International Journal of Pharmaceutics, 61 (1990) 225–234
Elsevier

IJP 02068

Formulation design of indomethacin gel ointment containing *d*-limonene using computer optimization methodology

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(Received 18 October 1989)
(Modified version received 14 November 1989)
(Accepted 29 November 1989)

Key words: Computer optimization; Adaptive least squares; Percutaneous absorption; Gel ointment; Indomethacin; *d*-Limonene

Summary

A computer optimization technique was applied for the designing of a formulation for a gel ointment of indomethacin (IMC) containing *d*-limonene as an absorption enhancer. The combined use of an adaptive least-squares (ALS) method with general optimization techniques was investigated for dealing with response variables which were given as non-quantitative data. Plasma concentrations of IMC absorbed from model formulations were determined in rats as prime response variables. Physicochemical characteristics such as the spreadability of the ointments and chemical stability of IMC in the formulations were also determined as response variables. These variables were predicted by multiple regression equations composed of a combination of formulation factors. On the other hand, irritation of the skin by each formulation and the appearance of the ointments were obtained as non-quantitative data. For these variables, the ALS method was applied to define discriminant equations composed of the combination of formulation factors. The regression and discriminant equations for response variables were assembled as the constrained optimization problem. This problem was solved on the basis of external transformation. Experimental results obtained for the optimum formulation agreed well with the predictions, indicating the usefulness of the optimization techniques in which the ALS method was incorporated.

Introduction

Transdermal drug delivery has recently been attracting considerable interest, as a new route for drug administration. Several reports are available concerning the investigation of absorption promoters in order to overcome the low permeability of drugs through the skin and to transport sufficient quantities of drugs into the living body (Cooper, 1984; Woodford and Barry, 1986; Okamoto et al., 1988; Sugibayashi et al., 1988). In a previous work (Okabe et al., 1989), we reported the promoting effect of cyclic monoterpenes present in essential oils on the percutaneous absorption of indomethacin (IMC). Absorption of IMC was remarkably enhanced on addition of hydrocarbons such as menthane, limonene, terpinene and terpinolene. In this study, we performed an investigation on the optimization of IMC formulations as gel ointment containing *d*-limonene as an absorption enhancer. *d*-Limonene was selected from

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among the other cyclic monoterpenes for pragmatic reasons. It is readily available as the main component in orange or lemon oils and its toxicity to or irritation of the skin is considered to be low. In addition, the fragrance of *d*-limonene is more acceptable than that of the other cyclic monoterpenes.

A computer optimization technique, based on response surface methodology, has been proven to be a useful approach for selecting pharmaceutical formulations (Fonner et al., 1970; Schwartz et al., 1973; Takayama and Nagai, 1989). The 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 systematic 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. In general, multiple regression analysis is utilized for the prediction of response variables. Finally, optimization algorithms are applied for deciding as to the best formulation. Despite the many advantages of computer optimization, its application is considered to be limited mainly to the design of solid dosage forms such as powders (Takayama et al., 1983, 1985; Takai et al., 1984) granules (Takayama and Nagai, 1989), tablets (Fonner et al., 1970; Schwartz et al., 1973; Fenyvesi et al., 1984; Harris et al., 1985; Franz et al., 1987) and capsules (Shek et al., 1980). In the designing of formulations of ointments, it is often necessary to consider 'non-quantitative' responses such as skin irritation and the appearance of ointments. This could be one of the main causes of the difficulty in applying the optimization technique to the designing of ointment formulation. Namely, multiple regression analysis cannot be employed in the prediction of non-quantitative response variables.

Moriguchi et al. (1977, 1981) recently developed a new classification method, the adaptive least-squares (ALS) technique, in the field of research on quantitative structure-activity relationships (QSAR). The biological potency of drugs has often been represented in the form of an activity rating. In this case, the usual QSAR techniques are not applicable. The ALS method was thus developed for making decisions regarding multicategory pattern classification by a single discriminant function for the case where activity data are given as an ordinal scale. In this study, we attempted to incorporate this method into the technique of computer optimization developed in the previous work (Takayama and Nagai, 1989). The applicability of this modification was examined by using the formulation design for IMC gel ointment in which some of the response variables were given as non-quantitative values.

Materials and Methods

Materials

IMC was purchased from Sigma. 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 were of reagent grade.

Preparation of gel ointments

The amounts of IMC (X_1), carboxyvinyl polymer (X_2), triethanolamine (X_3), ethanol (X_4) and *d*-limonene (X_5) were selected as causal factors. The composite orthogonal experimental design for five factors was applied to prepare the model formulations. Central experimental points were repeated 10 times for evaluating an experimental error (formulations 27–36). The experiments listed in Table 1 in coded form were transformed to the physical units as summarized in Table 2. The gel ointments were prepared as follows: IMC 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 air-tight conditions prior to use.

Determination of response variables

The response variables selected on the model formulations are determined as follows:

Percutaneous absorption of IMC (Y_1, Y_2 and Y_3) Plasma concentrations of IMC at 3, 6, and 24 h

TABLE 1

Composite experimental design for five factors

Formulation no.	Factor level in coded form				
	X_1	X_2	X_3	X_4	X_5
1	−1	−1	−1	−1	1
2	1	−1	−1	−1	−1
3	−1	1	−1	−1	−1
4	1	1	−1	−1	1
5	−1	−1	1	−1	−1
6	1	−1	1	−1	1
7	−1	1	1	−1	1
8	1	1	1	−1	−1
9	−1	−1	−1	1	−1
10	1	−1	−1	1	1
11	−1	1	−1	1	1
12	1	1	−1	1	−1
13	−1	−1	1	1	1
14	1	−1	1	1	−1
15	−1	1	1	1	−1
16	1	1	1	1	1
17	−2	0	0	0	0
18	2	0	0	0	0
19	0	−2	0	0	0
20	0	2	0	0	0
21	0	0	−2	0	0
22	0	0	2	0	0
23	0	0	0	−2	0
24	0	0	0	2	0
25	0	0	0	0	−2
26	0	0	0	0	2
27	0	0	0	0	0
28	0	0	0	0	0
29	0	0	0	0	0
30	0	0	0	0	0
31	0	0	0	0	0
32	0	0	0	0	0
33	0	0	0	0	0
34	0	0	0	0	0
35	0	0	0	0	0
36	0	0	0	0	0

TABLE 2

Levels of factors in physical units

Factor (%)	Factor levels in coded form				
	−2	−1	0	1	2
X_1	0.500	0.750	1.00	1.25	1.50
X_2	0.500	1.00	1.50	2.00	2.50
X_3	1.00	1.50	2.00	2.50	3.00
X_4	20.0	30.0	40.0	50.0	60.0
X_5	0.500	1.00	1.50	2.00	2.50

(Y_1, Y_2 and Y_3) in rats were employed as the response variables concerned with percutaneous absorption of IMC from the model formulations. The animal experiments were carried out as follows: male Wistar rats weighing 181–190 g were used. After anesthetization with urethane 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 electrical animal clipper. Glass cells (16 mm inner diameter, 10 mm height) containing the gel ointment under test (1.5 g) were attached to the shaved skin with cyanoacrylate-type adhesives. Blood samples (0.5 ml) were taken via the jugular vein at 3, 6, and 24 h after administration. Following centrifugation of 0.5-ml blood samples (Abbot Laboratories, centrifuge model LN 9527), the plasma sample (200 μl) was taken and thoroughly mixed with methanol (500 μl) containing an appropriate amount of *p*-hydroxybenzoic acid *n*-hexyl ester as an internal standard. The mixtures were again centrifuged for 5 min. The supernatant solutions were filtered using a disposable filter unit (Gelman Science Japan. Ltd., Ekikuro-Disk 3CR), and the concentration of IMC in the filtrate was determined by HPLC (Shimadzu HPLC model LC-3A). Ultraviolet (UV) detection was employed at 254 nm (Shimadzu UV 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 (25 : 75). The flow rate was 1.5 ml/min. Under these conditions, IMC showed a retention time of 6 min.

Spreadability of ointments (Y_4) The spreadability of model formulations was determined using a spreadmeter (Rigosha model 419). A volume of 0.45 ml of each ointment was placed in the sample hole on the spreadmeter. The diameter of the area over which each ointment had spread at 1 min after starting the measurement was read at 25 °C. The spread area for each ointment calculated from the diameter was used as an index of spreadability.

Stability of IMC in ointments (Y_5) For assessment of the thermal stability of IMC in ointments, the residual IMC content of each sample was measured after storage for 30 days at 40 °C. Ap-

prox. 0.5 g of sample ointment was accurately weighed, and agitated vigorously in 100 ml of a mixture of methanol-water (1 : 1). This solution (5 ml) was diluted to 50 ml with methanol containing an appropriate amount of *p*-hydroxybenzoic acid *n*-hexyl ester as the internal standard. Solutions were filtered using a disposable filter unit (Ekikuro-Disk 13C) and the concentration of IMC in the filtrate was determined via HPLC.

Skin irritation by ointments (L_1) Irritation evoked by model formulations on rat skin was visually judged immediately after the end of experiments on percutaneous absorption. The extent of irritation was assigned the scores 1 or 2 according to whether no change was detected on the skin surface or a slight erythema was observed, respectively.

Appearance of ointments (L_2) The visual appearance of model formulations was judged on a black plate under a fluorescent light. Appearance was assigned the scores 1 or 2 according to whether or not a yellowish, clear gel was visible.

Computer programs

The following computer programs, all written by the authors, were used in this study on an NEC PC-9800 series personal computer: STEPRA and ALCORA were used in the multiple regression analysis; they were combined for selecting the best combination of factors. ALSMET was employed in the ALS analysis and was written with reference to published data (Moriguchi and Komatsu, 1977; Moriguchi et al., 1981). THREED was for the three-dimensional graph which allowed a visual representation of the multi-dimensional equations. Finally, NOPCON was the constrained nonlinear optimization program in which the simplex method was incorporated, involving random number techniques and Andrews' plots (Takayama and Nagai, 1989).

Results and Discussion

Plasma concentrations of IMC at 3, 6 and 24 h (Y_1, Y_2 and Y_3) in rats were selected as prime response variables concerned with percutaneous absorption of IMC from the model formulations. The differences among the model formulations during the initial absorption stage could be explained by the change in the values of Y_1. On the other hand, the Y_2 and Y_3 values may reflect the apparent steady-state absorption of IMC. Furthermore, skin damage caused by application of ointments may be evaluated based on the differences between Y_2 and Y_3 values. The values observed for all the response variables are summarized in Table 3. A large deviation was observed for data in the case of percutaneous absorption of IMC. For example, the values of Y_2 (plasma concentration of IMC at 6 h) varied from 1.22 μg/ml (formulation 5) to 54.7 μg/ml (formulation 26). Similarly, results varying over a wide range were obtained in the case of physicochemical responses such as spreadability and stability of IMC. These results indicated that the change in factor levels significantly affected important characteristics of these formulations. The formation of a slight erythema was observed in the cases of model formulations 10, 11 and 13. The amounts of ethanol and *d*-limonene in the ointments were greater relative to those of the other formulations except no. 16, suggesting that the combination of these factors results in a cooperative effect on the occurrence of skin irritation. The use of an extremely high concentration of ethanol (formulation 24) led to failure when preparing the gel ointment. Therefore, this was omitted from the calculations for predicting each response variable except for the case of L_2 (the appearance of ointments).

Prediction of response variables

Since the response variables from Y_1 to Y_5 were determined as quantitative data, the following second-order polynomial equation was used for the prediction of each response variable:

$$Y = b_0 + \sum_{i=1}^{5} b_i X_i + \sum_{i=1}^{5} \sum_{j=i}^{5} b_{ij} X_i X_j \qquad (1)$$

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 or X_j is the causal factor level in coded form. The optimum regression equation which was

TABLE 3

Experimental values of response variables

Formulation no.	Y_1 (μg/ml)	Y_2 (μg/ml)	Y_3 (μg/ml)	Y_4 (cm^2)	Y_5 (%)	L_1	L_2
1	8.21	14.3	20.0	11.8	72.8	1	2
2	2.28	5.00	7.42	11.5	86.5	1	2
3	1.31	2.46	2.81	9.35	90.4	1	2
4	2.07	3.45	4.51	8.51	68.5	1	2
5	0.563	1.22	2.04	12.9	31.3	1	2
6	6.77	14.8	21.6	12.6	45.8	1	2
7	7.70	13.5	12.4	9.08	79.3	1	2
8	0.812	4.68	4.59	12.7	51.6	1	1
9	5.09	17.8	66.9	14.0	87.9	1	1
10	10.1	48.6	258	16.5	87.4	2	1
11	22.0	24.4	20.3	10.4	89.6	2	1
12	5.66	17.2	67.3	10.2	85.9	1	2
13	5.37	20.5	61.9	13.9	61.4	2	1
14	6.05	20.9	32.9	16.0	60.3	1	1
15	5.63	21.7	37.9	10.3	77.7	1	1
16	24.6	34.9	60.2	10.6	91.1	1	1
17	6.96	15.7	30.9	11.3	79.9	1	1
18	19.7	32.1	32.0	11.5	72.6	1	1
19	3.05	10.0	23.4	19.5	48.1	1	1
20	9.64	13.4	12.5	8.07	89.8	1	2
21	8.84	10.7	9.41	10.1	81.8	1	2
22	8.79	20.1	40.5	11.2	61.8	1	1
23	0.849	1.66	1.67	9.30	70.2	1	2
24	–	–	–	–	–	–	2
25	2.70	6.43	12.7	11.3	85.7	1	1
26	28.6	54.7	58.7	10.3	85.4	1	1
27	16.3	30.0	37.9	11.3	84.1	1	1
28	10.3	18.9	35.8	11.0	82.1	1	1
29	10.7	27.3	53.6	11.3	83.2	1	1
30	9.28	18.9	40.5	11.2	83.5	1	1
31	11.8	23.4	38.3	10.9	87.3	1	1
32	10.9	19.9	37.1	10.8	84.2	1	1
33	17.3	25.6	33.0	11.1	85.7	1	1
34	8.80	19.8	28.8	10.9	81.5	1	1
35	14.6	44.1	52.6	11.3	85.8	1	1
36	15.0	30.2	38.8	10.4	88.2	1	1

composed of the combination of statistically significant factors was obtained by a backward selective, stepwise, multiple regression analysis. The correlation coefficient, which was doubly adjusted with degrees of freedom, was used as an index for selection of the optimum combination of factors (Haga et al., 1976). The optimum regression equations obtained are summarized in Table 4. Logarithmic transformation was performed on the response variables Y_1, Y_2 and Y_3, since the predictability of these variables with raw data was rather poor. As a result, each response variable was accurately predicted, since the values of the multiple correlation coefficients, r, were satisfactory and the regression equations were significant with high F_0 values (mean square regression/mean square residual).

The ALS method was used in the classification of response variables, L_1 and L_2, which were given as the ordinal scale. Similarly to regression

TABLE 4

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

	$\log Y_1$	$\log Y_2$	$\log Y_3$	Y_4	Y_5
b_0 (constant)	1.08	1.37	1.56	11.1	83.1
b_1 (X_1)	0.0436	0.0598	0.0606	0.303	– [a]
b_2 (X_2)	0.0580	–	−0.106	−2.12	7.67
b_3 (X_3)	–	–	–	5.43	3.31
b_4 (X_4)	0.258	0.318	0.459	0.850	6.55
b_5 (X_5)	0.264	0.200	0.165	−0.232	–
b_{11} (X_1X_1)	–	–	–	–	–
b_{12} (X_1X_2)	−0.100	−0.0834	–	–	−4.16
b_{13} (X_1X_3)	0.0905	0.0539	–	0.285	–
b_{14} (X_1X_4)	0.0410	–	–	–	–
b_{15} (X_1X_5)	−0.0504	−0.0568	–	–	–
b_{22} (X_2X_2)	−0.102	−0.0849	−0.0822	0.730	−4.19
b_{23} (X_2X_3)	0.0813	0.127	0.146	–	6.32
b_{24} (X_2X_4)	0.102	–	–	−0.609	–
b_{25} (X_2X_5)	–	−0.0626	−0.125	−0.273	–
b_{33} (X_3X_3)	−0.0497	−0.0596	−0.0679	–	−3.48
b_{34} (X_3X_4)	–	–	−0.0518	−0.401	–
b_{35} (X_3X_5)	0.0629	–	0.0768	−0.493	5.57
b_{44} (X_4X_4)	−0.190	−0.147	−0.107	–	−1.24
b_{45} (X_4X_5)	−0.0835	−0.0851	−0.0970	0.335	–
b_{55} (X_5X_5)	−0.0500	–	–	–	–
r [b]	0.975	0.973	0.972	0.971	0.940
s [c]	0.129	0.114	0.140	5.37	13.2
F_0 [d]	21.9 [e]	32.9 [e]	30.8 [e]	2.52 [f]	3.13 [f]

[a] Not included in the optimum regression equation.
[b] Multiple correlation coefficient.
[c] Standard deviation.
[d] Observed F value.
[e] $p < 0.01$.
[f] $p < 0.05$.

analysis, the following equation was used to define a discriminant function of these response variables:

$$L = w_0 + \sum_{i=1}^{5} w_i X_i + \sum_{i=1}^{5} \sum_{j=i}^{5} w_{ij} X_i X_j \qquad (2)$$

where L is the score for the response variable, w_0 is a constant, and w_i and w_{ij} are the weighting coefficients of each monomial. In order to presume the most suitable combination of causal factors, the selection of factors was performed using a contribution index which was defined as the product of the weighting coefficient and the standard deviation (Moriguchi et al., 1981). The most suitable discriminant equations obtained are summarized in Table 5. Spearman's rank correlation coefficients, r_s, and numbers of misclassification were acceptable for both response variables. Interestingly, the discriminant equation for L_1 was expressed only as a function of X_4 and X_5, suggesting that the amounts of ethanol and *d*-limonene in the formulation and their interaction are significant factors in skin irritation. The score of each response variable was classified in the following manner: If $L_k \leqq 1$ (k: number of model formulations), then the k-th formulation was assigned to class 1; for $L_k > 1$, the k-th formulation was assigned to class 2. Although the experimental results for L_1 showed large bias, each score was well classified by the discriminant equation except for no. 16 (misclassified into class 2). In the case

TABLE 5

Most suitable discriminant equation for each response variable determined by the ALS method

	L_1	L_2
w_0 (constant)	0.585	0.523
w_1 (X_1)	– [a]	–
w_2 (X_2)	–	0.0833
w_3 (X_3)	–	−0.167
w_4 (X_4)	0.143	−0.232
w_5 (X_5)	0.112	–
w_{11} (X_1X_1)	–	–
w_{12} (X_1X_2)	–	–
w_{13} (X_1X_3)	–	−0.125
w_{14} (X_1X_4)	–	0.125
w_{15} (X_1X_5)	–	–
w_{22} (X_2X_2)	–	0.0855
w_{23} (X_2X_3)	–	−0.125
w_{24} (X_2X_4)	–	0.125
w_{25} (X_2X_5)	–	–
w_{33} (X_3X_3)	–	0.121
w_{34} (X_3X_4)	–	–
w_{35} (X_3X_5)	–	0.125
w_{44} (X_4X_4)	–	0.272
w_{45} (X_4X_5)	0.168	−0.125
w_{55} (X_5X_5)	–	−0.00439
r_s [b]	0.852	1
n (*miss*) [c]	35 (1)	36 (0)
V_e [d]	0.109	0.0549

[a] Not included in the most suitable discriminant equation.
[b] Spearman's rank correlation coefficient.
[c] Number of samples (number of misclassification).
[d] Apparent variance of error.

of L_2 (appearance of ointments), the classification was found to be perfect.

Mathematical optimization

Optimization of the IMC gel ointment containing *d*-limonene was performed according to the previously developed method (Takayama and Nagai, 1989). In general, optimization problems are described mathematically so as to minimize the objective function, $F(\boldsymbol{X})$, under the following inequality and/or equality constraints:

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

$$H_j(\boldsymbol{X}) = 0, \quad j = 1, 2, 3, \ldots, n \tag{4}$$

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

$$T(\boldsymbol{X},r) = F(\boldsymbol{X}) + r^{-1}\sum_{i=1}^{m}\Phi_i\left[G_i(\boldsymbol{X})\right]^2 + r^{-1}\sum_{j=1}^{n}\left[H_j(\boldsymbol{X})\right]^2 \tag{5}$$

when $G_i(\boldsymbol{X}) < 0, \quad \Phi_i = 1$

when $G_i(\boldsymbol{X}) \geqq 0, \quad \Phi_i = 0$

where $T(\boldsymbol{X},r)$ is the transformed unconstrained objective function which is obtained based on the external transformation, r is a perturbation parameter of $T(\boldsymbol{X},r)$ and Φ_i is a step function by which the objective function, $F(\boldsymbol{X})$, is penalized. The second and third terms in Eqn 5 act as penalty functions because their values increase abruptly when the values of $G_i(\boldsymbol{X})$ are negative or the $H_j(\boldsymbol{X})$ values deviate from zero. The meaning of the perturbation parameter, r, and the means of obtaining a global optimum solution are described fully in the previous paper (Takayama and Nagai, 1989).

The purpose of the optimization of IMC gel ointment containing *d*-limonene concerns the determination of a formulation giving sufficient percutaneous absorption of IMC and having acceptable values for the other characteristics. Thus, the regression and discriminant equations for each response variable listed in Tables 4 and 5 were assembled as a constrained nonlinear optimization problem as follows:

$$F(\boldsymbol{X}) = -(\log Y_1 + \log Y_2 + \log Y_3) \tag{6}$$

$$G_1(\boldsymbol{X}) = \log Y_3/\log Y_2 - 0.8 \geqq 0 \tag{7}$$

$$G_2(\boldsymbol{X}) = 1.2 - \log Y_3/\log Y_2 \geqq 0 \tag{8}$$

$$G_3(\boldsymbol{X}) = Y_4 - 8 \geqq 0 \tag{9}$$

$$G_4(\boldsymbol{X}) = 12 - Y_4 \geqq 0 \tag{10}$$

$$G_5(\boldsymbol{X}) = Y_5 - 90 \geqq 0 \tag{11}$$

$$G_6(\boldsymbol{X}) = 1 - L_1 \geqq 0 \tag{12}$$

$$G_7(\boldsymbol{X}) = 1 - L_2 \geqq 0 \tag{13}$$

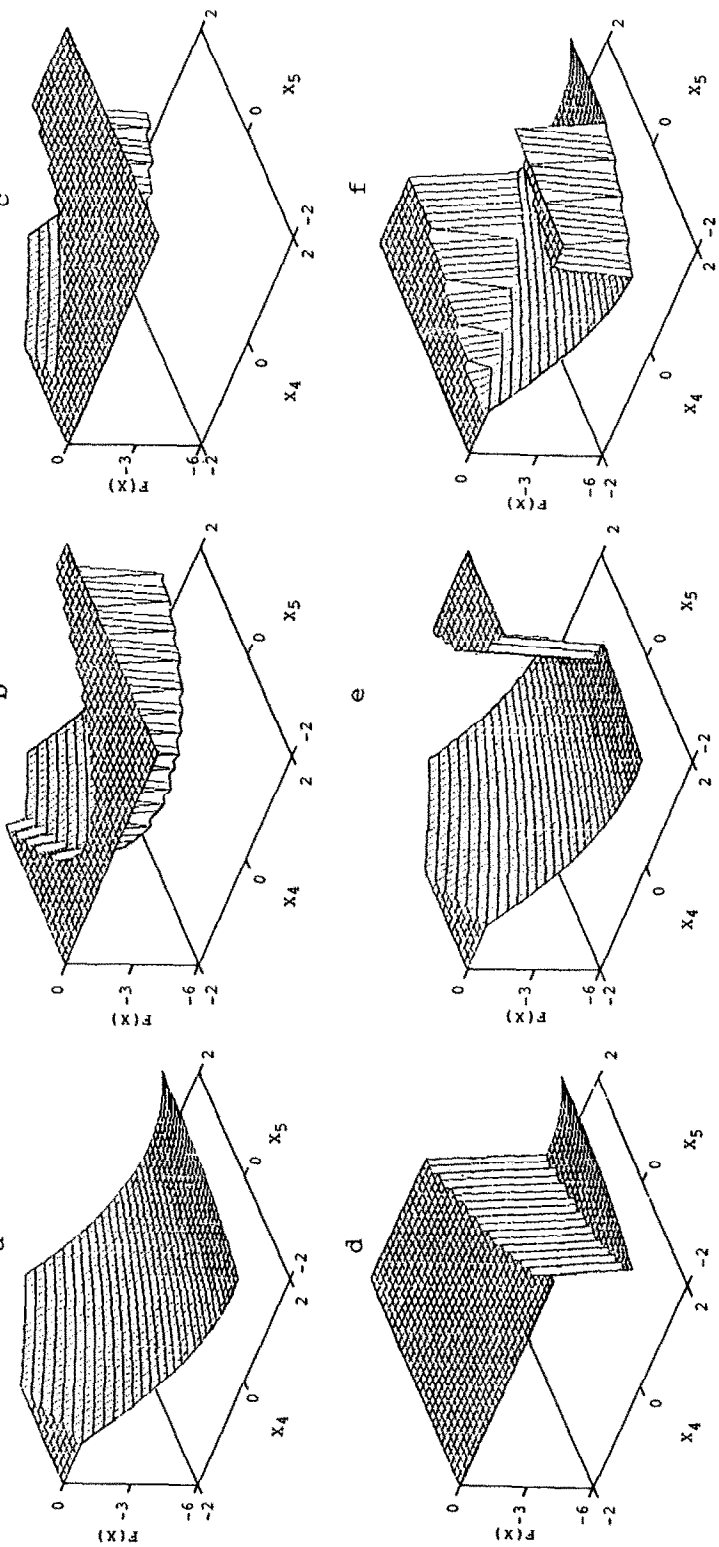

Fig. 1. Three-dimensional diagrams for the objective function, $F(X)$, under various constraints as a function of X_4 and X_5 at constant values of X_1, X_2 and X_3 ($X_1 = -0.346$, $X_2 = 0.0865$, $X_3 = 0.265$). (a) $F(X)$ without constraints; (b) $F(X)$ with Eqns 7 and 8; (c) $F(X)$ with Eqns 9 and 10; (d) $F(X)$ with Eqn 11; (e) $F(X)$ with Eqn 12; (f) $F(X)$ with Eqn 13.

where to minimize the objective function, $F(X)$, signifies that maximization of the percutaneous absorption of IMC is carried out. This function was employed as an overall index for the absorption of IMC. The ratio of plasma concentrations ($\log Y_3/\log Y_2$) was used as an indirect index evaluating skin damage due to application of the ointments. Namely, if the skin was irreversibly damaged with passage of time by application of the ointments, the values of Y_3 (plasma concentration of IMC at 24 h) would be abnormally enhanced as compared with the value of Y_2 (plasma concentration of IMC at 6 h). For example, the value of Y_3 was extremely large in formulation 10 and, in fact, the degree of irritation of this formulation to the skin was observed as a score 2 in the response variable L_1. In contrast, when the flux of IMC from ointments markedly decreased during the experiment on percutaneous absorption, the value of $\log Y_3/\log Y_2$ could be lowered. Accordingly, the set of expressions, Eqns 7 and 8, appears to be appropriate and significant with acceptable limit values. The limit values in Eqns 9 and 10 were selected as proper criteria, referring to the value of spreadability which had been measured in the commercial gel ointment of IMC. Other constraints used in this study were based on the experimental data obtained in the model formulations. Constraints, $-2 \leqq X_i \leqq 2$ $(i = 1\text{–}5)$, were also employed in order to restrict the values of each factor within the experimental region. A computer program for the optimization, NOPCON, was applied for this problem. Thus, $X_1 = -0.346$, $X_2 = 0.0865$, $X_3 = 0.265$, $X_4 = 0.399$, and $X_5 = 2.00$ were obtained in coded form as the optimum formulation for IMC gel ointment. Fig. 1 shows the three-dimensional diagrams for the objective function (Eqn 6) with and without constraints as a function of X_4 and X_5 at optimum values of the other factors. The optimum point of the objective function without any constraints was observed at higher values of X_4 and X_5. However, this point was positively affected by Eqns 9–12, as shown in the diagrams for the constrained objective function. When the constraint on Y_5 (Eqn 11) was included in the objective function, limiting the area was observed to span a wide range of X_4. On the other hand, the limiting area was obtained at higher values of X_4 and X_5 when the constraint on L_1 (Eqn 12) was introduced into the objective function, clearly indicating that the irritation of the skin by the ointments was due mainly to an increase in content of both the components ethanol and *d*-limonene in the formulation. The optimum formulation determined in coded form was transformed to physical units and the following concentrations were obtained: 0.914% for IMC, 1.54% for carboxyvinyl polymer, 2.13% for triethanolamine, 44.0% for ethanol and 2.50% for *d*-limonene. The predicted values of the response variables coincided well with the experimental data as summarized in Table 6. Fig. 2 shows the plasma concentration profiles of IMC absorbed from the optimum formulation of the ointment. The plasma concentration of IMC was satisfactorily predicted in spite of the fact that the function was composed of a simple combination of formulation factors.

TABLE 6

Response variables of the optimum formulation

Response	Predicted	Experimental [a]
$\log Y_1$	1.66	1.41 ± 0.16
$\log Y_2$	1.81	1.69 ± 0.08
$\log Y_3$	1.96	1.93 ± 0.17
$\log Y_3/\log Y_2$	1.08	1.14 ± 0.11
Y_4 (cm^2)	12.0	11.8 ± 0.3
Y_5 (%)	90.0	86.2 ± 0.8
L_1	1	1
L_2	1	1

[a] Represented as the mean ± S.D. for five determinations.

In future investigations, we intend to examine the clinical application of gel ointments of IMC containing *d*-limonene on the basis of the optimum formulation evaluated in this study. The possible differences in optimum formulations between humans and other animals will also be studied. However, with respect to preformulation studies, optimization of the gel ointment of IMC containing *d*-limonene could be reasonably well performed by means of the optimization method in which the ALS procedure has been incorporated. This type of method should be applicable to the solving of optimization problems in pharma-

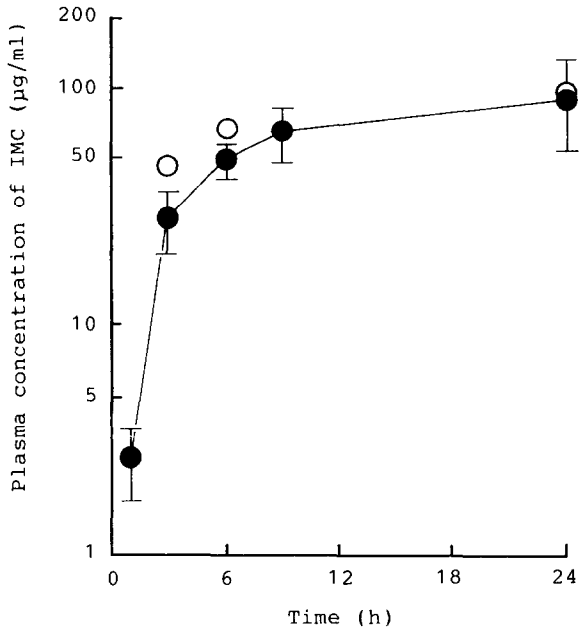

Fig. 2. Percutaneous absorption of IMC from the optimum formulation of gel ointment in rats. (○) Predicted; (●) experimental (mean ± S.D. for five determinations).

ceutical formulations in which some of the response variables are given as non-quantitative data.

Acknowledgement

This study was supported by a Grant-in-Aid for Scientific Research on Priority Area, New Functional Materials-Design, Preparation and Control (No. 01604025), from the Ministry of Education, Science and Culture.

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