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Simultaneous optimization for several characteristics concerning percutaneous absorption and skin damage of ketoprofen hydrogels containing *d*-limonene

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Summary

A simultaneous optimization technique for multi-objective problems was applied for the designing of a formulation for a gel ointment of ketoprofen (KPF) containing *d*-limonene and ethanol as absorption enhancers. The generalized distance function was employed for dealing with all the responses simultaneously. Pharmacokinetic parameters of KPF percutaneously absorbed from model formulations were determined in rats as prime response variables. The skin damage evoked by each formulation was microscopically judged and graded as the response variable concerned with safety to the skin. These response variables were predicted by multiple regression equations composed of a combination of the concentrations of *d*-limonene and ethanol as formulation factors. The regression equations for response variables were assembled as a simultaneous optimization problem. Experimental results obtained for the optimum formulation agreed well with the predictions, suggesting the usefulness and reliability of the optimization techniques based on a generalized distance function in which the impartiality among the responses was considered.

Introduction

Transdermal drug delivery has been considered to be a desirable route for drug administration. Many reports are available concerning the basic investigation of absorption promoters in order to improve the permeability of drugs through the skin (Cooper, 1984; Woodford and Barry, 1986; Okamoto et al., 1988; Sugibayashi et al., 1988; Quan et al., 1989, 1990). In previous works (Okabe et al., 1989, 1990), we reported the promoting effect of cyclic monoterpenes present in essential oils on the percutaneous absorption of anti-inflammatory drugs such as indomethacin (IMC) and ketoprofen (KPF). Percutaneous absorptions of these drugs were remarkably enhanced on addition of hydrocarbons such as menthane, limonene, terpinene and terpinolene. Recently we have also performed the optimization of the formulation for gel ointments of IMC containing *d*-limonene as an absorption enhancer

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(Takayama et al., 1990). The gel ointment of IMC containing *d*-limonene could be reasonably well optimized by means of the computer optimization method in which an adaptive least-squares procedure has been incorporated.

A computer optimization technique, based on response surface methodology (Box et al., 1978), has proven to be a useful approach for selecting pharmaceutical formulations (Fonner et al., 1970; Schwartz et al., 1973; Takayama et al., 1983, 1985, 1990; Fenyvesi et al., 1984; Takai et al., 1984; Akitoshi et al., 1985; Franz et al., 1987; McLeod et al., 1988; Takayama and Nagai, 1989). Basically, the optimization methods include factorial experimental design, multiple regression analysis, and mathematical optimization algorithms for seeking the best formulation under a set of restrictions. Factorial experimental designs can be applicable for preparing systemic model formulations which are composed of several causal factors. The response variables of these model formulations are predicted quantitatively from a combination of these factors by means of multiple regression analysis. Finally, optimization algorithms are applied for deciding the best formulation. As is typical in optimization problems, the individual optima for different responses are not the same. Therefore, the optimum formulation has to be taken as an acceptable one which will sufficiently satisfy the primary objective under a set of various constraints.

In a practical situation, however, it is rather difficult to select the primary objective from among various response variables. A further difficulty is how to make a set of proper and acceptable constraints even though the primary objective has been reasonably selected. A desirable way to solve this problem is to deal with all the responses simultaneously as multi-objective optimization problems. Khuri and Conlon (1981) introduced the simultaneous optimization technique based on the generalized distance function in which the equality among the responses was well considered. In this study, we attempted to investigate further improvement of this approach, considering its practical applicability to the design of pharmaceutical formulations. The method modified in this study was applied for the optimization of the formulation for a gel ointment of KPF containing *d*-limonene and ethanol as absorption promoters.

Theoretical

Single-objective optimization

In general, the optimization problems of pharmaceutical formulations can be regarded so as to minimize the objective function, $F(X)$, under the following inequality and/or equality constraints:

$$G_i(X) \geq 0 \quad i = 1, 2, 3, \ldots, m \tag{1}$$

$$H_j(X) = 0 \quad j = 1, 2, 3, \ldots, n \tag{2}$$

where $G_i(X)$ and $H_j(X)$ are the inequality and equality constraints, respectively. The constrained optimization problem defined above can be transformed to one that is unconstrained by adding a penalty function as follows:

$$T(X,r) = F(X) + r^{-1} \sum_{i=1}^{m} \Phi_i \{G_i(X)\}^2 + r^{-1} \sum_{i=1}^{n} \{H_i(X)\}^2 \tag{3}$$

when $G_i(X) < 0$ then $\Phi_i = 1$; and
when $G_i(X) \geq 0$ then $\Phi_i = 0$

where $T(X,r)$ is the transformed unconstrained objective function, r is a perturbation parameter of $T(X,r)$ and Φ_i is a step function by which the objective function, $F(X)$, is penalized. The second and third terms in Eqn 3 act as penalty functions because these values increase abruptly when the values of $G_i(X)$ are negative or the $H_i(X)$ values deviate from zero. The meaning of the perturbation parameter, r, and the means of obtaining a global optimum solution are described fully in a previous paper (Takayama and Nagai, 1989).

Multi-objective optimization

When the optimization problem includes several objectives, response variables should be in-

corporated into a single function in order to consider all the responses simultaneously. Derringer and Suich (1980) introduced general transformations based on the concept of desirability associated with a given response function. This transformation, a desirability function method, requires minimum and maximum acceptable values for every responses. The individual responses can be normalized to the desirability functions, d_i, which have values inside the interval [0,1] by using the distance between minimum and maximum acceptable values. The normalized functions are then combined into a multi-objective function, D_{total}, by means of the geometric mean of predicted values of each function:

$$D_{total} = (d_1 \times d_2 \times d_3 \times \ldots \times d_n)^{1/n} \quad (4)$$

The desirability function method has recently been applied to the preparation of nanoparticles composed of polyglutaraldehyde (McLeod et al., 1988) and the method was indeed quite useful to solve the practical optimization problems. However, one of the basic shortcomings of this approach is the subjectivity in the selection of the minimum and maximum acceptable values for each response. Namely, improper values of minima and/or maxima may lead to inaccurate solutions for the optimum formulation.

In order to avoid the problem of subjectivity in application of the desirability function method, we employed another approach based on the generalized distance between the predicted value of each response and the optimum one that was obtained individually (Khuri and Conlon, 1981):

$$S(X) = \left(\sum_{i=1}^{n} \left[w_i \{ FD_i(X) - FO_i(X) \} \right]^2 \right)^{1/2} \quad (5)$$

where $S(X)$ is the distance function generalized by the weighting coefficient, w_i, $FD_i(X)$ is the optimum value of each objective function, $F_i(X)$, optimized individually over the experimental region and $FO_i(X)$ is the predicted value of $F_i(X)$. As a proper and significant way to determine the w_i values, we introduced the following equation:

$$w_i = RA_i^2 / SD_i \quad (6)$$

where RA_i^2 is the coefficient of determination which was doubly adjusted with degrees of freedom (Haga et al., 1976) and SD_i is the standard deviation of observed values of each response variable. In general, the response variables of model formulations are predicted by a combination of causal factors by means of multiple regression analysis. Therefore, the values of RA_i^2 may well express the reliability of predicted values of each objective function, $F_i(X)$. On the other hand, the value of SD_i reflects the statistical variance of the observed values of each response. Thus, the weighting coefficient given in Eqn 6 is considered to be quite proper and acceptable from the point of the objectivity. Further improvement of the distance function given in Eqn 5 can be made available as follows:

$$S(X) = \left[\sum_{i=1}^{n} \left\{ w_i \left| FD_i(X) - FO_i(X) \right| \right\}^P \right]^{1/P} \quad (7)$$

where P is a parameter relating to the impartiality among the response variables ($P \geq 1$). When the P value is 1, the weighted distances of the predicted values of given responses from the individual optima can be treated impartially in Eqn 7. Increasing the P values leads to an enlargement in the importance of the response of which deviation from the optimum value was greater than that of the other responses. Thus, the user's preferability can be incorporated into the multi-objective function to a certain extent as a function of the P values in Eqn 7.

Materials and Methods

Materials

KPF was generously supplied by Rhône-Poulenc Japan, Ltd. Carboxyvinyl polymer, marketed as Hiviswako 105, was generously supplied by Wako Pure Chemical Industries, Ltd. *d*-Limonene, extra pure reagent grade, was purchased from Tokyo Chemical Industries. Other chemicals and solvents were of reagent grade.

TABLE 1

Composite spherical experimental design for two factors used to prepare the gel ointments

Formulation number	X_1	X_2	*d*-Limonene (%)	Ethanol (%)
1	−1	−1	1.0	30
2	−1	1	1.0	50
3	1	−1	3.0	30
4	1	1	3.0	50
5	$-\sqrt{2}$	0	0.6	40
6	$\sqrt{2}$	0	3.4	40
7	0	$-\sqrt{2}$	2.0	26
8	0	$\sqrt{2}$	2.0	54
9	0	0	2.0	40
10	0	0	2.0	40
11	0	0	2.0	40
12	0	0	2.0	40

Preparation of gel ointment

The amounts of *d*-limonene (X_1) and ethanol (X_2) were selected as causal factors. The composite spherical experimental design for two factors was applied to prepare the model formulations (Table 1). Central experimental points were repeated 4 times for evaluating experimental error (formulations 9–12). The factor levels in coded form were transformed to the physical units as summarized in Table 1. Based on the study previously reported (Okabe et al., 1990), the concentrations of KPF, carboxyvinyl polymer and triethanolamine in the gel ointments were fixed at 3.0, 1.5 and 2.0%, respectively. An appropriate amount of water was added to adjust the total weight of the gel ointments. The gel ointments were prepared as follows: KPF was dissolved in ethanol with *d*-limonene. Carboxyvinyl polymer and triethanolamine were separately dissolved in distilled water. Both components were then mixed thoroughly and the resulting gel ointment was stored at room temperature for 24 h under airtight conditions prior to use.

Percutaneous absorption from rat abdominal skin

Male Wistar rats weighing 180–200 g were used. After anesthetization with ethylcarbamate saline solution (25%; 3 ml/kg; i.p.), the rats were secured on their back and the hair on the abdominal skin was removed with an electric clipper. Glass cells (16 mm inner diameter, 10 mm height) were attached to the shaved skin with cyanoacrylate-type adhesives. The gel ointment (1.0 g) under test was applied and the glass cell was covered with Parafilm (American Can Company) to prevent evaporation of volatile components in the gel ointment. Blood samples (0.3 ml) were taken via the jugular vein at 1, 2, 4, 6, 8 and 10 h after the administration. Each sample was centrifuged, and the plasma (0.1 ml) was thoroughly mixed with methanol (0.3 ml) containing an appropriate amount of *p*-hydroxybenzoic acid *n*-butyl ester as an internal standard. The mixture was again centrifuged for 5 min, and the supernatant solution was filtered using a disposable filter unit (Gelman Science Japan, Ltd, Ekikuro-Disk 3CR). The concentration of KPF in the filtrate was determined by HPLC (Shimadzu HPLC model LC-3A). Ultraviolet detection was employed at 254 nm (Shimadzu U.V. detector model SPD 6A). The column (YMC-Pack A-302 S-5 120A ODS 4.6 mm × 150 mm; Yamamura Chemical Labs) was eluted at room temperature with a mobile phase consisting of 0.1% aqueous phosphoric acid-methanol (35:65). The flow rate was 1.0 ml/min. Under these conditions, KPF and the internal standard showed retention times of 5.5 and 7.5 min, respectively.

Intravenous administration of KPF in rats

The dose of 2.5 mg/kg in pH 7.2, 1/15 M phosphate buffer solution (2.5 ml/kg) was administered by bolus injection via the jugular vein of male Wistar rats weighing 180–200 g. Blood samples (0.3 ml) were taken via the jugular vein at appropriate intervals. The concentration of KPF in the samples was determined by HPLC.

Evaluation of skin damage

Irritation evoked by model formulations on rat skin was microscopically judged after the end of experiments on percutaneous absorption. The application site on the skin for each formulation was excised from the rats. The separated skins were fixed in 10% neutral carbonate-buffered formalin for at least 24 h before routine process-

ing and then cut vertically against the skin surface at the central region in 4 mm width. Each section was dehydrated using a graded series of ethanol solutions and embedded in paraffin wax. Tissues were divided into small pieces (about 3 μm in thickness) and stained with hematoxylin and eosin. All sections were examined by optiphoto light microscopy (Optiphoto microscope, Nikon).

Computer programs

The computation was carried out on a desk-top digital computer (PC-9801 RX, NEC Corp., Tokyo). The curve fitting program MULTI (Yamaoka et al., 1981) was applied to estimate the pharmacokinetic parameters. In the optimization study, the computer programs, written by the authors, were used for the multiple regression analysis, the contour diagrams and the non-linear optimization under the constraints (Takayama and Nagai, 1989).

Results and Discussion

Percutaneous absorption

First, KPF was intravenously injected into rats in order to investigate the percutaneous absorption of KPF from the model formulations pharmacokinetically. The plasma concentrations of KPF after i.v. administration declined in a biexponential manner. Therefore, the pharmacokinetic parameters of KPF were calculated according to the two-compartment open model. The parameters calculated are listed in Table 2.

The pharmacokinetics of percutaneous absorption has been widely discussed and several effective models have been developed for understanding the absorption behavior of drugs through the skin (Naito and Tsai, 1981; Guy et al., 1982; Naito et al., 1985; Kubota and Ishizaki, 1986; Guy and Hadgraft, 1987; Yamada and Tanigawara, 1987; Sato et al., 1988a,b; Tojo, 1988; Ogiso et al., 1989). However, many of these mathematical models are rather complicated and relatively many parameters are required to predict the plasma concentrations. In this study, we employed the simple model illustrated in Fig. 1 on the assumption of a constant penetration rate through the skin after the initial induction period (lag time). The plasma concentration of KPF can be given as follows:

TABLE 2

Pharmacokinetic parameters of ketoprofen after intravenous administration (2.5 mg / kg)

Parameter	Value
α (h^{-1})	3.30 ± 0.61
β (h^{-1})	0.329 ± 8.088
$t_{1/2}$ (h)	2.24 ± 0.61
k_{12} (h^{-1})	1.53 ± 0.34
k_{21} (h^{-1})	0.882 ± 0.194
k_{10} (h^{-1})	1.22 ± 0.25
V_1 (ml)	24.9 ± 4.1
V_2 (ml)	43.9 ± 11.7

Abbreviations: α and β, hybrid first-order rate constants; $t_{1/2}$, elimination half-life at β phase; k_{12}, rate constant from the central to tissue compartment; k_{21}, rate constant from the tissue to central compartment; k_{10}, elimination rate constant from the central compartment; V_1 and V_2, distribution volume of central and tissue compartments. Each value represents the mean ± SD of 5 animals.

$$C = \frac{R_p}{V_1 k_{10}} \left\{ 1 + \frac{\beta - k_{10}}{\alpha - \beta} e^{-\alpha(t - t_L)} + \frac{k_{10} - \alpha}{\alpha - \beta} e^{-\beta(t - t_L)} \right\} \quad (8)$$

where C is the plasma concentration, R_p is the apparent penetration rate, t is a time and t_L is the lag time. The details of other parameters, such as α, β, V_1 and k_{10}, are the same as those

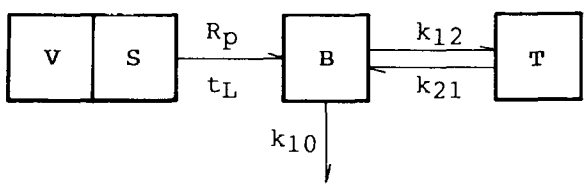

Fig. 1. Schematic representation of percutaneous absorption and elimination of drugs. V, vehicle; S, skin; B, central compartment; T, tissue compartment; R_p, penetration rate; t_L, lag time; k_{12}, rate constant from the central to tissue compartment; k_{21}, rate constant from the tissue to central compartment; k_{10}, elimination rate constant from the central compartment.

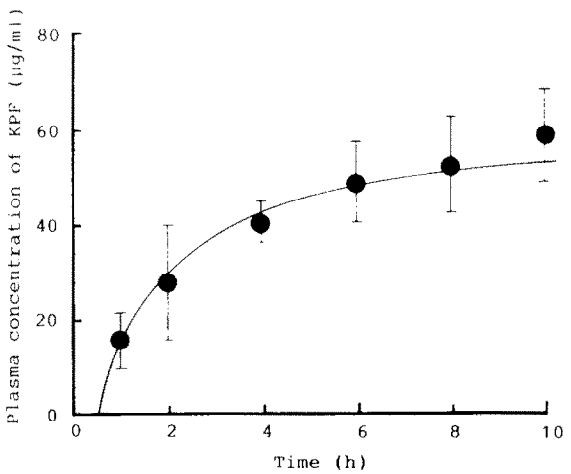

Fig. 2. Plasma concentration of KPF absorbed from central formulations (nos 9–12). Each point represents the mean ± SD for central formulations (4 determinations). Solid line was obtained by using $R_p = 1.67$ mg/h and $t_L = 0.56$ h in Eqn 8.

given in Table 2. As shown in Fig. 2, the plasma concentrations of KPF percutaneously absorbed from central formulations (nos 9–12 in Table 1) coincided well with the theoretical ones that were calculated by Eqn 8. The results indicate that the simple equation for the constant infusion of drugs with a lag time seemed applicable in predicting the plasma concentration of KPF percutaneously absorbed from the model formulations.

Skin damage

In general, the skin is described in terms of three tissue layers: the stratified, avascular, cellular epidermis, the underlying dermis of connective tissue, and the subcutaneous fat layer. In addition, the highly vascularized dermis and the epidermis support several skin appendages: errine, apocrine, sebaceous glands, and hair follicles (Okum and Edelstein, 1976; Chien, 1982; Mehregan, 1986). In this study, the influence of each formulation on skin irritation was investigated after the end of the percutaneous absorption experiment. Histopathological findings of the above-mentioned three tissue layers were discussed in detail. For example, a microscopic photo of rat skin at 10 h after application of formulation no. 2 is shown in Fig. 3a. The three layers of skin tissue (epidermis, dermis and hypodermis) showed almost no change at 10 h after application. On the other hand, rat skin treated with formulation no. 12 showed moderate liquefaction in the epidermis, subcutaneous edema and collagen fiber swelling in the dermis (Fig. 3b). In addition, inflammatory cell infiltration was observed in the dermis and hypodermis. The findings of the histopathological study with all of the model formulations were graded as summarized in Table 3. The histopathological scores evoked by model formulations showed a large deviation and the most severe skin damage under test was observed by application of the central formulations (nos 11 and 12).

Regression analysis

The penetration rate (R_p) and lag time (t_L) were employed as the response variables concerned with percutaneous absorption of KPF from the model formulations. On the other hand, a total irritation score (*TIS*) as an index of skin damage was obtained by the summation of the irritation scores listed in Table 3. The values observed for all the response variables are summarized in Table 4. A large deviation was observed for data in the cases of penetration rates and total irritation scores. These results indicate that the change in factor levels, the concentrations of *d*-limonene and ethanol, significantly affected important characteristics of these formulations. For example, excellent permeation of KPF was obtained by the application of central formulations (nos 9–12). However, the damages evoked by these formulations were more severe relative to those of the other formulations.

The following second-order polynomial equation was used for the prediction of each response variable:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{11} X_1^2 + b_{12} X_1 X_2 + b_{22} X_2^2 \quad (9)$$

where Y is the response variable, b_0 is a constant, b_i and b_{ij} are the coefficients of each monomial, and X_i is the causal factor level in coded form. The optimum regression equation which was composed of the combination of statis-

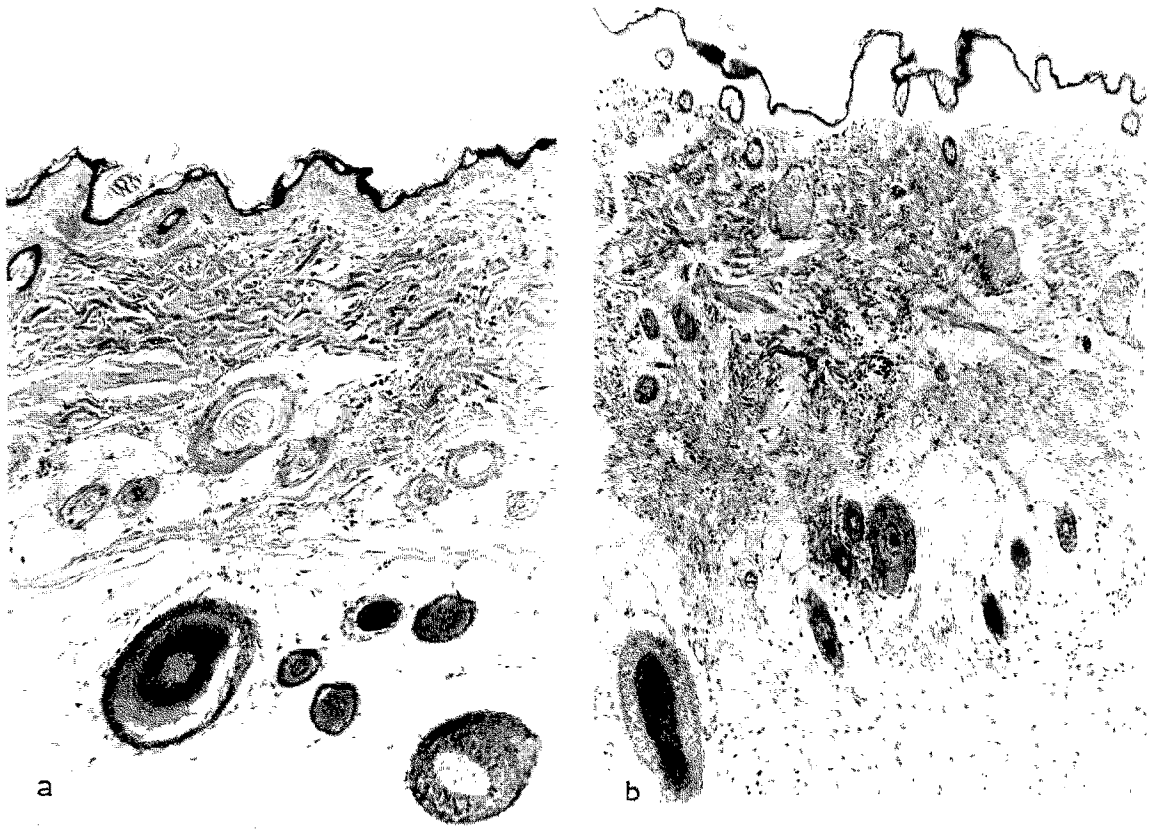

Fig. 3. Microscopic photos of rat skin at 10 h after application of model formulation no. 2 (a) and model formulation no. 12 (b). H & E stain ×100.

TABLE 3

Histopathological findings of rat abdominal skin at 10 h after application of model formulations

Histopathological finding	Formulation number											
	1	2	3	4	5	6	7	8	9	10	11	12
Epidermis												
liquefaction	2	0	3	1	0	3	3	1	2	3	3	3
Subepidermis												
edema	2	0	1	1	0	3	2	0	3	3	3	3
Dermis												
collagen fiber swelling	2	2	2	3	2	2	3	3	2	2	2	3
inflammatory cell infiltration	1	0	0	2	0	1	1	1	0	1	1	1
Hypodermis												
inflammatory cell infiltration	1	0	2	1	0	2	1	0	2	2	2	2
collagen fiber swelling	0	0	0	0	0	0	2	0	0	0	0	1
focal edema	0	0	0	0	0	3	0	0	4	3	3	2
Skin appendages												
degeneration	0	0	3	1	0	2	1	0	2	2	3	2

Irritation score: 0, no change; 1, very slight; 2, slight; 3, moderate; 4, marked.

TABLE 4

Experimental values of response variables

Formulation number	R_p [a] (mg/h)	t_L [b] (h)	*TIS* [c]
1	0.427	0.999	8
2	0.0851	0.656	2
3	1.37	−0.0210	11
4	0.309	1.01	9
5	0.0341	1.00	2
6	1.29	0.568	16
7	0.799	0.687	13
8	0.383	0.962	5
9	1.96	0.477	15
10	1.63	0.506	16
11	1.63	0.747	17
12	1.47	0.495	17

[a] Penetration rate.
[b] Lag time.
[c] Total irritation score.

TABLE 5

Optimum regression equation for each response variable determined by multiple regression analysis

	R_p	t_L	*TIS*
b_0 (constant)	1.67	0.556	16.3
b_1 (X_1)	0.368	−0.160	3.72
b_2 (X_2)	−0.249	0.135	−2.41
b_{11} (X_1X_1)	−0.525	0.0781	−4.00
b_{12} (X_1X_2)	−0.180	0.344	− [a]
b_{22} (X_2X_2)	−0.560	0.0983	−4.00
r [b]	0.973	0.948	0.964
s [c]	0.212	0.130	1.88
F_0 [d]	21.7 [e]	10.7 [e]	23.3 [e]
RA^2 [f]	0.867	0.744	0.857

[a] Not included in the optimum regression equation.
[b] Multiple correlation coefficient.
[c] Standard deviation of residual.
[d] Observed F value.
[e] $p < 0.01$.
[f] Doubly adjusted r^2 with degrees of freedom.

tically 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 31 kinds of regression equations. The coefficient of determination, which was doubly adjusted with degrees of freedom, was used as an index for selection of the optimum combination of factors. The optimum regression equations obtained are summarized in Table 5. As a result, each response variable was accurately predicted, since the values of the multiple correlation coefficient, r, were satisfactory and the regression equations were significant with high F_0 values (mean square regression/mean square residual). Fig. 4 shows the contour diagrams for each response variable as a function of X_1 and X_2 under the restrictions of the experimental region ($X_1^2 + X_2^2 \leq 2.0$). The individual optima were given as follows: $R_p = 1.78$

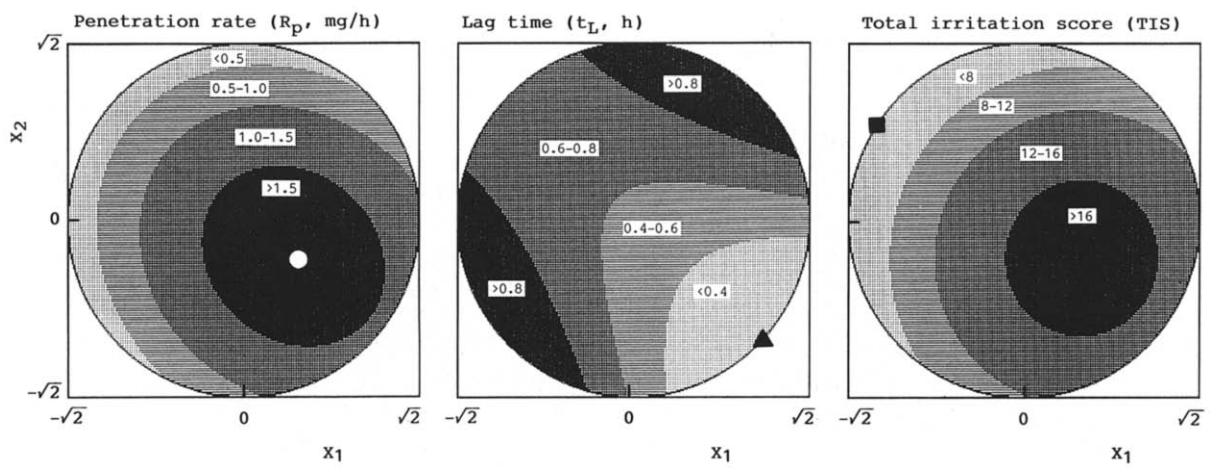

Fig. 4. Contour diagrams of response variables as a function of X_1 and X_2. ○, optimum point of R_p; ▲, optimum point of t_L; ■, optimum point of *TIS*.

mg/h at $X_1 = 0.417$ and $X_2 = -0.301$; t_L = 0.0936 h at $X_1 = 1.04$ and $X_2 = -0.960$; *TIS* = 1.97 at $X_1 = -1.18$ and $X_2 = 0.772$. The optimum point of R_p was defined as being at about the center of the graph, but this position leads to the worst result for *TIS*. Therefore, it is obvious that a formulation with a high penetration rate of KPF causes considerable damage to the skin. As for the lag time, the combination of larger values of X_1 (*d*-limonene) and smaller values of X_2 (ethanol) shortened the induction period for the absorption of KPF, though the deviation among the observed values was relatively small (within 1 h).

Mathematical optimization

The optimization of the KPF gel ointment containing *d*-limonene was performed according to the method stated in the theoretical section. The regression equations for each response summarized in Table 5 were combined into the generalized distance function, $S(\boldsymbol{X})$, given in Eqn 7. Simultaneous optima for the three responses can be directly obtained by minimizing $S(\boldsymbol{X})$ under the constraints of the experimental region. In this study, the influence of P values in Eqn 7 (impartiality parameter among the responses) on the simultaneous optima was investigated in detail. Fig. 5 shows the contour diagrams of $S(\boldsymbol{X})$ as a function of X_1 and X_2 at $P = 2$ and $P = 3$, respectively. The simultaneous optimum at $P = 2$ was found to be close to the individual optima of R_p and t_L and far from that of *TIS*. When $P = 1$ was employed, the optimum point hardly changed and the contour diagram of $S(\boldsymbol{X})$ was almost the same as that obtained with $P = 2$. On the other hand, the optimum formulation was greatly affected by the change of P values from 2 to 3, as shown in Fig. 5. Thus, the optimum formulation surpassing the safety to the skin can be obtained by the increase in P values, though the percutaneous absorption was somewhat lowered. Further increase in P values (e.g., $P = 4$ or $P = 5$) resulted in little change in the simultaneous optima.

As the optimum formulation at $P = 3$, $X_1 = -0.647$ and $X_2 = 0.600$ were obtained in coded forms. These values were transformed into physical units and the following results were obtained: 1.35% as the concentration of *d*-limonene and 46.0% as the concentration of ethanol. Fig. 6 shows the plasma concentration of KPF percutaneously absorbed from the optimum formulation. The plasma concentration of KPF was satisfactorily predicted in spite of the fact that the functions of R_p and t_L were composed of a simple combination of formulation factors. Although the skin section after application of the optimum

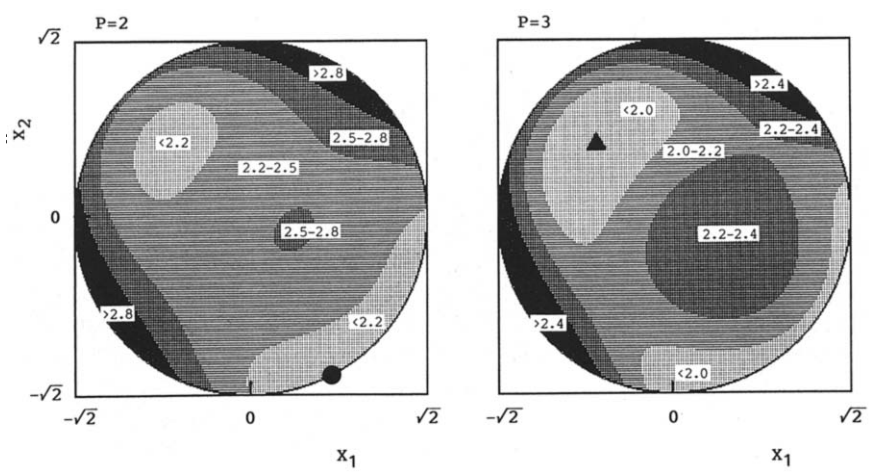

Fig. 5. Contour diagrams of combined objective function, $S(\boldsymbol{X})$, as a function of X_1 and X_2. ●, optimum point of $S(\boldsymbol{X})$ at $P = 2$; ▲, optimum point of $S(\boldsymbol{X})$ at $P = 3$.

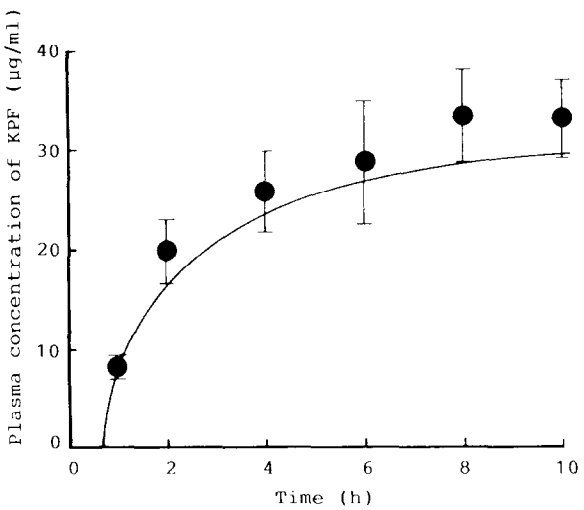

Fig. 6. Plasma concentration of KPF absorbed from the optimum formulation ($P = 3$). Solid line means plasma levels of KPF simulated by using predicted values of R_p and t_L listed in Table 6. Each point represents the mean ± SD of 5 animals.

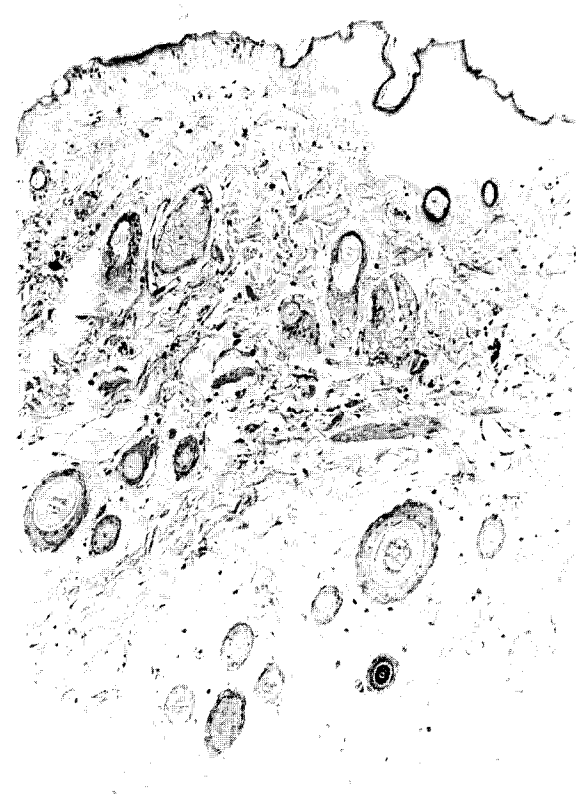

Fig. 7. Microscopic photo of rat skin at 10 h after application of the optimum formulation ($P = 3$). H & E stain ×100.

TABLE 6

Response variables of the optimum formulation ($P = 3$)

Response	Predicted	Experimental [a]
R_p (mg/l)	0.931	1.02 ± 0.14
t_L (h)	0.675	0.718 ± 0.075
TIS	9.33	9.25 ± 1.26

[a] Represented as the mean + SD for 5 determinations.

formulation showed epidermal liquefaction and subepidermal edema (Fig. 7), the damage due to application of the optimum formulation may not be serious as a whole. Comparing the results obtained with model formulations such as nos 3, 6, 9, 10, 11 and 12, the extent of percutaneous absorption with the optimum formulation was somewhat low; however, the skin damage evoked by the optimum formulation was obviously improved. As summarized in Table 6, the predicted values of the response variable coincided well with the experimental data, suggesting usefulness and reliability of the optimization method introduced in this study. With respect to the preformulation studies, optimization of the gel ointment of KPF could be reasonably well performed by means of the simultaneous optimization method based on the generalized distance function in which the impartiality among the responses is well considered.

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