Simultaneous Optimization Based on Artificial Neural Networks in Ketoprofen Hydrogel Formula Containing *O*-Ethyl-3-butylcyclohexanol as Percutaneous Absorption Enhancer

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ABSTRACT: The influence of the amounts of additives including 1-*O*-ethyl-3-*n*-butylcyclohexanol (OEBC), diisopropyl adipate (DIA), and isopropanol (IPA) on the penetration rate (R_p) of ketoprofen from hydrogels through rat skin *in vivo* was investigated. Skin irritation evoked by the application of hydrogels was evaluated based on a microscopic observation of skin cross-sections. Both optimization techniques incorporating an artificial neural network (ANN) and a second-order polynomial regression analysis were applied to the optimization of ketoprofen hydrogel formulations. Findings indicated that the R_p and total irritation score (TIS) of the skin were predicted quantitatively as a function of quantities of OEBC, DIA, and IPA, employing ANN. In contrast, the prediction ability of the polynomial regression equation was somewhat poorer compared with that of ANN. The observed results of R_p and TIS in the optimal formulation coincided well with the predictions in the simultaneous optimization technique incorporating ANN.
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Keywords: percutaneous absorption; absorption enhancers; hydrogel formulation; simultaneous optimization; artificial neural network

INTRODUCTION

Transdermal drug delivery has been considered to be an ideal route for drug administration. However, the stratum corneum barrier against foreign substances is the most difficult to overcome. The use of penetration enhancers is valuable and important for achieving therapeutic plasma levels for many drugs.[1] Diisopropyl adipate (DIA) has been reported to enhance the permeation of diclofenac,[2] indomethacin,[3] and hydrocortisone butyrate[4] through the skin. In a previous study,[5] a large number of chemical enhancers were synthesized by using L-menthol as a lead compound. Structure–activity and structure–toxicity relationship studies using several structure parameters revealed that the lipophilicity of molecules, the molecular size, and the affinity to the skin tissues were important factors for exhibiting enhancement activity as well as skin damage. Among these chemicals, 1-*O*-ethyl-3-*n*-butylcyclohexanol (OEBC, Figure 1) was found to be a promising compound with potent enhancement activity and relatively low skin irritation.[5] In addition, several studies have reported that the lipophilic penetration enhancer combination with a short-chain alkanol, such as propylene glycol and isopropanol (IPA), could produce a synergistic enhancement and decrease the skin irritation.[2,6–8] Furthermore, the enhancement potency of a branched alkanol, such as isopropanol, was stronger than that of *n*-alkanol.[9] The aim of the

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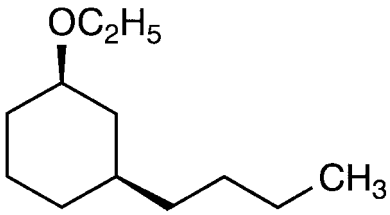

Figure 1. Chemical structure of 1-*O*-ethyl-3-butylcyclohexanol (OEBC).

present study was to evaluate the combined effect of penetration absorption as well as skin irritation in the ketoprofen hydrogel formulation incorporating OEBC, DIA, and IPA.

In the development of transdermal preparations, an important issue is to design an optimized pharmaceutical formulation that has appropriate penetration absorption with concomitant acceptable skin irritation. For this purpose, a computer optimization technique, based on a response surface method (RSM), has been widely used.[10] The prediction of pharmaceutical responses based on the second-order polynomial equation, commonly used in RSM, however, is often limited to low levels. This limitation may result in the poor estimation of optimal formulations. To overcome the shortcomings in RSM, we developed a multiobjective simultaneous optimization technique in which an artificial neural network (ANN) was incorporated.[11–13] ANN is a learning system based on a computational technique that can simulate the neurological processing ability of the human brain.[14] Application of ANN in the field of pharmaceutical development has gained interest in recent years.[15–17] The basic concept of simultaneous optimization of several pharmaceutical responses based on ANN has been fully described previously.[11–13] The optimization technique incorporating ANN was applied to the development of ketoprofen hydrogel, which has optimal percutaneous absorption as well as acceptable skin damage.

EXPERIMENTAL SECTION

Materials

Ketoprofen was purchased from Sigma (St. Louis, MO). Hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (HEC), and diisopropyl adipate (DIA) were obtained from Tokyo Kasei Chemical Industries (Tokyo, Japan). Isopropanol (IPA) was purchased from Wako Pure Chemical Industries (Osaka, Japan). OEBC was synthesized following the method described previously,[5] and was characterized by elemental analysis, nuclear magnetic resonance spectroscopy (NMR; Jeol GSX 270F, Tokyo, Japan), and gas chromatography (GC; Shimadzu GC-7A, Kyoto, Japan). The purity of this compound was a $> 99\%$.

Experimental Design and Hydrogel Preparation

To easily optimize the formulation and evaluate the influence of each additive on penetration absorption and skin irritation, a three-factor spherical second-order composite experimental design was used.[10] As shown in Table 1, 16 types of ketoprofen model formulations composed of HPC and HEC as gel bases, OEBC and DIA as penetration enhancers, and IPA and water as solvents were prepared. The quantities of OEBC (X_1), DIA (X_2) and IPA (X_3) were selected as causal factors. The amounts of ketoprofen, HPC, and HEC were fixed at 3, 1, and 1%, respectively. The total amount of each hydrogel was adjusted to 100% by the addition of water. HPC and HEC were dissolved in water. Ketoprofen was dissolved in IPA containing OEBC and DIA, separately. Then, the both components were mixed well, and the resulting hydrogels were stored in air-tight containers at room temperature prior to use.

The three-factor spherical second-order composite experimental design shown in Table 1 requires a total of 16 experiments including a repetition of the center experiment. The first eight experiments represented a factorial design for three factors at two levels, resulting in 2^3 trials. Three additional levels were selected: zero represented the base level midway between the aforementioned levels and the positive and negative 1.732 values represented the extreme values. Each experimental point in this design was located at the same distance from the center experiment. In our previous studies,[2,5] OEBC and DIA showed better enhancement activity and moderate irritation to the skin at 1 and 2.5%, respectively, in the hydrogel formulations; therefore, the amounts of 1% OEBC, 2.5% DIA, and 30% IPA were used as the base levels, and 0–2, 0–5, and 20–40%, respectively, as the extreme values.

Percutaneous Absorption Study

Male Wistar rats weighing 180–200 g were anesthetized with a 25% carbamic acid ethyl ester

Table 1. Experimenal Design and Model Formulations of Ketoprofen Hydrogels[a]

Formulation	X_1	OEBC (%)	X_2	DIA (%)	X_3	IPA (%)
1	−1	0.42	−1	1.06	−1	24.23
2	−1	0.42	−1	1.06	1	35.77
3	−1	0.42	1	3.94	−1	24.23
4	−1	0.42	1	3.94	1	35.77
5	1	1.58	−1	1.06	−1	24.23
6	1	1.58	−1	1.06	1	35.77
7	1	1.58	1	3.94	−1	24.23
8	1	1.58	1	3.94	1	35.77
9	−1.732	0	0	2.50	0	30.00
10	1.732	2.00	0	2.50	0	30.00
11	0	1.00	−1.732	0	0	30.00
12	0	1.00	1.732	5.00	0	30.00
13	0	1.00	0	2.50	−1.732	20.00
14	0	1.00	0	2.50	1.732	40.00
15	0	1.00	0	2.50	0	30.00
16	0	1.00	0	2.50	0	30.00

[a]The amounts of ketoprofen, HPC, and HEC were fixed at 3, 1, and 1%, respectively. The total amount of each hydrogel was adjusted to 10.00 g by addition of water.

solution (3 mL/kg, intraperitoneally) and secured on their backs. Their abdominal hair was gently removed with an electric clipper. A glass cell with a 16-mm inner diameter (i.d.) and 10 mm in height, was attached on the shaved abdominal skin with cyanoacrylate type adhesive (Aron Alpha A, Sankyo Company, Ltd., Tokyo, Japan) and filled with the test hydrogel (1 mL) under occlusive conditions. Blood samples (500 μL) were taken via the jugular vein at 1, 2, 4, 6, and 8 h after topical application. Each blood sample was centrifuged for 1 min at 13,000 rpm, and the plasma sample (100 μL) was mixed with methanol (300 μL) containing *p*-hydroxybenzoate-*n*-butyl ester (3 μL/mL) as an internal standard. The mixture was centrifuged (13,000 rpm, 1 min) again to precipitate the denatured proteins. Then, the supernatant solution was filtered through a disposable filter unit (Sample prep LCR4(T)-LG, Japan Millipore, Yonezawa, Japan). The concentration of ketoprofen in the filtrate was analyzed using a high-performance liquid chromatography (HPLC) system (Shimadzu, LC-5A, Kyoto, Japan) equipped with a variable wavelength ultraviolet (UV) monitor (Shimadzu, SPD-6A). The other analytical conditions were as follows: column, YMC-Pack ODS-A, 150×4.6 mm i.d. (YMC, Tokyo, Japan); UV detection, 254 nm; mobile phase, 0.057% phosphoric acid/methanol (35:65); and flow rate, 1 mL/min. The retention times of ketoprofen and internal standard were 6.6 and 9.3 min, respectively.

To evaluate the percutaneous absorption of experimental formulations through rat skin, the rate of penetration (Rp) of ketoprofen was estimated from a pharmacokinetic model based on the assumption that the rate of penetration of ketoprofen absorbed from the hydrogel is constant after a lag time:[11]

$$C = \frac{R_p}{V_d k_{10}} \left\{1 + \frac{\beta - k_{10}}{\alpha - \beta} e^{-\alpha(t - t_L)} + \frac{k_{10} - \alpha}{\alpha - \beta} e^{-\beta(t - t_L)}\right\} \quad (1)$$

where C is the plasma concentration, Rp is the rate of penetration, t is time, t_L is the lag time, Vd is the distribution volume of the central compartment, k_{10} is the elimination rate constant from the central compartment, and α and β are the hybrid first-order rate constants. The mean values of V_d, k_{10}, α, and β, estimated previously, were used to determine Rp values.[11]

Evaluation of Skin Irritation

Irritation evoked by model formulations on rat skin was microscopically judged at the end of experiments on penetration absorption. The site of application of each formula on the skin was excised and fixed in 10% neutral carbonated-buffered formalin for at least 24 h before routine processing. Then the skin was cut vertically at the

central region at a width of 4 mm. Each section was dehydrated using a graded series of ethanol solution and embedded in paraffin wax. Tissues were divided into small pieces (~3 μm in thickness) and stained with hematoxylin and eosin. All sections were examined by light microscopy (Optiphoto, Nikon, Tokyo, Japan).

The microscopic findings were graded as five levels of irritation from no change (level 0) to a marked change (level 4) including the liquefaction of epidermis, edema of subepidermis, collagen fiber swelling, and inflammatory cell infiltration in both the dermis and hypodermis, as well as degeneration of skin appendages.[18] The total irritation score (TIS) was obtained by summation of each irritation score and used as an index of skin damage caused by the application of ketoprofen hydrogel.[18]

Data Analysis and Computer Programs

Details of simultaneous optimization methods incorporating ANN were fully described previously.[13] A typical flow of the optimization technique incorporating ANN is shown in Figure 2. The multi-objective simultaneous optimization, the last process in Figure 2, was performed according to the generalized distance function method:[13]

$$S(X) = \left[\sum_{i=1} \left\{\frac{\mathrm{FD}_i(X) - \mathrm{FO}_i(X)}{\mathrm{SD}_i}\right\}^2\right]^{1/2} \quad (2)$$

where $S(X)$ is the distance function generalized by the standard deviation, SD_i, of the observed values for each response variable, $FD_i(X)$ is the optimum values of each response optimized individually over the experimental region, and $FO_i(X)$ is the simultaneous optimum value.

In approximations of response variables based on the classical response surface method, the second-order polynomial regression equation defined in eq. 3 was employed:

$$F(X) = \beta_0 + \sum_{i=1} \beta_i X_i + \sum_{i=1} \sum_{j=1} \beta_{ij} X_i X_j \quad (3)$$

where $F(X)$ is the predicted response variable, β_0 is a constant, and β_i and β_{ij} are coefficients of each monomial, and X_i and X_j are the causal factors. The optimum regression equation composed of the combination of statistically significant factors was obtained by investigating the overall combination of factors. The best combination of factors for the prediction of each response was selected from among $2_m - 1$ kinds of regression equations (where m is the number of all factors including single, square, and interaction terms). The coefficient of determination, which was doubly adjusted with degrees of freedom (r^{**2}), was used as an index for selection of the optimum combination of factors:

$$r^{**2} = 1 - \frac{(n-1)(n+p+1)}{(n+1)(n-p-1)}(1-r^2) \quad (4)$$

where n is the number of data pairs, p is the number of factors, and r is a multiple correlation coefficient.

Computer programs executable in Windows 2000, incorporating ANN as well as the polynomial regression analysis and written by us, were used for the simultaneous optimization of ketoprofen hydrogel formulation. An extended Kalman filter algorithm was employed as a training algorithm for ANN.[19] Unlike the conventional algorithms, such as the conjugate gradient method, we can greatly reduce the number of training by using the extended Kalman filter;[19] thus, the iteration of training was set at the most to 1000 to avoid over-training problems. To optimize the structure of ANN, a simulated

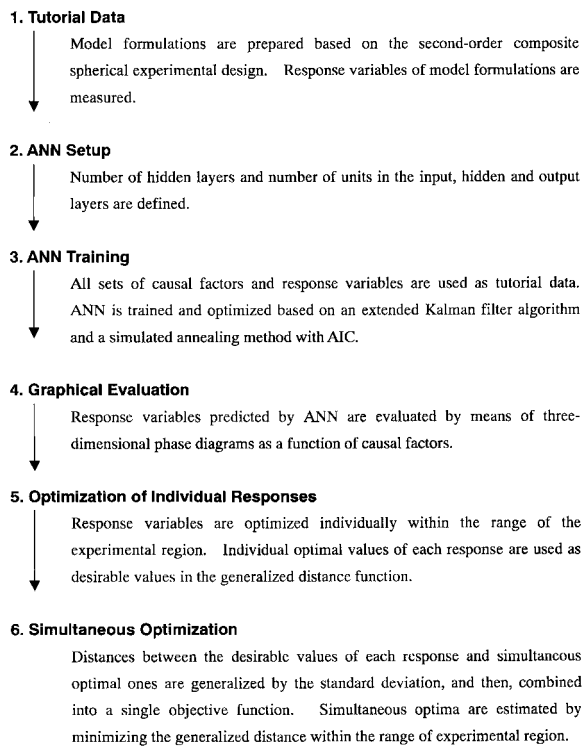

Figure 2. Flow of the multi-objective simultaneous optimization incorporating ANN and the second-order polynomial regression equation.

annealing technique was employed.[20] As a judging standard to evaluate the optimality of ANN, Akaike's information criterion (AIC) was used:

$$\mathrm{AIC} = n \times \ln(\mathrm{SS}) + 2 \times nw \qquad (5)$$

where n is the number of data pairs, nw is the number of weights in ANN, and SS is the residual sum of squares.

The curve-fitting program MULTI[21] was modified to be executable in Windows 2000 and used for estimating R_p values in eq. 1 along with the standard deviation, SD, of the estimate.[21]

RESULTS AND DISCUSSION

Percutaneous Absorption

The values of R_p in the model formulations are listed in Table 2. A significant difference in R_p values was seen in the model formulations. The highest penetration rate (formulation 6) was ~18-fold greater than that of the lowest (formulation 1), suggesting that the concentration of additives greatly affected the absorption of ketoprofen through rat skin. Comparing formulations 9, 10, 15, and 16, it is evident that increasing the amount of OEBC from 0 to 2% led to an increase in the penetration rate. In contrast, the penetration rate decreased when the concentration of DIA increased from 0 to 5% (formulations 11, 12, 15, and 16). Although previous studies indicated that DIA induced an enhancement action to the percutaneous absorption of many drugs,[2–4] such DIA activity was not observed in this mixture system containing OEBC and IPA. In addition, the penetration rate increased with increasing amounts of IPA (from 20 to 40%; formulations 13, 14, 15, and 16).

Skin Damage

To evaluate the influence of additives on the skin irritation, the skin 8 h after application of the ketoprofen hydrogels was pathologically investigated. For example, formulation 3 gave almost no irritation except very slight inflammatory cell infiltration in epidermis, as can be seen in Figure 3A. On the other hand, formulation 11 was highly irritating, which can easily be seen in the photo shown in Figure 3B of the rat skin

Table 2. Experimental Values of Response Variables

Formulation	R_p (μg/h)	TIS	Formulation	R_p (μg/h)	TIS
1	52.0 (11.3)[a]	2	9	111.1 (18.3)	1
	40.0 (5.3)	1		62.0 (29.4)	1
	44.6 (6.5)	2		74.5 (10.8)	1
2	414.4 (30.9)	4	10	387.6 (22.5)	8
	271.2 (19.5)	4		619.9 (26.8)	9
	267.1 (27.4)	4		287.3 (39.5)	7
3	119.0 (19.3)	1	11	628.4 (28.8)	11
	118.7 (10.7)	1		627.2 (41.3)	10
	80.9 (21.4)	1		585.2 (41.4)	12
4	122.6 (14.7)	1	12	280.4 (15.2)	2
	196.6 (8.7)	2		224.2 (9.7)	2
	170.5 (10.8)	2		188.1 (10.0)	1
5	275.6 (10.3)	3	13	218.2 (16.6)	2
	249.3 (8.5)	4		342.2 (26.5)	2
	269.0 (12.1)	5		185.8 (8.3)	2
6	605.5 (14.7)	8	14	500.1 (23.7)	6
	806.8 (75.5)	9		441.7 (51.1)	8
	1098.6 (92.4)	9		574.6 (74.5)	9
7	290.4 (20.7)	4	15	368.2 (17.1)	6
	338.7 (51.9)	3		266.8 (3.9)	4
	224.8 (10.1)	3		440.7 (31.8)	7
8	503.4 (29.8)	9	16	418.3 (17.7)	6
	636.7 (51.8)	9		438.9 (39.7)	6
	661.0 (44.6)	11		474.5 (60.2)	5

[a] SD value of R_p estimated in eq. 1.[21]

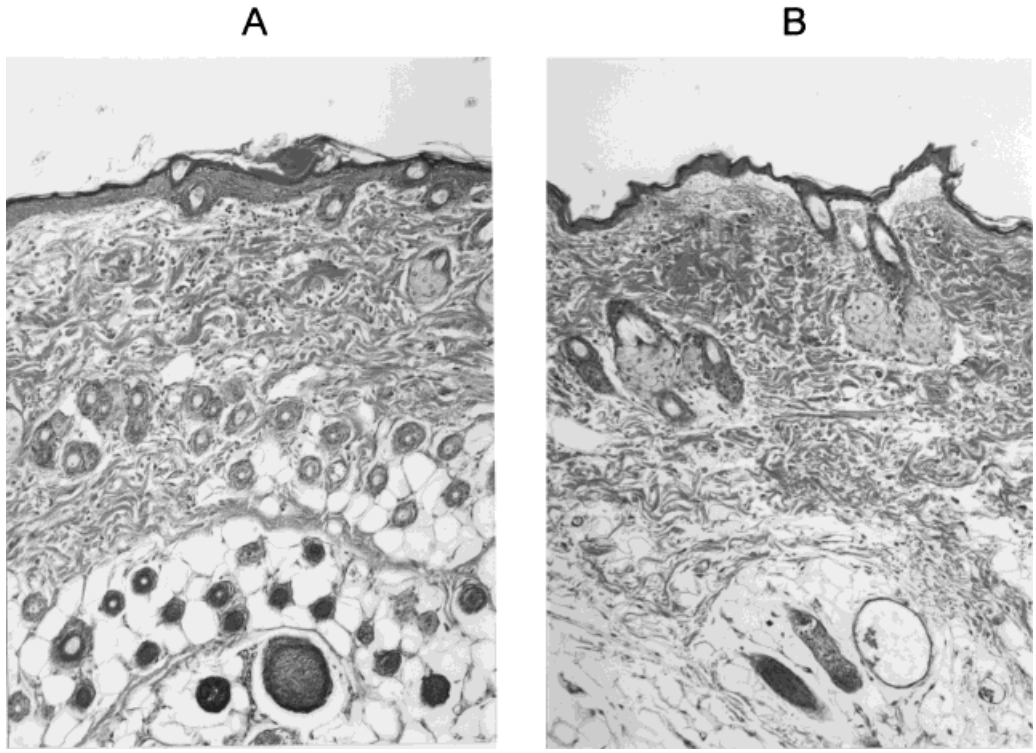

Figure 3. Microscopic photos of rat skin 8 h after application of model formulations (H & E stain, ×100; A, formulation 3; B, formulation 11). Photo A shows almost no irritation except very slight inflammatory cell infiltration in epidermis (level 1). Photo B shows moderate epidermis liquefaction (level 3), subepidermis edema (level 3), slight collagen fiber swelling in dermis (level 2) and hypodermis (level 2), and very slight inflammatory cell infiltration in dermis (level 1).

exposed to formulation 11. The photo shows moderate epidermis liquefaction and subepidermis edema and slight collagen fiber swelling of dermis and hypodermis. Findings of total irritation score (TIS) of rat skin are summarized in Table 2. TIS values of these formulations showed significant differences (from 1 to 12), demonstrating that skin damage caused by ketoprofen hydrogel were greatly influenced by the amounts and types of additives. It is generally accepted that penetration enhancers causes skin damage accompanied by an increase in the transdermal penetration rate.[1] The same phenomena were seen in the present study. Compared with formulations 9, 10, 15, and 16, and formulations 13, 14, 15, and 16, respectively, the TIS values of the skin increased with increasing amounts of penetration enhancer OEBC (0–1%) and solvent IPA (20–40%) in hydrogel formulation, as observed from the R_p values. In contrast, lower skin irritation was observed when the hydrogel incorporated large amounts of DIA (formulations 11, 12, 15, and 16).

Prediction of Response Variables

It is impossible to understand and clarify the combined effect of causal factors on the response variables using a normal analysis based on a one-factor-at-a-time experiment. Therefore, ANN was applied to the prediction of response variables as a function of causal factors. The second-order polynomial regression equation was used for comparing the prediction ability.

Three causal factors corresponding to different levels of OEBC, DIA, and IPA were used as each unit of the input layer of ANN. Response variables, R_p and TIS, were predicted individually by the different sets of ANN. A single layer was employed as the hidden layer in ANN. A set of causal factors and response variables (48 data pairs) was used as tutorial data for ANN. To optimize the structure of ANN, the simulated annealing technique[20] was applied, employing AIC as a judging standard. The AIC values of ANN that were trained for the prediction of R_p and TIS as a function of the number of units in the

hidden layer are shown in Figure 4. To achieve reasonable prediction of both R_p and TIS, 3 units were appropriate as the number of units in the hidden layer. The result suggested that 12 unknown parameters (3 input units, 3 hidden units, and 1 output unit; i.e., $3 \times 3 + 3 \times 1 = 12$) were required to fit as the weights of ANN for the prediction of both R_p and TIS. On the other hand, the second-order polynomial equation, defined in eq. 3, requires estimation of 10 unknown parameters at the most as regression coefficients of the polynomial equation. To enable an impartial comparison of the prediction ability between ANN and the second-order polynomial equation, we employed the coefficient of determination, which was doubly adjusted with degrees of freedom (r^{**2}), defined in eq. 4. As a result, predicted values of R_p and TIS based on the ANN coincided well with the experimental values ($r = 0.936$ and $r^{**2} = 0.793$ for R_p; $r = 0.978$ and $r^{**2} = 0.927$ for TIS). However, approximations of R_p and TIS values based on the second-order polynomial regression equation (Table 3) were somewhat poorer ($r = 0.898$ and $r^{**2} = 0.751$ for R_p; $r = 0.906$ and $r^{**2} = 0.759$ for TIS).

To estimate the significant levels of each causal factor in the prediction of responses in ANN, we developed a novel method based on a Monte Carlo approach. First, a large number of factor sets (X_n) except the factor under test (X_{test}) are generated by means of an arithmetic random number within the range of the experimental region. However, X_{test} is generated sequentially within the range of the experimental region for at least 100 steps. Namely, a large number of data sets for X_n (at least $100 \times n$ sets) are generated randomly at every step of the X_{test} values. The values of each response are estimated at every step of X_{test}, and then, the standard deviation is calculated.

Table 3. Optimal Regression Equation for Each Response Variable Determined by Multiple Regression Analysis

Regression Coefficient	R_p	TIS
b_0 (constant)	389	5.73
b_1 (X_1)	140	2.10
b_2 (X_2)	−69.5	−1.35
b_3 (X_3)	119	1.70
b_{11} (X_1X_1)	−49.5	−0.591
b_{12} (X_1X_2)	—[a]	0.417
b_{12} (X_1X_3)	69.9	1.00
b_{22} (X_2X_2)	—	—
b_{23} (X_2X_3)	−59.0	—
b_{33} (X_3X_3)	—	−0.480
r[b]	0.898	0.906
s[c]	106	1.53
F_0[d]	28.4[e]	26.1[e]

[a]Not included in the optimum regression equation.
[b]Multiple correlation coefficient.
[c]Standard deviation of residual.
[d]Observed F value.
[e]$P < 0.01$.

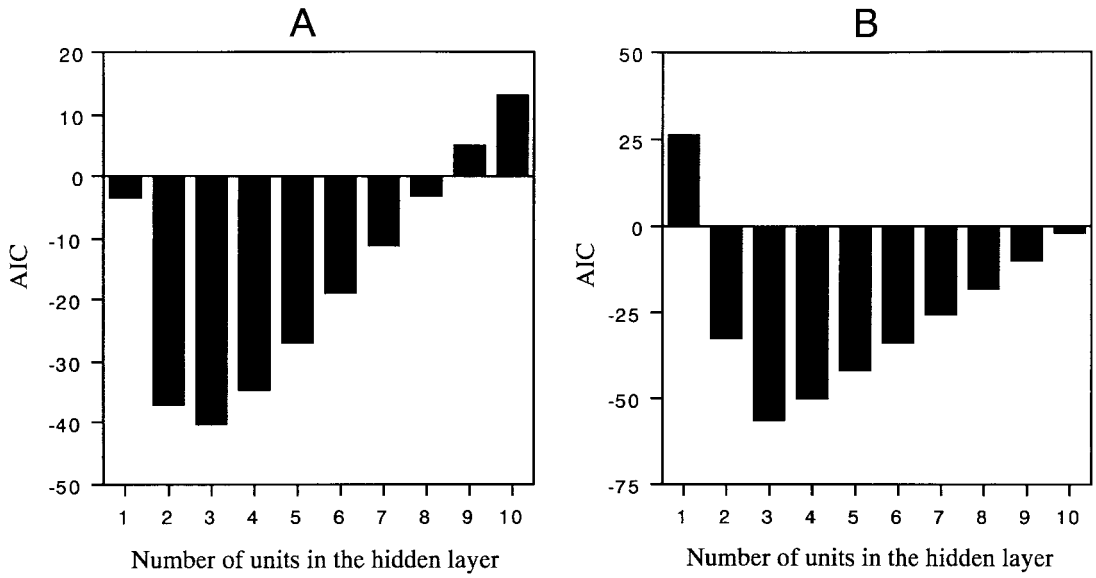

Figure 4. Akaike's information criterion (AIC) values of ANN trained for the prediction of penetration rate (Rp) and total irritation score (TIS) as a function of the number of units in the hidden layer: (A) Rp; (B) TIS.

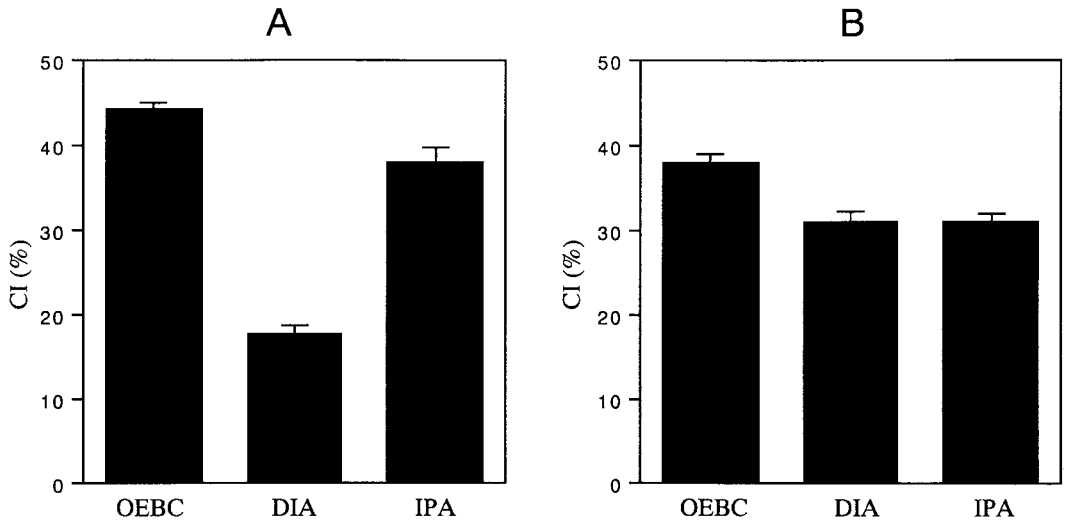

Figure 5. Contribution index (CI) values of causal factors in ANN prediction of penetration rate (Rp) and total irritation score (TIS). The mean ± SD for 10 trials.

Finally, the mean of standard deviations in the response values calculated at all steps of X_{test} is defined as the contribution index (CI). CI values are affected only by the change in X_{test}, because the effect of X_n variation on the response variables is thought to be equivalent in every step of the X_{test} values. It would be possible to compare the CI values directly for understanding the degrees of contribution of each factor in the prediction of responses. As depicted in Figure 5, the CI value of OEBC in the prediction of R_p was the highest among the causal factors, suggesting that the quantities of OEBC is the most important to promote percutaneous absorption of ketoprofen in the hydrogel formulation. In contrast, DIA is less effective for the enhancement of percutaneous absorption. In the prediction of TIS, the degrees of contribution were comparable among all factors.

The response surfaces generated by ANN and a second-order polynomial equation are shown in Figures 6 and 7, respectively, as a function of X_1 (the amounts of OEBC) and background factors [sum of the amounts of DIA (X_2) and IPA (X_3)]. The response surfaces generated by ANN clearly demonstrated the combined effect of OEBC and the other factors on both R_p and TIS values.

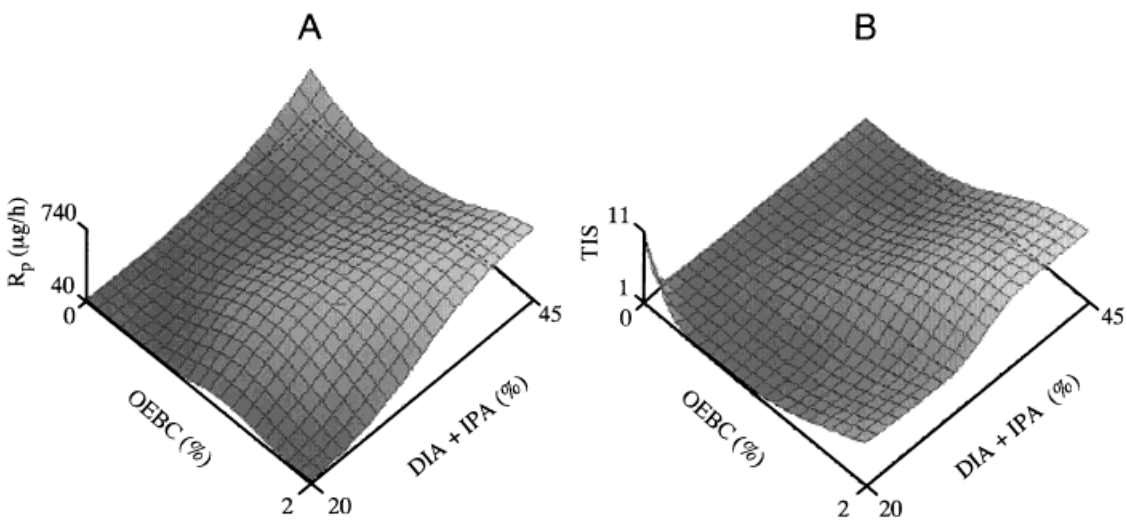

Figure 6. Response surfaces of penetration rate (Rp) and total irritation score (TIS) predicted by ANN as a function of amounts of OEBC and background factors (DIA + IPA): (A) Rp; (B) TIS.

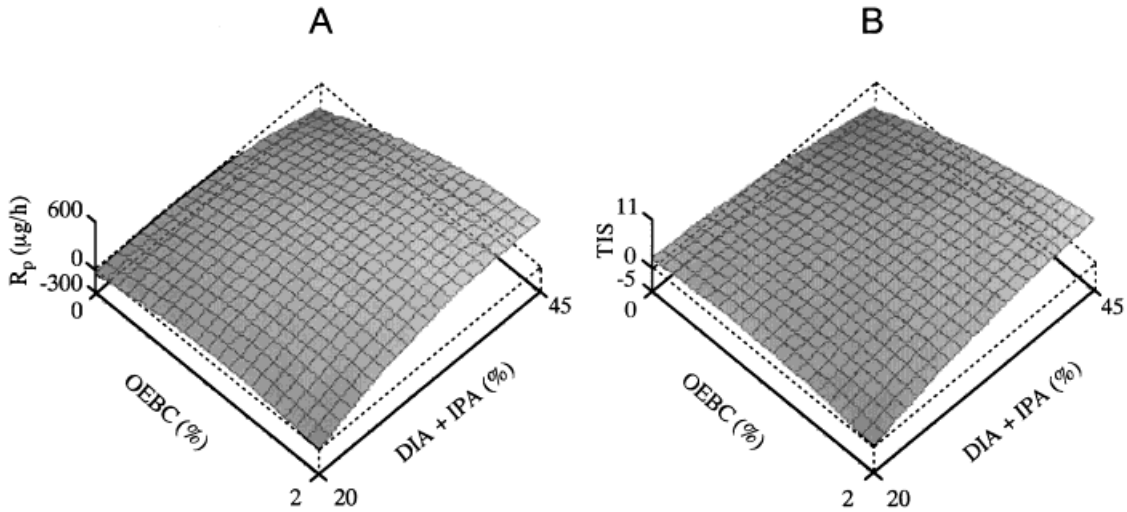

Figure 7. Response surfaces of penetration rate (Rp) and total irritation score (TIS) predicted by the second-order polynomial regression equation as a function of amounts of OEBC and background factors (DIA + IPA): (A) Rp; (B) TIS.

Furthermore, the nonlinear relationship between the factors and the responses was represented well with the response surface predicted by ANN (Figure 6). However, a second-order polynomial equation exhibited relatively plain surfaces (Figure 7). Furthermore, polynomial equation analysis predicted negative values in the boundary region of the experimental limits that were out of physical reality. Generally, the quantitative relationships between causal factors and response variables *in vivo* are thought to be complex and nonlinear. It was suggested that ANN was more useful than the polynomial equation in cases when approximations of such relationships were required.

Simultaneous Optimization

Simultaneous optimization of ketoprofen hydrogel was performed according to the generalized distance function defined in eq. 2. The optimal values of individual response variables, $FD_i(X)$, were calculated before simultaneous optimization was carried out (i.e., the individual maximum R_p and the minimum TIS values, respectively). Results of the simultaneous optima are given in Table 4. The concentration of OEBC predicted by ANN was 2-fold greater than that estimated by the polynomial equation. However, no significant difference was seen between ANN and the polynomial regression equation in the optimal concentrations of DIA and IPA. The R_p and TIS values predicted by ANN coincided well with the experimental values, whereas the approximations based on the polynomial equation were somewhat poorer (Table 5). Furthermore, the optimal formulation predicted by ANN exhibited the most

Table 4. Optimal Formulations Predicted by ANN and the Second-Order Polynomial Regression Equation

Factor, %	ANN	Polynomial Equation
OEBC	1.15	0.610
DIA	1.15	0.923
IPA	31.1	36.7

Table 5. Predicted and Experimental Values of Response Variables of Optimal Formulations Based on ANN and the Second-Order Polynomial Regression Equation

	ANN		Polynomial Equation	
Response	Prediction	Experimental[a]	Prediction	Experimental[a]
R_p (μg/h)	621	623±73	506	399±31
TIS	4	4±1	6	4±1

[a]Mean±SD for three determinations.

prominent activity on the percutaneous absorption of ketoprofen despite the finding that the skin damage was sufficiently low. It is obvious that it would be impossible to reach the exact combination of causal factors using a normal analysis based on a one-factor-at-a-time experiment.

CONCLUSIONS

It is generally recognized that the strong percutaneous absorption action can be obtained by increasing the concentrations of the chemical enhancers, but this increase often causes significant skin damage. However, the findings in the present study clearly demonstrate that strong enhancement action as well as the low skin damage can be attained by seeking the optimal combination of enhancers and other additives in the formulation. In future investigations, the possible differences in the optimum formulations between human and other animals should be evaluated. However, with respect to preformulation studies, optimization of ketoprofen hydrogel containing OEBC, DIA, and IPA was successfully accomplished by means of the optimization technique incorporating ANN.

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REFERENCES

1. Cooper ER. 1984. Increased skin permeability for lipophilic molecules. J Pharm Sci 73:1153–1156.
2. Okuyama H, Ikeda Y, Kasai S, Inamori K, Takayama K, Nagai T. 1999. Influence of diisopropyl adipate on percutaneous absorption and subcutaneous tissue penetration of diclofenac from alcoholic gel ointment. Yakuzaigaku 59:75–83.
3. Inagi T, Muramatsu T, Terada H. 1986. Interaction of indomethacin with the vehicle component diisopropyl adipate. Chem Pharm Bull 34:1228–1234.
4. Ozawa Y, Yamahira T, Sunada H, Nadai T. 1988. Influence of fatty acid-alcohol esters on percutaneous absorption of hydrocortisone butyrate propionate. Chem Pharm Bull 36:2145–2151.
5. Obata Y, Sato H, Li CJ, Takayama K, Higashiyama K, Nagai T, Isowa K. 2000. Effect of synthesized cyclohexanol derivatives using L-menthol as a lead compound on the percutaneous absorption of ketoprofen. Int J Pharm 198:191–200.
6. Goodman M, Barry BW. 1989. Lipid-protein-partitioning (LPP) theory of skin enhancer activity: Finite dose technique. Int J Pharm 57:29–40.
7. Arellano A, Santoyo S, Martin C, Ygartua P. 1998. Influence of propylene glycol and isopropyl myristate on the *in vitro* percutaneous penetration of diclofenac sodium from carbopol gels. Eur J Pharm Sci 7:129–135.
8. Goldberg-Cettina M, Liu P, Nightingale J, Kurihara-Bergstrom T. 1995. Enhanced transdermal delivery of estradiol *in vitro* using binary vesicles of isopropyl myristate and short-chain alkanols. Int J Pharm 114:237–245.
9. Kim YH, Ghanem AH, Mahmoud H, Higuchi WI. 1992. Short chain alkanols as transport enhancers for lipophilic and polar/ionic permeants in hairless mouse skin: Mechanism(s) of action. Int J Pharm 80:17–31.
10. Khuri AI, Cornell JA. 1978. Response surface: design and analysis. New York: Marcel Dekker. pp. 116–140.
11. Takayama K, Nagai T. 1991. Simultaneous optimization for several characteristics concerning percutaneous absorption and skin damage of ketoprofen hydrogels containing *d*-limonene. Int J Pharm 74:115–126.
12. Takahara J, Takayama K, Isowa K, Nagai T. 1997. Multi-objective simultaneous optimization based on artificial neural network in a ketoprofen hydrogel formula containing *O*-ethylmenthol as a percutaneous absorption enhancer. Int J Pharm 158:203–210.
13. Takayama K, Fujikawa M, Nagai T. 1999. Artificial neural network as a novel method to optimize pharmaceutical formulations. Pharm Res 16:1–6.
14. Achanta AS, Kowalski JG, Rhodes CT. 1995. Artificial neural networks: Implications for pharmaceutical sciences. Drug Dev Ind Pharm 21:119–155.
15. Hussain AS, Yu X, Johnson RD. 1991. Application of neural computing in pharmaceutical product development. Pharm Res 8:1248–1252.
16. Hussain AS, Johnson RD, Vachharajani N, Ritschel WA. 1993. Feasibility of developing a neural network for prediction of human pharmacokinetic parameters from animal data. Pharm Res 10:466–469.

17. Smith BP, Brier ME. 1996. Statistical approach to neural network model building for gentamicin peak prediction. J Pharm Sci 85:65–69.
18. Quan D, Takayama K, Mitsuzono T, Isowa K, Nagai T. 1991. Influence of novel percutaneous absorption enhancers, cyclohexanone and piperidone derivatives, on histopathology of rat skin. Int J Pharm 68:239–253.
19. Murase H, Koyama S, Honami N, Kuwabara T. 1991. Kalman filter neuron training. Bull Univ Osaka Pref Ser B 43:91–101.
20. Kirkpatrick S, Gelatt CD, Vecchi MP. 1983. Optimization by simulated annealing. Science 200:671–680.
21. Yamaoka K, Tanigawara Y, Nakagawa T, Uno T. 1981. A pharmacokinetic analysis program [MULTI] for microcomputers. J Pharmacobio-Dyn 4:879–885.