Response Surface Method: A Novel Strategy to Optimize Iontophoretic Transdermal Delivery of Thyrotropin-releasing Hormone

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Purpose. To maximize the iontophoretic transdermal delivery rate of thyrotropin-releasing hormone (TRH) facilitated by periodically monophase-pulsed current across excised skin.

Methods. The pH of the buffer, the ionic strength in the solution, the frequency of the periodically monophase-pulsed current and the current on/off ratio were chosen as the key variables. A response surface method was applied to optimize the transdermal delivery rate of TRH under different operational conditions.

Results. The optimum operating conditions were achieved via experimentation based on the response surface method by systematically adjusting the pH of the buffer, the ionic strength in the solution, the current amplitude, frequency and the active temporal ratio of the pulsed current. The rate of permeation of TRH crossing the skin during iontophoresis varied from two to ten-fold, depending on operating conditions.

Conclusions. Only a few steps, two in this work, were needed to reach the optimal. The response surface near the region of the maximal point was thoroughly described with a quadratic function. A maximal transdermal rate of permeation of TRH, 103.2 $\mu g\ h^{-1}\ cm^{-2}$, was obtained when the donor solution was at pH = 7.0, ionic strength = 0.037, and with a periodically monophase-pulsed current iontophoresis with duty cycle = 75%. The effect of pulse frequency was not statistically significant.

KEY WORDS: optimization; iontophoretic transdermal delivery; thyrotropin releasing hormone (TRH); response surface method.

INTRODUCTION

The response surface method has shown great potential as an appropriate model with the minimal number of experiments, especially for an unknown structure with a single response or multiple responses in a multiple-variable system. It is used in many pharmaceutical processes, such as for optimizing granulation (1, 2), formulations of capsules and tablets (3), or oral solution (4). It has also been successfully applied to optimize the transdermal delivery of pilocarpine during iontophoresis (5). Most peptide drugs have more than one dissociation constant. The degree of dissociation and the drug's charge depend on the buffer environment. Peptide drugs are also zwitterionic, i.e. they can carry opposite charges depending on the pH of the buffer solution. The conditions are more complicated for iontophoretic transdermal delivery of peptide drugs than for such delivery of nonpeptide drugs. Most peptide drugs have varied degrees of protonation, depending on the pH of the bulk solution. At pH 8, TRH is 98% unprotonated. With the buffer at a low pH, the ratio of protonated TRH increases and attains 99% protonated ion at pH 4. A greater degree of protonation intuitively provides a greater rate of permeation. The charge and degree of dissociation of the drug affect the effectiveness of iontophoresis. At a low ionic strength, an increased degree of protonation in TRH increases the rate of permeation. The flux of TRH in a buffer at pH 4 is greater than that at pH 8 when the ionic strength is 0.1M. However, at a greater ionic strength, the trend is reversed (7).

Other than the pH and ionic strength of the donor buffer, the mode of current is a key factor in transdermal delivery of a peptide drug. An electrical field with iontophoresis in a continuous mode may cause electrochemical polarization in the skin, which prevents the drug from penetrating. Depolarization of the skin during iontophoresis with a pulsed current results in a significantly increased amount of transdermal delivery of TRH. The frequency, amplitude, and the temporal ratio of the pulsed current are the most important parameters influencing the rate of permeation of peptide or protein through skin under iontophoresis. The key variables that effectively influence the rate of delivery of TRH during monophase-pulsed iontophoresis are the pH of the buffer, the ionic strength in the solution, the current amplitude, the frequency of the periodically monophase-pulsed current and the active temporal ratio. In this work, the response surface method with experimentation to optimize the transdermal delivery of TRH as facilitated by periodically monophase-pulsed current iontophoresis was investigated. The relationships between the rate of permeation and the parameters of operation and the interactions of variables were assessed.

MATERIALS AND METHODS

Thyrotropin-releasing hormone(TRH) was purchased from the Sigma Chemical Company (St. Louis, MO, U.S.A.). Other chemicals used in the experiments and analysis were reagent grade (the Sigma Chemical Company, or Wako Chemicals, Japan). Unless otherwise stated, all chemicals were used as received. Water was obtained from a purification system (Millipore Inc., Milli-Q, Milford, MA).

Iontophoretic Permeation Tests in Vitro

Valia-Chien side-by-side cells (model VSC-1, Crown Glass, Somerville, NJ) and coiled platinum electrodes were used and kept at 37 ± 1 °C. An area (0.64 cm^2) of rabbit pinna skin was exposed in the donor and receptor compartments of this diffusion cell. The volume of each cell was 3.5 ml. The TRH concentration in the donor solution was 1 mg/ml. During iontophoresis, 200 μl of receptor solution per hour were taken for instantaneous analysis. Each experiment was duplicated.

Rabbit inner pinna skin was used in all the experiments because its transdermal permeation characteristics are similar to those of human cadaver skin (8). Skin was freshly excised from New Zealand white rabbits, about 3–5 kg, obtained from an animal center in the College of Medicine. The outermost

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layers of skin (epidermis skin) were taken from the animals immediately after they were sacrificed and were used within one hour.

Various buffers and ionic strengths were used in donor and receptor cells. In order to simulate body fluid, a Sorensen phosphate buffer (pH 7.4) generally served as the receiver solution. TRH permeated from the donor side through the skin to the receptor side. Phosphate buffer was also used in the donor solution, but the pH and buffer capacity were adjusted with weak acid (acetic acid or phosphoric acid) and NaOH (0.1 M) to meet the requirements of each experiment. In some cases, phosphate-citrate buffer was also used in an acidic environment as the donor solution. For comparison with results of Burnette and Marrero (9), phosphate-citrate buffer (pH 4), consisting of Na_2HPO_4 (0.083 M), citric acid (0.059 M), NaCl (0.427 M), and phosphate-citrate buffer (pH 8), consisting of Na_2HPO_4 (0.195 M), citric acid (0.0028 M), NaCl (0.041 M) were also used.

Determination of TRH

TRH was assayed using capillary zone electrophoresis (P/ACE System 2100, Beckman Instruments, Fullerton, CA). The capillary cartridge contained a capillary of fused silica of length 57 cm (50 cm to detector) and internal diameter 75 μm. A UV detector at 214 nm was used, and the temperature of the capillary was maintained at 30 ± 0.1 °C. The separation buffer contained phosphate (pH 7.4) at an ionic strength 0.02 M. Before each injection, the column was preconditioned by flushing first with NaOH (0.1 M) for 1 min, then with deionized water for 1 min, and finally with separation buffer for 2 min. The sample was injected into the column under pressure for 5 seconds, corresponding to approximately 30 nl. All the separations were performed for 10 min at a constant voltage of 15 kV. The retention period of TRH was about 7.55 min. The sample signals were analyzed using software (Beckman System Gold, version 8.1).

Optimizing Method

The response surface method was described in detail in a previous paper (5) and by Wehrle and Stamm (6). For an unknown structural system, such as transdermal iontophoretic delivery, the mechanism of the system or the relationships of the variables are difficult to deduce. The response surface method is designed to analyze the output response and to establish the relationships between the response and the variables efficiently via systematic experimentation. The steps were outlined below.

1. Identify the known experimental region $a_i \leq x_i \leq c_i$, $i = 1, \ldots, n$. Select a starting point, $\mathbf{x}^0$, within this region. With $\mathbf{x}^0$ as its center, array an orthogonal first-order response surface design within a selected design radius. Place $n_c = n/2 \geq 2$ points at the design center, $\mathbf{x}^0$ (coded as the 0-vector).

2. Perform experiments at each of N experimental design points, and record the responses y_j^l, $j = 1, \ldots, m$; $l = 1, \ldots, N$. Using multiple linear regression, fit linear models of the form

$$\hat{y}_j = \hat{\beta}_{j,o} + \sum_{i=1}^{n} \hat{\beta}_{j,i} x_i, \qquad j = 1, \ldots, m \tag{1}$$

3. Decide the path of steepest ascent, and conduct design points along this direction to find a local maximum.

4. Apply an appropriate mathematical programming technique to locate the next center point in the search.

5. Repeat steps 1–4 until an "optimum" solution is located (Fig. 1).

6. Add design points (central composite design) to complete a second-order response surface design to test this optimum solution.

7. Execute the experimental trial at each point in a second-order response surface experimental design covering the known region, and use multiple linear regression to fit second-order regression models to the resulting data. The second-order fitted response surface has the form

$$\hat{y} = \hat{\beta}_o + \sum_{i=1}^{n} \hat{\beta}_i x_i + \sum_{i=1}^{n} \hat{\beta}_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j>i}^{n} \hat{\beta}_{ij} x_i x_j + \epsilon, \tag{2}$$

in which y is the response, and x_i is a dependent variable; β_o is equivalent to the response at the central point; β_i shows the degree of effect of x_i on y; β_{ij} shows the interaction of x_i and x_j; ϵ is the vector of errors.

8. Use statistics to examine whether this optimum is a local or absolute optimum.

9. Analyze the effects of factors. From the final high-order surface response, the degree of the effect of x_i on y, and the interaction of x_i and x_j are determined according to the coefficients of β_i and β_{ij}. Student's t test serves to verify both the significance of these coefficients and the validity of this model.

Data regression and statistical analysis were analyzed using the Metlab software (The Math Works Inc., Mass. USA).

RESULTS AND DISCUSSION

Because the rate of transdermal permeation of a hydrophilic drug is low, and the half life of peptide/protein is short, prolonged administration is required in order to attain the therapeutic level. For some treatments using peptide/protein drugs, pulsatile administration is more efficient (10, 11). With iontophoresis, the protocols of treatment are easily designed and manipulated. In the present study, the main objective function of the system was chosen as the maximal rate of delivery of TRH during transdermal iontophoresis in the first six hours.

With a four-variable fractional factorial design, 2^{4-1} designed experiments were conducted with the pH of the buffer (x_1), the ionic strength of the donor solution (x_2), the frequency of applied pulsed current (x_3), and the duty cycle of the applied pulsed current(x_4) as independent variables. The current ampli-

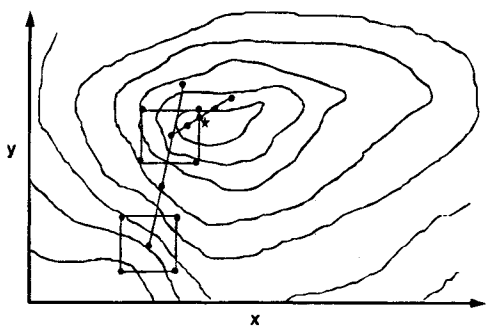

Fig. 1. Sequential search by using steepest ascent method (a first-order gradient projection search).

Table 1. Experimental Conditions of the First Set of the Two-level Factorial Design in the Iontophoretic Transdermal Delivery of TRH

Factor	pH (x_1)	ionic strength (x_2)	frequency 10^{x_3}	duty (x_4)	Permeation rate ($\mu g/hr/cm^2$)
center	6	0.1	1000	50	
unit	0.5	0.05	$10^{\pm 0.5}$	10	
	6.5	0.15	3000	60	
expl	(+1)	(+1)	(+1)	(+1)	7.9991
	6.5	0.15	300	40	
exp2	(+1)	(+1)	(−1)	(−1)	7.5298
	6.5	0.05	3000	40	
exp3	(+1)	(−1)	(+1)	(−1)	13.1642
	6.5	0.05	300	60	
exp4	(+1)	(−1)	(−1)	(+1)	45.4571
	5.5	0.15	3000	40	
exp5	(−1)	(+1)	(+1)	(−1)	9.8890
	5.5	0.15	300	60	
exp6	(−1)	(+1)	(−1)	(+1)	9.8404
	5.5	0.05	3000	60	
exp7	(−1)	(−1)	(+1)	(+1)	52.0100
	5.5	0.05	300	40	
exp8	(−1)	(−1)	(−1)	(−1)	20.9346

tude during iontophoresis was 0.6 mA. Because TRH began to protonate at pH 8 and became completely protonated at pH 4, the midpoint was chosen as a starting point for the pH value. When Burnette & Marrero (9) undertook iontophoresis for 12 h, a minimal buffer capacity of 0.6 M was required. Decreasing the duration of current applied, ionic strength 0.1M sufficed to maintain the variation of buffer pH within 1 pH unit during iontophoresis within 6 hr in the preliminary experiments. The starting point for design of the first two-level factorial experiment is summarized as follows:

donor solution pH 6 ± 0.5;
donor solution ionic strength 0.1 ± 0.05M;
pulse frequency 10^3 Hz, with increment $10^{\pm 0.5}$ Hz (i.e. $10^{2.5} \cong 300$Hz, $10^{3.5} \cong 3000$Hz);
current duty cycle 50 ± 10%.

Table 1 shows the conditions of eight experiments and their cumulative amount of transdermal permeation of TRH during iontophoresis with a pulsed current for six hours. The flux of TRH varied according to operating conditions. The donor solution at a low ionic strength ($x_2 = 0.05$) yielded greater permeation than it did at a high ionic strength ($x_2 = 0.15$). The variation was 7.5 to 52 $\mu g\ h^{-1}\ cm^{-2}$—an almost seven-fold variation. A low pH also increased the flux because a large portion of TRH was protonated. The flux of TRH, Y($\mu g/h\ cm^2$), was obtained according to a linear least-squares method:

$$Y = 20.9 - 2.3x_1 - 12.0x_2 - 0.1x_3 + 8.0x_4. \quad (3)$$

Equation 3 shows the result of mutiple regression based on all the independant variables. In order to check the validity of equation 3, forward stepwise regression was used. One-sided F test shows that the model which included only x_2 and x_4, $Y = 20.9\text{-}12.04\ x_2 + 8.0\ x_4$, had more than 95% statistical significance ($F = 7.56 > F(0.05, 2,5) = 5.7861$). If a 10% level was used for including variables, pH could be added to

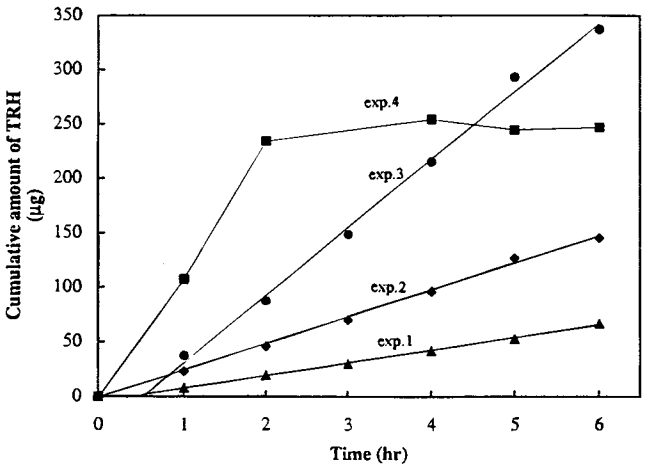

Fig. 2. Cumulative amount of TRH in the receptor cell versus the iontophoretic duration for the first path of steepest ascent in the factorial step-determining block. Experimental conditions are listed below:

expl: pH = 6.0, ionic strength = 0.10, frequency = 3000, and duty cycle = 50%.
exp2: pH = 5.9, ionic strength = 0.07, frequency = 2997.5, and duty cycle = 55%.
exp3: pH = 5.8, ionic strength = 0.04, frequency = 2995.0, and duty cycle = 60%.
exp4: pH = 5.7, ionic strength = 0.01, frequency = 2992.5, and duty cycle = 65%.

the model. $Y = 20.9\text{-}12.04\ x_2 + 8.0\ x_4 - 2.3\ x_1$ showed more than 90% statistical significance ($F = 4.486 > F(0.1, 3,4) = 4.19$). When x_3 included, the level of confidence that the regression equation predicts the responses better than the mean equals 76.3%. Equation (3) indicates that ionic strength (x_2) was the most important parameter controlling the transdermal iontophoretic permeation of TRH. Each variation of ionic

Table 2. Experimental Conditions of the Second Set of the Two-level Factorial Design in the Iontophoretic Transdermal Delivery of TRH

Factor	pH (x_1)	ionic strength (x_2)	frequency (10^{x_3})	duty (x_4)	Permeation rate ($\mu g/hr/cm^2$)
center	5.8	0.04	1000	60	
unit	1	0.01	0	10	
	6.8	0.05		70	
expl	(+1)	(+1)	1000	(+1)	99.2818
	6.8	0.05		50	
exp2	(+1)	(+1)	1000	(−1)	30.4187
	6.8	0.03		50	
exp3	(+1)	(−1)	1000	(−1)	72.2152
	6.8	0.03		70	
exp4	(+1)	(−1)	1000	(+1)	81.5601
	4.8	0.05		50	
exp5	(−1)	(+1)	1000	(−1)	73.9824
	4.8	0.05		70	
exp6	(−1)	(+1)	1000	(+1)	39.8792
	4.8	.0.03		70	
exp7	(−1)	(−1)	1000	(+1)	55.1866
	4.8	0.03		50	
exp8	(−1)	(−1)	1000	(−1)	47.4655

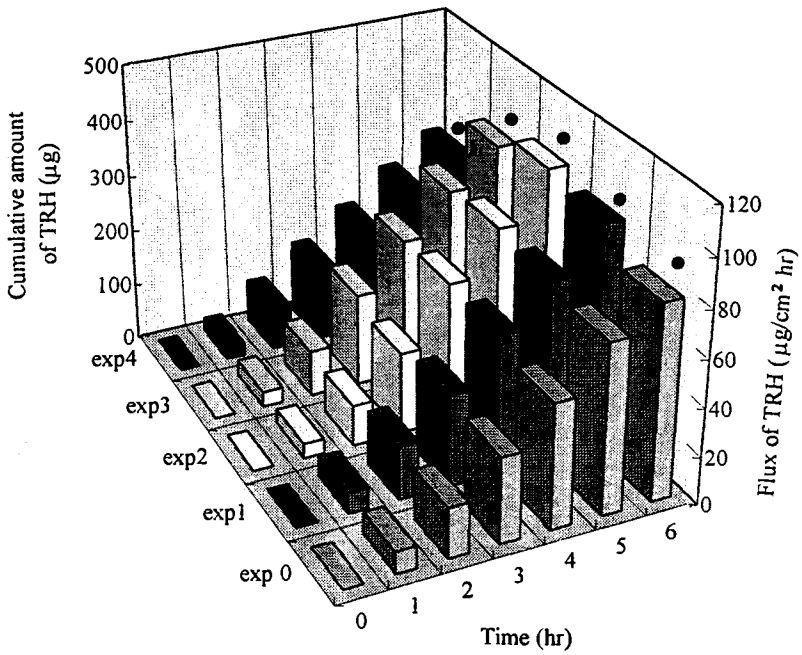

Fig. 3. Cumulative amount of TRH in the receptor cell versus the iontophoretic duration for the second path of steepest ascent in the factorial step-determining block. Experimental conditions are listed below:

exp0: pH = 5.8, ionic strength = 0.04, frequency = 1000, and duty cycle = 60%.
exp1: pH = 6.2, ionic strength = 0.039, frequency = 1000, and duty cycle = 65%.
exp2: pH = 6.6, ionic strength = 0.038, frequency = 1000, and duty cycle = 70%.
exp3: pH = 7.0, ionic strength = 0.037, frequency = 1000, and duty cycle = 75%.
exp4: pH = 7.4, ionic strength = 0.036, frequency = 1000, and duty cycle = 80%.

strength by one unit (0.05 M) caused a variation of 12 $\mu g\ h^{-1}\ cm^{-2}$ of the permeation flux of TRH. Next in importance was the duty cycle. The effect of frequency on transdermal iontophoretic drug delivery was insignificant, even in a large range of variation, 300~3000 Hz, as shown in Table 1.

Equation (3) also provides the path of steepest ascent toward the optimal objective function. Following procedure 3 outlined in the optimizing method, four additional experiments along the direction of steepest ascent were conducted to find a local maximum. A large rate of permeation was attained upon decreasing the donor pH, upon decreasing the ionic strength, or upon increasing the current duty cycle. Because of the small influence of frequency on TRH permeation, the frequency was kept constant for the succeeding experiments. From the starting point x^0 = (6, 0.1, 3000, 50), four more experiments along the direction of steepest ascent, $\nabla g(x^0)$, were conducted. The experimental conditions and results are shown in Figure 2. The greatest amount of average flux during six hours of iontophoresis (90.3 $\mu g\ h^{-1}\ cm^{-2}$) was in the third of this batch of experiments, at pH = 5.8, ionic strength = 0.04 M, and duty cycle = 60%. Because this value exceeded all the results in the first set of factorial designed experiments, it tended correctly to the maximum.

Table 2 presents the new two-level factorial experimental design and their corresponding rate of permeation with the third experiment (Exp 3) in the second batch as the center point: pH = 5.8, ionic strength = 0.04 M, and duty cycle = 60%, so x^1 = (5.8, 0.04, 1000, 60) was the starting point. Increments were (± 1, ± 0.01, ± 0, ± 10). Because the frequency was kept constant, full-factorial (2^3) design was conducted during this screening. The results appear in Table 2. In this region, Exp 1 had a maximal cumulative amount of permeation. The highest permeation rate of TRH was near 99.28 $\mu g\ h^{-1}\ cm^{-2}$, better than in the other experiments. The corresponding response surface in the first order obtained according to the method of least squares was as follows:

$$Y = 62.5 + 8.4x_1 - 1.6x_2 + 6.5x_4. \quad (4)$$

In these region (experiments), the influence of pH on the rate of the transdermal delivery of TRH by iontophoresis changed to a positive effect ($8.4\ x_1$) instead of a negative effect ($-2.3\ x_1$).

Along the second path of steepest ascent, $\nabla g(x^1)$, four experiments were performed in steps to test for the possibility of a local maximum. In these four experiments, the rate of permeation increased and then decreased as shown in Figure 3. The local optimal objective function was located at the second experiment, for which pH = 6.6, ionic strength = 0.038 M, and duty cycle = 70%. The optimal operating condition was certainly located in this region. To understand further the response surface of this region, a model of higher order was needed. Additional experiments near the center, x^1 = (5.8, 0.04, 1000, 60), including 6 star-shaped points and 3 set around the central point (where the star point was selected along each axis with increment ± 1.7 unit around the central point; hence, the central composite points were constructed on a spherical surface with radius 1.7, and x^1 as the center) were conducted to construct

Table 3. Experimental Conditions of Central Composite Designs for Determining the Response Surface of Second Order

Factor	pH (x_1)	ionic strength (x_2)	frequency	duty (x_4)	Permeation rate ($\mu g/hr/cm^2$)
center	5.8	0.04	1000	60	
unit	1	0.01	0	10	
	7.5	0.04		60	
exp9	(+1.7)	(+0)	1000	(+0)	62.2768
	4.1	0.04		60	
exp10	(−1.7)	(+0)	1000	(+0)	34.0817
	5.8	0.057		60	
exp11	(+0)	(+1.7)	1000	(+0)	31.0430
	5.8	0.023		60	
exp12	(+0)	(−1.7)	1000	(+0)	59.9229
	5.8	0.04		77	
exp13	(+0)	(+0)	1000	(+1.7)	83.1050
	5.8	0.04		43	
exp14	(+0)	(+0)	1000	(−1.7)	30.1156
	5.8	0.04		60	
exp15	(+0)	(+0)	1000	(+0)	96.5282
	5.8	0.04		60	
exp16	(+0)	(+0)	1000	(+0)	90.3171
	5.8	0.04		60	
exp17	(+0)	(+0)	1000	(+0)	83.9684
	5.8	0.04		60	
exp18	(+0)	(+0)	1000	(+0)	89.7350

the detailed response surface. With a central composite design about the central point, eight more experiments about the center were conducted. The experimental design and operating conditions are presented in Table 3. According to the experimental results and mathematical regression, the response surface of the second order was

$$Y = 89.72 + 8.05x_1 - 4.79x_2 + 10.01x_4 - 11.74x_1^2 - 12.67x_2^2 - 8.82x_4^2 - 4.91x_1x_2 + 12.57x_1x_4 + 1.71x_2x_4. \quad (5)$$

Optimal operating conditions were obtained by differentiating this equation. In the iontophoretic transdermal delivery of TRH with periodically pulsed current, the best operating conditions were pH = 7.0, ionic strength = 0.037 M, and duty cycle = 75%. The corresponding maximal rate of permeation of TRH delivered within six hours was 103.2 $\mu g\ h^{-1}\ cm^{-2}$.

To gain an understanding of the nature of the response surface, canonical analysis, described by Box and Draper (12), was used. Equation (5) becomes transformed to the canonical form

$$Y = 103.2 - 11.5\ Z_1^2 - 18.0\ Z_2^2 - 3.4\ Z_4^2 \quad (6)$$

Because all the coefficients of Z_i^2 are negative, an absolute maximal rate of permeation of TRH crossing the skin under iontophoresis was obtained. According to the covariance analysis, Table 4, F. = 3.2735 > F(0.05, 9, 5) = 3.1789; hence, the response quadratic model obtained for the response surface to describe the experimental data had 95% confidence. Statistical tests show that a response surface of second order was adequate to describe this complicated system regardless of the complexity of the transdermal iontophoresis, skin disparity, and experimental variation. There was a significant increase in the rate of permeation of TRH from 7.52 $\mu g\ h^{-1}\ cm^{-2}$ to 103 $\mu g\ h^{-1}\ cm^{-2}$, nearly a fourteen-fold increase after a systematic search using the response surface method. The experiments on the optimal point were conducted in triplicate for the final reconfirmation. Figure 4 shows the cumulative amount of TRH at the best operating conditions under iontophoresis, where the cumulatively permeating amount of TRH increased linearly with time.

Table 4. Covariance Analysis of the Resulting Quadratic Response Model

Source of variation	Sum of square	Degree of freedom	Mean square	F
Regression	7536.61	9	837.40	*3.2735*
Residual	2302.30	9	255.81	
Lack of fit	2223.21	6	370.54	*14.0544*
Pure error	79.09	3	26.36	

*R^ 2 = 0.7660, F(0.05,9,9) = 3.1789, F(0.05,6,3) = 8.9407.

CONCLUSIONS

Optimization via experiments based on the response surface method had been applied to seek the optimal operating conditions of the iontophoretic transdermal delivery of TRH under a periodically pulsed current mode. After two steps of search, the maximum rate of permeation of TRH crossing the skin was achieved at a low ionic strength, moderate pH and large current duty cycle, pH (x_1) = 7.0, ionic strength (x_2) = 0.037, and current duty cycle (x_4) = 75%, under the constraint of a pulsed current density of less than 1 mA/cm^2.

These results suggest that the response surface method is an effective method for maximizing the iontophoretic transdermal delivery rate of peptides. Another attractive feature of the response method is that conclusions can be drawn at each stage of the experiment, and that experiments can be terminated

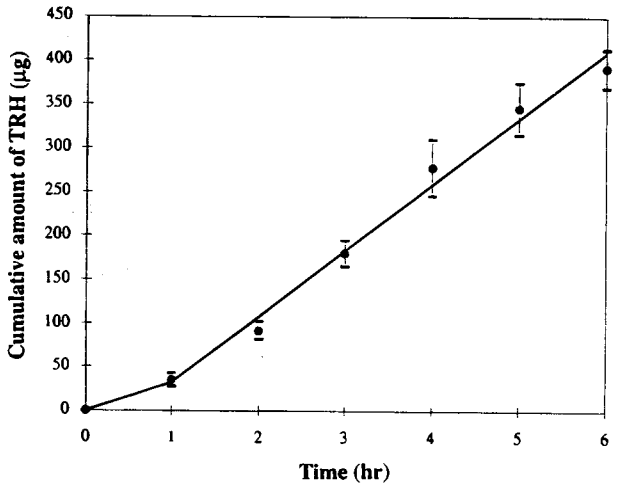

Fig. 4. Plot of the concentration of TRH in the receptor cell versus the iontophoretic duration at the maximal rate of permeation in transdermal iontophoresis facilitated by periodically pulsed current (mean ±standard error, n = 3).

whenever further investigation appears uneconomic. With this systematic search, an absolute maximal rate of delivery (corresponding to optimal operating conditions) was easily obtained. This method can also serve to find the best operating conditions of the transdermal delivery of other active drugs facilitated by periodically monophase-pulsed current iontophoresis to fulfill therapeutic requirements.

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REFERENCES

1. P. Wehrle, P. Nobelis, A. Cuine, and A. Stamm. Response Surface Methodology: An Interesting Statistical Tool for process Optimization and Validation: Example of Wet Granulation in a High-Shear Mixer. *Drug Dev. Ind. Pharm.*, **19**:1637–1653 (1993).
2. D. Vojnovic, M. Moneghini, and F. Rubessa. Optimization of Granulation in a High Shear Mixer by Mixture Design. *Drug Dev. Ind. Pharm.*, **20**:1035–1047 (1994).
3. B. Iskandarani, J. H. Clair, P. Patel, P. K. Shiromani, and R. E. Dempski, R.E., Simultaneous Optimization of Capsule and Tablet Formation Using Response Surface methodology. *Drug Dev. Ind. Pharm.*, **19**:2089–2101 (1993).
4. E. Senderak, H. Bonsignore, and D. Mungan. Response Surface methodology as an Approach to Optimization of an Oral Solution. *Drug Dev. Ind. Pharm.*, **19**:405–424 (1993).
5. Y. Y. Huang, S. M. Wu, C. Y. Wang, and T. S. Jiang. A strategy to optimize the operation conditions in iontophoretic transdermal delivery of pilocarpine, *Drug Dev. Ind. Pharm.*, **21**:1631–1648 (1995).
6. P. Wehrle and A. Stamm. Statistcal tools for process control and quality improvement in the pharmaceutical industry, *Drug Dev. Ind. Pharm.*, **20**:141–164 (1994).
7. Y. Y. Huang and S. M. Wu. Transdermal iontophoretic delivery of thyrotropin releasing hormone across excised rabbit pinna skin, *Drug Dev. Ind. Pharm.*, **21**: in press (1996).
8. M. Corbo, P. R. Wang, J. K. Li, and Y. W. Chien. Effect of propranolol on the myocardial contractility of normotensive and spontaneously hypertensive rabbits: relationship of pharmacokinetics and pharmacodynamics. *J. Pharmacokinetics and Biopharmaceutics*, **17**:551–570 (1989).
9. R. R. Burnette and D. Marrero. Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin. *J. Pharm. Sci.*, **75**:738–743 (1986).
10. J. B. Jaspan, E. Lever, K. S. Polonsky, and E. V. Cauter. In Vivo Pulsatility of Pancreatic Islet Peptides, *Am. J. Physiol.* **251**:E215–E226 (1986).
11. D. S. Weigle. Pulsatile Secretion of Fuel-Regulatory Hormones. *Diabetes.* **36**:764–775 (1987).
12. G. E. Box and N. R. Draper. *Emperical model building and response surfaces.* Wiley, New York, (1987).