant current or a periodically pulsed current as output. A periodically pulsed current was used in all experiments. A constant current of 0.6 mA was maintained in all experiments. The frequency and duty cycle

were varied according to the experimental conditions.

Skin was mounted between the diffusion cells. In the donor side, pilocarpine (1 mg/ml) was loaded in the donor buffer. Pilocarpine permeates from the donor side through the skin to the receiver side. To simulate the body fluid, a Sorensen phosphate buffer (pH 7.4) was used as the receiver solution. Phosphate buffer was used also in the donor solution, but the pH and buffer capacity were adjusted with weak acid and NaOH (0.1 N) to meet the requirements of each experiment. In some cases, acetate buffer was also used in an acidic environment for the donor solution.

Rabbit inner pinna skin was used in all experiments. Skin was freshly excised from New Zealand white rabbits, about 3–5 kg, obtained from our animal center in the College of Medicine. The outermost layers of skin (epidermis skin) were taken from the animals immediately after being sacrificed and were used as soon as possible.

A sample (100 μ l) from the receptor cell was withdrawn at preset intervals to determine the rate of permeation of pilocarpine. The same volume of fresh buffer was added each time that a sample was taken. Samples were analyzed immediately by capillary electrophoresis.

3.2. Determination of the pilocarpine

Pilocarpine was assayed on a micellar electrokinetic capillary chromatograph in capillary electrophoresis, (P/ACE System 2100, Beckman Instruments, Palo Alto, CA). The capillary cartridge contained a capillary of fused silica of length 57 cm (but 50 cm to detector) and internal diameter 75 μ m. On-line UV detection was monitored at 214 nm and the temperature of the capillary was maintained at $30 \pm 0.1^\circ\text{C}$. A separation buffer was prepared using borate buffer (pH 9.0) with sodium dodecyl sulfate (SDS, 2.5%) and an ionic strength of 0.002 M. Before each injection, the column was preconditioned by flushing with NaOH (0.1 M) for 2 min, with deionized water for 2 min, and finally with separation buffer for 2 min. The sample was introduced into the column using pressure injection for 5 s,

corresponding to approximately 30 nl. All separations were performed for 15 min using a constant high voltage of 20 kV. The retention time of pilocarpine was about 10 min. The sample signals were analyzed with Beckman System Gold, version 8.1 software.

4. Results and discussion

The response surface method was applied to seek the maximal rate of iontophoretic permeation of pilocarpine crossing skin. As in the diagnosis of cystic fibrosis (Gibson and Cooke, 1959; Nielsen and Flensberg, 1987), or in the development of non-invasive monitoring of the concentration of glucose in plasma (Rao et al., 1993), a quick pharmacological response and much perspiration were needed. In the experiments of Gibson and Cooke (1959), iontophoresis of pilocarpine proceeded for only 5 min. Other applications or other drugs may required a prolonged application. Here, our quantitative objective function was defined as 'the maximum permeation of drug in the first hour'. In order to prevent the polarization of the skin, a periodic monophasic current was preferably used in iontophoresis. For iontophoresis with a monophasic pulsed current, Chien (1993) concluded that in iontophoretic experiments with insulin, greater flux of permeation was obtained with a periodically pulsed wave of current with frequency of 2000 Hz and duty cycle 50% (i.e. the ratio of current on and off equals 1). In solution, pilocarpine is subject to serious degradation when the pH exceeds 7. To avoid such degradation, the pH of the donor solution was restricted below this condition. The starting points for design of the first two-level factorial experiment was as follows: donor solution pH was 4, with increment ± 1 pH; donor solution ionic strength 0.012 M, with increment ± 0.008 M; pulse frequency 2000 Hz, with increment ± 1000 Hz; current duty cycle 50%, with increment $\pm 10\%$.

With four variables, 2^{4-1} experiments were conducted. Table 1 presents the conditions of the first eight experiments and the corresponding transdermal cumulative amount of permeation of pilo-

Table 1
Experimental conditions of the first two-level factorial design in iontophoretic transdermal delivery of pilocarpine

Factor	pH(x_1)	μ (x_2)	f (x_3)	Duty(x_4)	Cum. amount for 1 h (μ g)
Center Unit	4	0.012	2000	50	
	1	0.008	1000	10	
Experiment 1	3	0.004	1000	40	218.69
	(-1)	(-1)	(-1)	(-1)	
Experiment 2	3	0.004	3000	60	324.24
	(-1)	(-1)	(1)	(1)	
Experiment 3	3	0.02	1000	60	66.71
	(-1)	(1)	(-1)	(1)	
Experiment 4	3	0.02	3000	40	67.31
	(-1)	(1)	(1)	(-1)	
Experiment 5	5	0.004	1000	60	918.27
	(1)	(-1)	(-1)	(1)	
Experiment 6	5	0.004	3000	40	623.85
	(1)	(-1)	(1)	(-1)	
Experiment 7	5	0.02	1000	40	320.52
	(1)	(1)	(-1)	(-1)	
Experiment 8	5	0.02	3000	60	496.41
	(1)	(1)	(1)	(1)	

carpine in the first hour under pulsed current iontophoresis. The rate of permeation of pilocarpine varies greatly depending on operating conditions, from 66.71 μ g/h to 918.27 μ g/h — more than 10-fold variation. A donor solution with a higher pH (pH 5, open symbols in Fig. 2) yields greater permeation than a lower pH value (pH 3, closed symbols in Fig. 2). A smaller ionic strength yields a greater flux. The surface response Y (objective function, the maximum permeation of drug in the first hour, μ g) of first order was obtained with the least squares method.

$$Y = 379.5 + 210.3x_1 - 141.8x_2 - 1.5x_3 + 71.9x_4 \quad (2)$$

Eq. (2) indicates that pH is the most important parameter in controlling the transdermal iontophoretic permeation of pilocarpine. Each variation of pH value caused 210.3 μ g/pH variation of

pilocarpine permeation. The ionic strength has obviously a negative effect on the drug permeation. A large ionic strength implies that more ionic species compete with the drug ion with the same opportunity so as to diminish the rate of permeation of drug. The influence of frequency on the transdermal iontophoretic drug delivery is insignificant, shown in Fig. 2 or from the coefficient of x_3 in Eq. (2) that at only 1.5 is far smaller than the others. In this batch of experiments, the amplitude of the current was maintained constant. A large duty cycle signifies that much work is done by the external electrical field and implies that much more ions are carried by the electric field.

Eq. (2) also provides the path of steepest ascent toward the optimal objective function. A large rate of permeation is attained on decreasing the donor pH, on decreasing the ionic strength, or on increasing the current duty cycle. Because of the

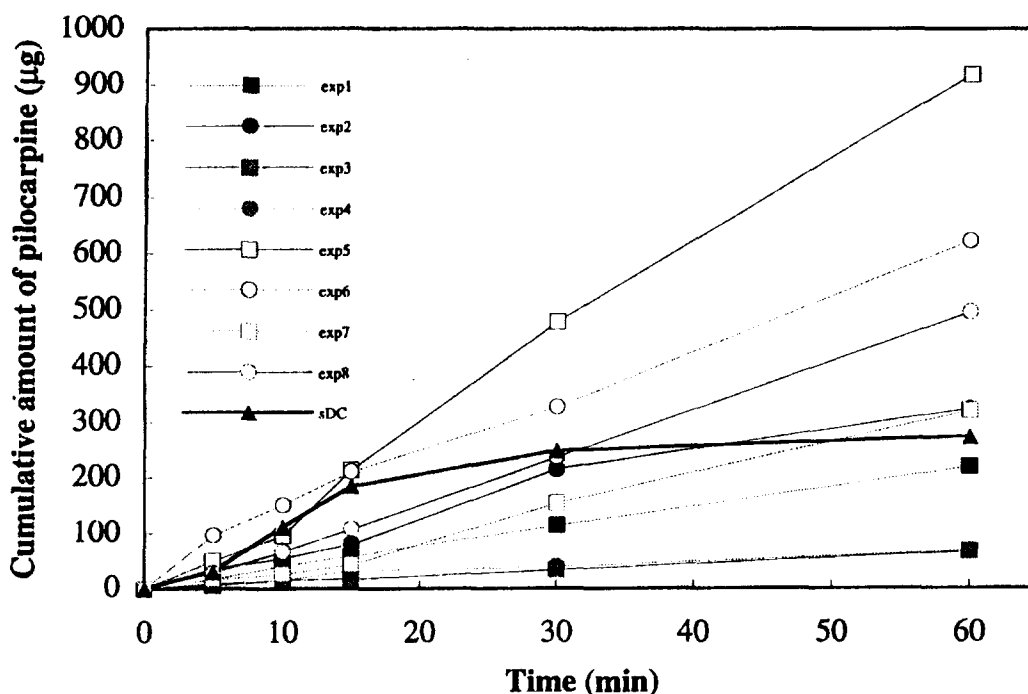


Fig. 2. Cumulative amount of pilocarpine within the first hour for the first factorial direction determining block (corresponding to experimental conditions in Table 1).

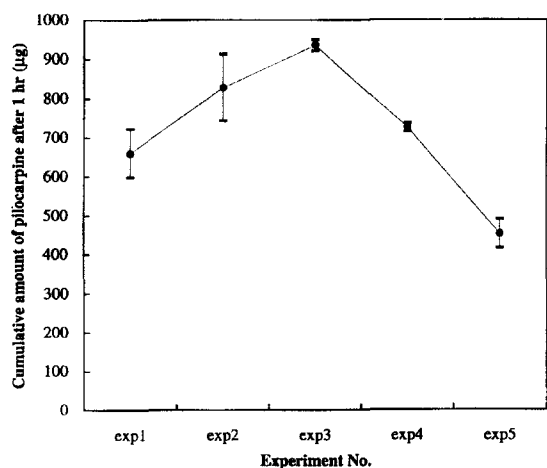
small influence of frequency on pilocarpine permeation, the frequency was maintained constant for the succeeding experiments. From the starting point $x^0 = (4, 0.012, 2000, 50)$, we designed five other experiments along the direction of steepest ascent, $\nabla g(x^0)$. The experimental conditions and results are shown in Fig. 3. The greatest amount of cumulative permeation was in Experiment 3 of this batch experiments, pH 5.5, ionic strength 0.0045 M, and duty cycle 56%. Because this point (5.5, 0.0045, 2000, 56) was outside the range of the original two-level factorial experimentation design ($4 \pm 1, 0.012 \pm 0.008, 2000 \pm 1000, 50 \pm 10$), another new starting point was chosen and the procedures were repeated as stated above (Fig. 1).

Table 2 presents the new two-level factorial experimental design with Experiment 3 in the second batch, pH 5.5, ionic strength 0.0045 M, and duty cycle 56%, $x^1 = (5.5, 0.0045, 2000, 56)$ as starting point. Increments were ($\pm 0.3, \pm 0.0015, \pm 0, \pm 10$). Because the frequency was main-

tained constant, only 2^{3-1} experiments were needed in this batch of screening. The results appear in Fig. 4. In this region, Experiment 1 had a maximal cumulative amount of permeation. The total amount of pilocarpine was 1182.42 $\mu\text{g/h}$, better than in other experiments. The corresponding response surface in first order obtained according to the method of least squares was as follows:

$$Y = 973.2 + 34.3x_1 - 69.8x_2 + 109.7x_4 \quad (3)$$

Along this path of steepest ascent, $\nabla g(x^1)$, four steps of experiments were performed to test the possibility of a local maximum. In these four experiments, the rate of permeation increased minutely and decreased as shown in Table 3. There was no significant variation of permeation amount among Experiments 1 to 3, because such variation may be caused by experimental error or skin disparity. The optimal objective function is considered to be at the starting point. The optimal operating condition is certainly located in this



Factor	pH (x1)	μ (x2)	f (x3)	duty (x4)
exp1	4.5	0.0095	2000	52
exp2	5.0	0.007	2000	54
exp3	5.5	0.0045	2000	56
exp4	6.0	0.002	2000	58
exp5	6.5	0	2000	60

Fig. 3. Cumulative amount of pilocarpine for the first factorial step determining block.

region. To understand further the response surface of this region, we needed a model of high order. Extra experiments near the center or a star-shaped experimental design should be conducted to construct the detailed response surface.

Table 2

Experimental conditions of the second two-level factorial design in iontophoretic transdermal delivery of pilocarpine

Factor	pH (x ₁)	/m/ (x ₂)	f (x ₃)	Duty (x ₄)	Cum. amount for 1 h (/m/g)
Center Unit	5.5	0.0045	2000	56	
	0.3	0.0015	0	10	
Experiment 1	5.2 (-1)	0.003 (-1)	2000	66 (1)	1118.42
Experiment 2	5.2 (-1)	0.006 (1)	2000	46 (-1)	759.38
Experiment 3	5.8 (1)	0.003 (-1)	2000	46 (-1)	967.47
Experiment 4	5.8 (1)	0.006 (1)	2000	66 (1)	1047.41

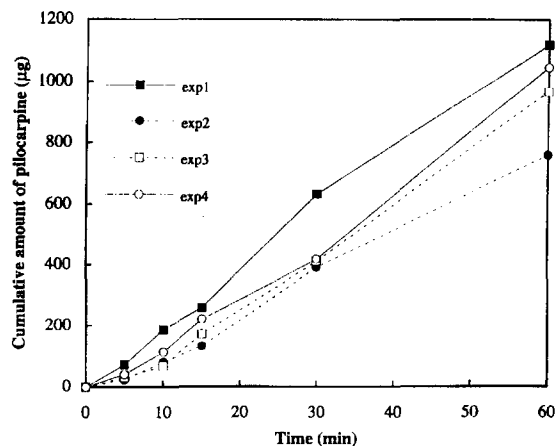


Fig. 4. Cumulative amount of pilocarpine within the first hour for the second factorial direction determining block (corresponding to experimental conditions in Table 2).

By using a central composite design about the central point, we chose eight other experiments about the center. The experimental design and operating conditions are presented in Table 4. From the experimental results and mathematical regression, the response surface of second order is

$$\begin{aligned}
 Y = & 995.79 + 4.24x_1 + 20.81x_2 + 44.87x_4 \\
 & - 13.05x_1^2 - 54.22x_2^2 - 13.21x_4^2 + 64.88x_1x_2 \\
 & - 90.59x_1x_4 + 30.03x_2x_4
 \end{aligned}
 \tag{4}$$

Table 3
Experimental design of the second factorial step determining block along the direction of steepest ascent

Factor	pH(x_1)	$\mu(x_2)$	$f(x_3)$	Duty(x_4)	Cum. amount for 1 h (μg)
Center	5.5	0.0045	2000	56	
Unit	0.3	0.0015	1000	10	
Slope	1	-2	0	3.2	
Proportion	0.3	-0.003	0	32	
Scale	0.1	-0.001	0	10	
Experiment 0	5.5	0.0045	2000	56	949.59
Experiment 1	5.6	0.0035	2000	66	954.58
Experiment 2	5.7	0.0025	2000	76	969.20
Experiment 3	5.8	0.0015	2000	86	747.71

Optimal operating conditions were obtained by differentiating this equation. In iontophoretic transdermal delivery of pilocarpine with periodically pulsed current, the best operating conditions are pH 5.98, ionic strength 0.0055 M, and duty cycle of 59% and the corresponding maximal amount of pilocarpine delivered in the first hour is 1011.4 μg .

To gain an understanding of the nature of the response surface, canonical analysis, described by Box and Draper (1987), was used. Eq. (4) becomes transformed to the canonical form

$$Y = 1011.4 - 24.1Z_1^2 + 34.2Z_2^2 - 90.5Z_4^2 \quad (5)$$

Because the coefficients of Z_i^2 are not all negative, only a local maximum was obtained in these experiments. This point is a saddle point, not an absolute maximum. Statistical tests recommend that a response surface of second order is inadequate to describe this complicated system because of the complexity of transdermal iontophoresis, skin disparity, and experimental variation. Although the objective function of the absolute maximum rate of delivery in iontophoretic transdermal delivery of pilocarpine was not achieved, a local large rate of permeation of pilocarpine crossing skin under iontophoresis was obtained. There is a significant increase in the cumulative amount of pilocarpine from 271.37 $\mu\text{g}/\text{h}$ (constant current iontophoresis, bold line in Fig. 2) to 1011.4 $\mu\text{g}/\text{h}$, nearly a

4-fold increase after systematic searching with the response surface method (Fig. 5).

5. Conclusions

Iontophoresis is a satisfactory method of enhancing the permeation of drugs through the skin. It is complicated and not well known. Many physicochemical and electric variables are involved in operating this system. Four experimental variables — pH, ionic strength, current frequency, and duty cycle — were selected as key parameters from results of previous preliminary tests in this complex system. Optimization via experiments based on the response surface method was applied in seeking optimal operating conditions of iontophoretic transdermal delivery of pilocarpine under a periodically pulsed current mode. The maximum rate of permeation of pilocarpine crossing skin was achieved at a small ionic strength, moderate pH and large current duty cycle: $pH(x_1) = 5.98$, ionic strength (x_2) = 0.0055, and current duty cycle (x_4) = 59%, under the constraint of pulsed current density less than 1 mA/cm^2 .

The response surface method is a powerful, efficient and systematic method of optimization via experiments for a complicated unknown system, as in transdermal iontophoresis. Only two steps were executed to approach the maximum objective function for this complicated system and satisfactory results were achieved.

Table 4

Experimental conditions of central composite designs to determine the second order response surface

Factor	pH(x_1)	$\mu(x_2)$	f(x_3)	Duty(x_4)	Cum. amount for 1 h (μg)
Center	5.5	0.0045	2000	56	
Unit	0.3	0.0015	0	10	
	4.9	0.0045		56	
Experiment 5			2000		906.18
	(-2)	(0)		(0)	
	6.1	0.0045		56	
Experiment 6			2000		923.13
	(2)	(0)		(0)	
	5.5	0.0015		56	
Experiment 7			2000		708.37
	(0)	(-2)		(0)	
	5.5	0.0075		56	
Experiment 8			2000		791.61
	(0)	(2)		(0)	
	5.5	0.0045		36	
Experiment 9			2000		854.17
	(0)	(0)		(-2)	
	5.5	0.0045		76	
Experiment 10			2000		1153.25
	(0)	(0)		(2)	
	5.5	0.0045		86	
Experiment 11			2000		931.73
	(0)	(0)		(3)	
	5.5	0.0045		56	
Experiment 12			2000		921.27
	(0)	(0)		(0)	
	5.5	0.0045		56	
Experiment 13			2000		977.91
	(0)	(0)		(0)	
	5.5	0.0045		56	
Experiment 14			2000		884.23
	(0)	(0)		(0)	
	5.5	0.0045		56	
Experiment 15			2000		1042.21
	(0)	(0)		(0)	

The response surface method is much more efficient than the traditional method. Another attractive feature of the response method is that conclusions can be drawn from the first experiments. The experiments can be terminated whenever further experiments appear uneconomic.

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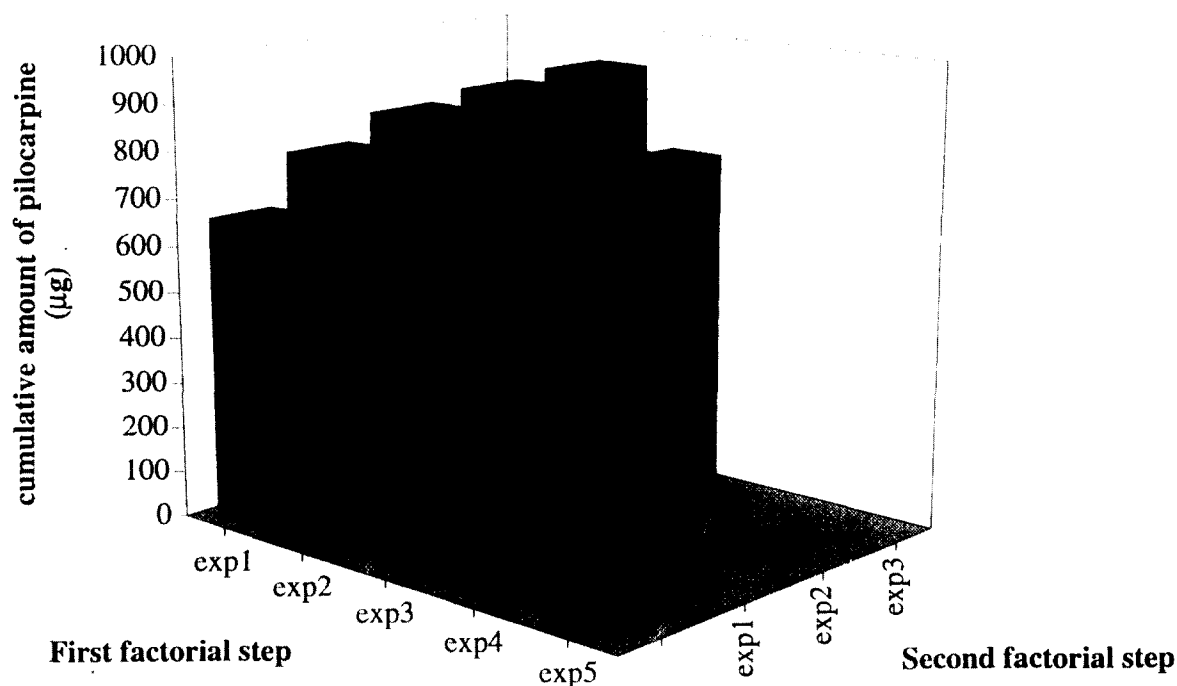


Fig. 5. Two steps in approaching the maximal rate of permeation of pilocarpine in transdermal iontophoresis facilitated by periodically pulsed current.

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