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Studies on Computer Optimization Techniques for Hydrophilic Vehicle Compositions

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Abstract □ The inflence of hydrophilic vehicles on percutaneous absorption rate of griseofulvin was studied using intact skin of full thickness of hairless rat. The *in vitro* absorption rates were used as the characteristics for deciding the optimum formula of ointment vehicles. The optimum formula of vehicle compositions for maximum absorption rate was obtained from the polynomial regression equation and the two graphical techniques, contour graph and partial derivative graph. It was composed of sodium lauryl sulfate (1.65 W/W%), white petrolatum (16.5 W/W%), propylene glycol (12.0 W/W%), and stearyl alcohol (19.6 W/W%). The experimental value obtained from the optimum formula and the prediction value were 33.99 and 33.87 $\mu g/\sqrt{min}$, respectively. From these results, it was believed that optimum formula for semisolid dosage forms could be obtained from the application of the optimization technique used in this study.

Keywords □ griseofulvin, hydrophilic vehicles, one-half factorial design, rat skin membrane, absorption rate, contour graphs, optimum formula.

The absorption of drugs depends primarily on the physiologic state of the skin[1] and the physicochemical properties of the drug and the vehicle in which the drug is incorporated. Therefore, the rate-determining step in percutaneous absorption through intact skin is the diffusion across the stratum corneum. Diffusion through the skin is usually a passive process governed by Fick's law of diffusion.[2] And the permeability coefficient depends on the diffusivity of the molecule through the skin barrier, the effective partition coefficient of the drug between skin barrier and vehicle, and the effective thickness of the skin barrier. Fundamentally, the driving force or the rate of transfer across the membrane is the concentration of the applied drug. Katz, *et al.*[3] found the relation between the partition coefficient of a drug and its ability to penetrate through the human skin. The partition coefficient can be defined as the drug concentrations found at equilibrium in the stratum corneum and the vehicle, respectively.[4] A low partition coefficient value indicates a high degree of affinity between the drug and vehicle and reflects the tendency of the drug to remain in the vehicle.

Then, the partition coefficient of a drug is roughly proportional to the drug's solubility in the two immiscible phases, *i.e.*, the skin and the vehicle. Therefore, the percutaneous absorption of a drug can be more easily altered by modifying the solubility of a particular drug in the vehicle, by altering the composition of the vehicle, or by modifying the structure of the drug rather than by attempting to improve the solubility of the drug in the skin barrier. Dempski[5] and Poulsen[6] demonstrated the effect of altering vehicle composition on the release of dexamethason and fluocinolone acetonide and its acetate ester, respectively. Many organic solvents, such as dimethyl sulfoxide, dimethyl formamide, N-methyl-2-pyrrolidinone and polyethylene glycol, *etc.*, have been studied for the percutaneous absorption enhancement.[7-12]

The experimental methods for percutaneous absorptions can be divided into two general types, *in vitro* and *in vivo*. *In vitro* experiment involves the excised skin of an animal or human subject and the artificial or mimetic membrane in the diffusion chamber.[13-15] *In vivo* experiment employs the skin of the living animal or human subject *in situ*.[16-25] Dempski, *et al.*[5] attemped to correlate *in vitro* release data with *in vivo* penetration studies using a practical topical vehicle. Scheuplein[26-27] reported mechanism of percutaneous absorption and routes of skin penetration. However, as above mentioned, ointments had been developed in terms of the stability, compatibility, and partition coefficient of a drug, rather than considering the influence which

the ratio of components of vehicle itself may have on the bioavailability of a drug. A component of vehicle composition may have the influence on enhancing or hindering the movement of the drug through skin.[28] Katz and Poulsen[29] discussed three factor interaction, *i.e.*, drug-vehicle-skin, and reported the design of a dosage form that not only had good physical stability and patient acceptability, but also that provided the optimum environment for the release of the drug from the vehicle and its penetration through the skin barrier.

In view of pharmaceutical field, many applications of optimization technique for the drug design and the process analysis system of solid dosage forms were published but few reported on the semi-solid dosage forms.[30-36]

The purpose of this experiment was to measure the influence of hydrophilic vehicle on *in vitro* percutaneous absorption rate of griseofulvin using intact skin of full thickness of hairless rat and to find out the optimum formula of vehicle compositions for maximum absorption rate by means of computer optimization technique. In this study, the optimization technique reported by Schwartz, *et al.*[31,34] was applied with some modifications, and hydrophilic ointment, appearing in official compendiums[37-39] as oil in water emulsion form, was selected as a representative vehicle. Their compositions were shown in Table I.

THEORY

Optimization problems may be broadly classified as either unconstrained or constrained. Unconstrained optimization involves the maximization or minimization of a function in which no restrictions or limits have been placed on the controllable variables or the functions of the controllable variables. The constrained optimization has any constraints which mean restrictions imposing on the function of the controllable variables, and it was used in this study.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing problems where several independent variables influence a dependent variable or response and optimizing this response. It is assumed that the independent variables denoted by X_1, X_2, $\cdots$ X_k, are continuous and controllable by the experimenter with negligible error and the response is assumed to be a random variable.

In this paper, in order to obtain maximum level of *in vitro* absorption rate as a function of the controllable variables, it was performed a set of statistically designed experiments as shown in Table II. The resulting data were used to derive a mathematical model which can be used for optimization of vehicle compositions. The experimental design is dependent on the number of variables involved in the study, *i.e.*, for four independent variables, the modified half-factorial design requires a total of 17 experiments as shown in Table II. In Table II, formulation No. 1 represents the experiment for midway point of the two base levels. And 8 formulations, i.e., from No. 2 to No. 9, represent one half-factorial design for four factor at two levels coded as +1 and −1.

In general, a one-half-factorial design of 2^k design is called a 2^{k-1} fractional factorial design. It may be constructed by first partitioning the full 2^k

Table I. Comparison of hydrophilic ointment (oil in water emulsion) in official compendiums

Component (w/w%)	KP IV	JP XI	USP XXI
Sodium lauryl sulfate	1.5	a*	1.0
White petrolatum	25.0	25.0	25.0
Propylene glycol	12.0	12.0	12.0
Stearyl alcohol	22.0	20.0	25.0
H_2O	q · s	q · s	q · s

a*: Tween 60 4.0 (w/w%) and glyceryl monostearate 1.0 (w/w%).

Table II. Experimental design for four factors

Formulation No.	Factor level in coded form			
	X_1	X_2	X_3	X_4
1	0	0	0	0
2	1	1	1	1
3	-1	-1	-1	-1
4	1	1	-1	-1
5	1	-1	1	-1
6	-1	1	1	-1
7	1	-1	-1	1
8	-1	1	-1	1
9	-1	-1	1	1
10	$\sqrt{3}$	0	0	0
11	$-\sqrt{3}$	0	0	0
12	0	$\sqrt{3}$	0	0
13	0	$-\sqrt{3}$	0	0
14	0	0	$\sqrt{3}$	0
15	0	0	$-\sqrt{3}$	0
16	0	0	0	$\sqrt{3}$
17	0	0	0	$-\sqrt{3}$

design into two blocks, using the highest-order interaction, as the defining contrast. Each block is then a 2^{k-1} fractional factorial design. The defining relation of the 2^{k-1} is I = ±ABC····K, where the sign on the generator depends on the fraction chosen. In practice we would randomly select which reaction to run.[40,41]

A 2^{k-1} design may be generated by another method. First, write down the treatment of combinations for a full 2^{k-1} factorial and then add the Kth factor by identifying its plus and minus levels with the plus and minus signs of the highest-order interaction ABC·····(K-1). Therefore, the 2^{4-1} fractional factorial is obtained by writting down the full 2^3 factorial and equating the factor D to the ABC interaction. The alternate fraction would be obtained by equating the factor to the -ABC interaction. This approach is illustrated in Table III, and in this study was selected the case of D = ABC.

For the remainder of this study, two additional levels were selected; the positive and negative $\sqrt{3}$ values represented the extreme values. The proper name for this design is "a four factor, orthogonal, central, composite, second-order design" and the type of predictor equation from such a study is second-order polynomial having the following form;

$$Y=a_o+\sum_{i=1}^{n} a_i X_i+\sum_{i=1}^{n} a_{ii} X_i^2+\sum_{i}^{n}\sum_{i<j}^{n} a_{ij} X_i X_j \quad \text{(Eq. 1)}$$

where;

Y; response (Absorption rate)

a; regression coefficient for second-order polynomial

X_i; independent variables

In the above polynomial equation 1, Y values, as absorption rates, were obtained from the equation by T. Higuchi(42):

$$Q=\sqrt{Dt\,(2C-C_s)\,C_s} \quad \text{(Eq. 2)}$$

Table III. The 2^{4-1} Design with defining relation I = ABCD

A	B	C	D = ABC	D = -ABC
−	−	−	−	+
+	−	−	+	−
−	+	−	+	−
−	−	+	+	−
+	+	−	−	+
+	−	+	−	+
−	+	+	−	+
+	+	+	+	−

where Q is the amount of drug absorbed to the sink at time t per unit area of contact, D is the diffusion coefficient of drug in the vehicle, C is the total drug concentration expressed in units per cubic centimeters, and C_s is the solubility of drug as units per cubic centimeters in ointment. Equation 2 predicts that plots of the amounts of drug absorbed with t will give a straight line passing through the origin. But, the origin as intercept may not be observed in some cases because of the lag time phenomenon.

Under the following assumptions that: (a) the diffusion coefficient is constant with respect to both time and position in the base; (b) the drug alone is allowed to diffuse out of the base; (c) the drug is rapidly removed upon reaching the base-membrane interface, and the receiving phase is a "perfect sink"; and (d) C is substantially greater than C_s in the case of suspensions; Equation 2 for the amount of drug absorbed becomes:

$$Q=2A\sqrt{t} \quad \text{(Eq. 3)}$$

where A is a constant, depending on the initial drug concentration and on a function describing the change of D. According to equation 3, plots of the amount of drug absorbed against the square root of times should be linear. Such a linear relationship should hold until C exceeds the effective solubility, C_s.

In this paper, the absorption rates of griseofulvin, as Y values, were calculated from the slopes of straight lines plotted by *in vitro* absorption experiments of ointments prepared according to the formulations listed in Table II. And the four independent variables were sodium lauryl sulfate (X_1), white petrolatum (X_2), propylene glycol (X_3), and stearyl alcohol (X_4), and *in vitro* percutaneous absorption rate was taken as the dependent variable (response). The statistical codes and physical amounts for independent variables are shown in Table IV.

EXPERIMENTAL METHOD

Materials

Table IV. Physical amount and Statistical code for experiments

Factor (w/w%)	Factor level in coded form				
	$-\sqrt{3}$	-1	0	1	$\sqrt{3}$
X_1(Sodium lauryl sulfate)	0.635	1.0	1.5	2.0	2.365
X_2(White petrolatum)	16.35	20.0	25.0	30.0	33.65
X_3(Propylene glycol)	5.08	8.0	12.0	16.0	18.92
X_4(Stearyl alcohol)	8.16	14.0	22.0	30.0	35.84

Griseofulvin (Sigma Chem. Co., USA), sodium lauryl sulfate (Kokusan Chem. Co., Japan), white petrolatum (Fluka AG., Switzerland), propylene glycol, stearyl alcohol(Junsei Chem. Co., Japan), acetonitrile (LC grade, Merck, W. Germany) and h_2O (LC grade, Wako, Japan) were used, and the other were all reagent grade.

Apparatus

HPLC system (Waters Associates, U.S.A) consisting of data module, Model 730, solvent delivery system, Model 510, and Lambda Max detector, Model 481 was used. Computer (Cyber and IBM PC), constant temperature water bath (Haake, type FS 2, W. Germany), stirrer (Caframo, type RZR 50, Canada), microtome cryostat (American Optical, type cryo cut II, USA) were used. The absorption of griseofulvin through rat skin membrane was investigated using diffusion cell, a specially designed acryl cell (Fig. 1).

Skin specimens

Male Sprague-Dawley rats weighing 230 to 270 g were sacrificed by overdosing ether. Full thickness skin membrane was taken from the abdominal skin of the hairless rat which was cut with an electric clipper, and shaved with an electric shaver. A rectangular section of abdominal skin, 5.5 centimeters in each dimension, was excised with surgical scissors and lifted easily from the basement membrane[17] because the skin was not firmly attached to the viscera. Adhering fat and other visceral debris were removed carefully from the undersurface with tweezers.

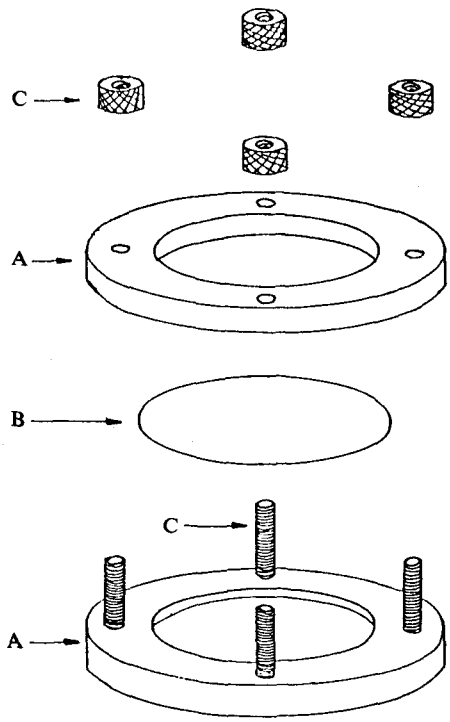


Fig. 1. Diffusion cell used for absorption experiments.
A: Acryl cell body and plate; Internal diameter; 50 mm; Height of cell body; 10 mm
B: Abdominal skin membrane of rat
C: Stainless steel bolts and nuts

Thickness of the skin membrane

In order to measure the thickness of skin membrane excised and lifted, it was rapidly frozen to -20 °C and the frozen skin was cut off using the microtome cryostat set at 15 μm. Skin sample obtained from cutting by the microtome cryostat was stained with hematoxyline and its thickness was measured under 100 × microscope.

Preparation of ointments

The formulas of ointments are listed in Table II. The ointment base was prepared according to KP IV, and 0.5 w/w % griseofulvin was added to warm base(75 °C). The mixture was cooled to room temperature with stirring. Each ointment was used after storage for 2 days in an incubator at 30 °C.

In vitro absorption of griseofulvin

The donor part of the diffusion cell was filled with an ointment and the excess was removed with the edge of a spatula to produce an even, uniform surface of constant dimension. The skin membrane was placed on the ointment with the stratum corneum facing the donor compartment and carefully pressed to ensure complete contact of the membrane with ointment. The acryl cell was assembled and tightly secured with bolts and nuts.

The donor part (exposed skin area, 19.625 cm^2) was placed in the bottom of a 500 m*l* beaker and the beaker was placed in a controlled 37 °C water bath throughout the experiment. 300 m*l* of normal saline, receiving phase, previously equilibrated to 37 °C was carefully layered over the vehicle, and it was stirred immediately using a two-blade propeller connected to a 100 rpm motor. At appropriate intervals, 10 m*l* of the receiving phase were withdrawn, and replaced with the same volume of prewarmed normal saline. The sampling solution was filtered with 0.45 μm microfilter (Millipore, USA) and assayed by HPLC system.

Analytical method

Liquid chromatography has been useful for the

analysis of griseofulvin in drug substance and in plasma.[43-46] A high performance liquid chromatograph (Waters Associates, USA) equipped with a pump (model 510), an injector, an UV detector (LC spectrophotometer model 481), a data module (model 730) and an automated gradient controller was used as the following conditions; column, reversed-phase μ Bondapak C_{18} (Waters Associates. Radial-PAK cartridges No. 85721); detector, UV spectra at 295 nm; mobile phase, acetonitrile H_2O(1 : 1); flow rate, 2.2 m*l*/min; sensitivity, 0.005 AUFS; injected sample volume, 100 μ *l*.

The drug concentrations of sample solutions were computed from the peak height corresponding to the calibration curve obtained from the standard solution.

Data treatment

Each of *in vitro* absorption experiment was repeated at least five times. The average data were plotted versus the square root of time and a straight line was obtained. Absorption rates of ointments by each formulation were computed from the slopes of the straight lines obtained. These data were inputted into the computer and the polynomial regression analysis was performed with the aid of SPSS, a statistical package for social science. The regression coefficients of a polynomial equation were calculated from the computer and an optimum formulation for maximum absorption rate was obtained from the resulting second-order polynomial equation.

RESULTS AND DISCUSSIONS

Thickness of intact skin membrane

The thickness of membrane was an important factor in absorption process by passive transport. As it was known in the expanded form of Fick's law,[4] the absorption rate was inversely proportional to the thickness of skin membrane.

The thickness of rat's intact skin membrane was found to be approximately 357.9 ± 7.6 μm from the results of the microscopic measurements of 50 rats. Therefore, it was considered as a constant in this study.

In vitro absorption of griseofulvin

The *in vitro* absorption amounts of griseofulvin from each of ointments prepared according to the experimental formulations listed in Table II were measured by HPLC system. It was known that the absorption amounts were approximately proportional to the square root of time.[42,47,48] As shown in Fig. 2, straight lines were obtained by plotting the absorption amount versus the square root of time, but all of them did not pass through the origin. These results indicated a lag time in all cases. It was believed that this phenomenon was associated with the presence of the skin membrane separating the bulk phase and sink. Consequently, this lag time resulted from slow membrane diffusion as several examples reported in the literature also.[47,48] Fig. 2 shows the absorption profiles of griseofulvin for some of 17 formulations in Table V. Absorption rates (μg/$\sqrt{\text{min}}$) of the drug were obtained from the slopes of straight lines shown in Fig. 2. Their values were calculated by a linear regression method and listed in Table V.

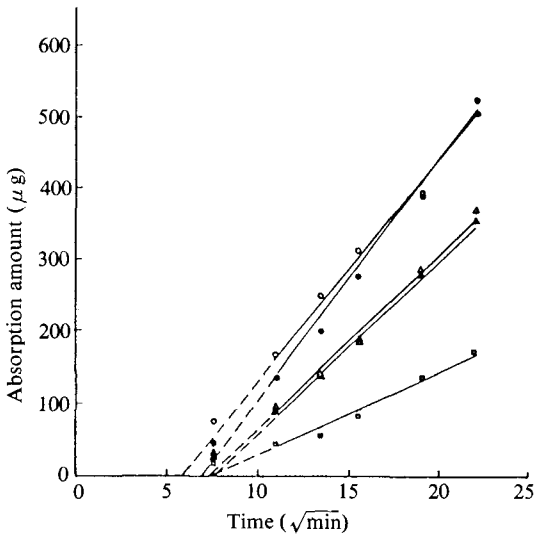


Fig. 2. Absorption profiles of griseofulvin obtained from some of 17 formulations through rat skin membrane. △, □, ▲, ○, ● : formulation No. 1, 2, 5, 12 and 13, respectively.

Polynomial regression equation for absorption rates of drug from ointments

In order to predict characteristics of the model ointment formulations, the amounts of sodium lauryl sulfate (X_1), white petrolatum (X_2), propylene glycol (X_3) and stearyl alcohol (X_4) were selected as the independent variables and the *in vitro* absorption rate of griseofulvin from ointments through skin membrane was selected as the dependent variable (Y). The overall combinations of four independent variables were investigated via computer analysis at the point of statistical significance in order to determine the optimum regression model, *i.e.*, first, second or more higher-order

Table V. Comparison of absorption rates for experimental and calculation values by computer

Formulation No.	Absorption rates ($\mu g/\sqrt{min}$)		
	Calculation values	Experimental values	R^{2*}
1	28.730	25.441	0.993
2	13.0365	12.224	0.980
3	18.0425	16.325	0.991
4	21.4665	20.438	0.980
5	25.8475	25.151	0.995
6	17.3595	17.153	0.996
7	16.5055	14.242	0.988
8	18.5535	16.781	0.978
9	20.7885	19.384	0.983
10	19.8728	21.424	0.987
11	18.9583	20.694	0.994
12	28.8076	27.793	0.993
13	33.5013	35.772	0.991
14	19.274	19.876	0.987
15	18.2071	20.885	0.997
16	13.2921	15.704	0.992
17	19.2814	20.160	0.989

* by linear regression method.

polynomial regression equation. And the polynomial regression analysis was performed with the aid of the computer using SPSS, a statistical package for social science. The optimum regression equation for the dependent variable was selected on the basis of statistical significance from 16,383 (2^{14}-1) equations, overall combinations of four factors and five levels. Coefficient of multiple determination (R^2) were used as an index for the selection of the optimum combination of independent variables and the result of regression analysis was summarized in Table VI. The coefficient of multiple determination of second-order polynomial regression model obtained from this experiment was 0.904. From this analysis, it was considered that second-order polynomial regression model was suitable for optimum regression equation. Calculation values for absorption rates of griseofulvin from each ointment of 17 formulations in Table II were obtained from the second-order polynomial regression equation and listed in Table V. The physical meaning of the regression equation was elucidated by means of graphical approach.

Graphical approach

Table VI. Parameters for Second-order polynomial regression equation of absorption rates determined by multiple regression analysis

Coefficient	Coefficient values
a_0	28.730
$a_1(X_1)$	0.264
$a_2(X_2)$	-1.346
$a_3(X_3)$	0.308
$a_4(X_4)$	-1.729
$a_{11}(X_1^2)$	-3.105
$a_{12}(X_1X_2)$	-0.6165
$a_{13}(X_1X_3)$	-0.08
$a_{14}(X_1X_4)$	-2.714
$a_{22}(X^2)$	0.803
$a_{23}(X_2X_3)$	0
$a_{24}(X_2X_4)$	0
$a_{33}(X_3^2)$	-3.33
$a_{34}(X_3X_4)$	0
$a_{44}(X_4^2)$	-4.148
R^{2*}	0.904
F^{**}	4.27

*Coefficient of multiple determination
**Level of significance

In the optimization procedure, two graphical techniques, that is, the contour graph and the partial derivative graph, were quite useful to explain the significance of a regression analysis.[30-36] Contour of the solid geometrical surface generated by the change in a response produced by continuous variations in values of two interacting factors was known as a response surface contour. In the contour graph, a three-dimensional situation might be plotted two dimensionally by computer.

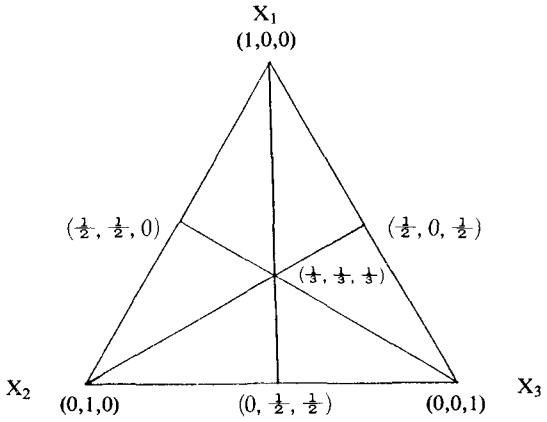
For the present work, four independent variables (X_1, X_2, X_3 and X_4) were used. Therefore, the contour graph was plotted in a triangular coordinate by changes in a response produced by continuous variations in values of three interaction factors at the fixed value of one factor. In triangular coordinate, the three sides of a triangle represented axes of three independent variables and the dimension units for each of axes were statistical coded forms. As shown in Scheme 1, generally, a given point P (X_1, X_2, X_3) in a triangular coordinate had always a value of 1 as the total sum of values of X_1, X_2 and X_3. That is, the central point in Scheme 1 had $\frac{1}{3}$, $\frac{1}{3}$ and $\frac{1}{3}$ for X_1, X_2 and X_3, respectively. In this study, the computer graphic program, programed with basic language in our laboratory, was

used for the purpose of drawing contour graphs.[49,50]

Four types of contour graphs were drawn by computer for three interacting factors with keeping one factor at constant level. Figure 3 showed the contour graphs for absorption rates of griseofulvin as a function of X_2, X_3 and X_4 at various fixed levels of X_1 (sodium lauryl sulfate). As shown in Fig. 3, the maximum position of absorption rate of griseofulvin was defined at the center of the graph. It was known that the optimum position of absorption rate moved to the center region on the contour graph. The center area at the axis of X_4 was the point where the absorption rate was at a maximum. Moreover, from five contour graphs of Fig. 3 drawn respectively at five fixed levels of X_1 ($-\sqrt{3}$, -1, 0, 1 and $\sqrt{3}$), it was observed that the maximum position was in the region of $X_1 = 0$ and $X_1 = 1$.

Fig. 4 was the results of the contour graphs as a function of X_1, X_3 and X_4 at various, fixed levels of X_2 (white petrolatum). The fixed values of X_2 were $-\sqrt{3}$, -1, 0, 1, and $\sqrt{3}$, respectively. As shown in figure 4, the area of maximum response appeared in Fig. 4(a) among five contour graphs. Since the optimum point was observed at the value of $-\sqrt{3}$ coded unit of X_2, a relation of white petrolatum amount to the absorption rate of griseofulvin might be explained as follows. When the amount of white petrolatum was small, the absorption rate of griseofulvin was high, because the degree of affinity between the drug and white petrolatum as the tendency of the drug to remain in the vehicle was very high. A larger X_2 value was worse for absorption rate of griseofulvin.

The contour graphs drawn at various, fixed levels ($-\sqrt{3}$, -1, 0, 1, and $\sqrt{3}$) of X_3 (propylene glycol) were represented in Fig. 5 and their shapes were all the same. The maximum position appeared in Fig. 5(c) drawn at the value of $X_3 = 0$. Therefore, it was known that the optimum amount of propylene glycol for maximum absorption rate of griseofulvin exited in the formulations of ointments. Also, it could be observed that the max-



Scheme 1. Triangular coordinate for three independent variables (X_1, X_2 and X_3) at a fixed level of X_4.

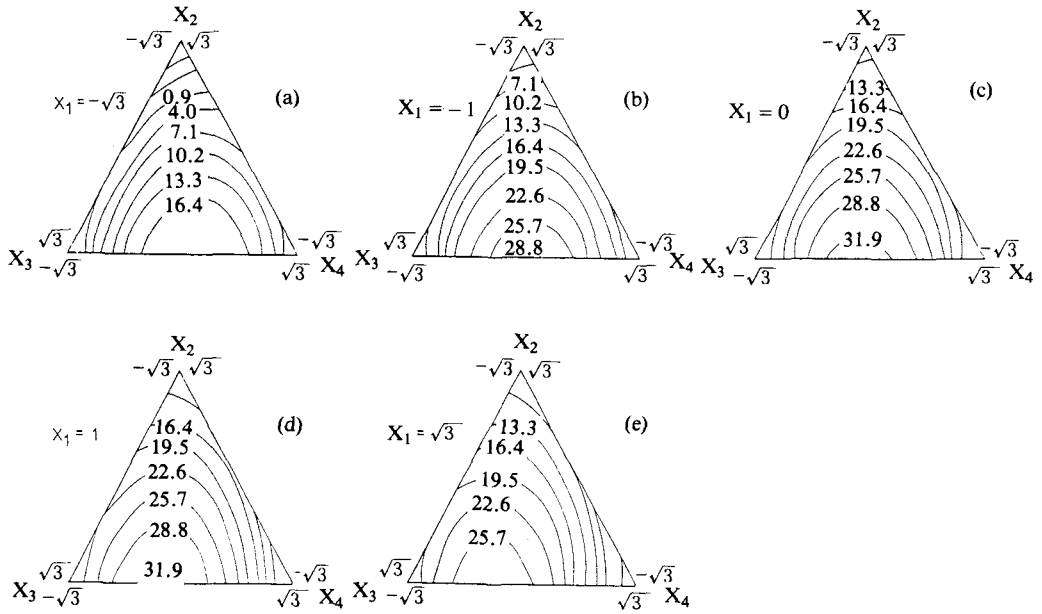


Fig. 3. Contour plots of absorption rate as a function of X_2, X_3, and X_4 at various, fixed level of X_1.

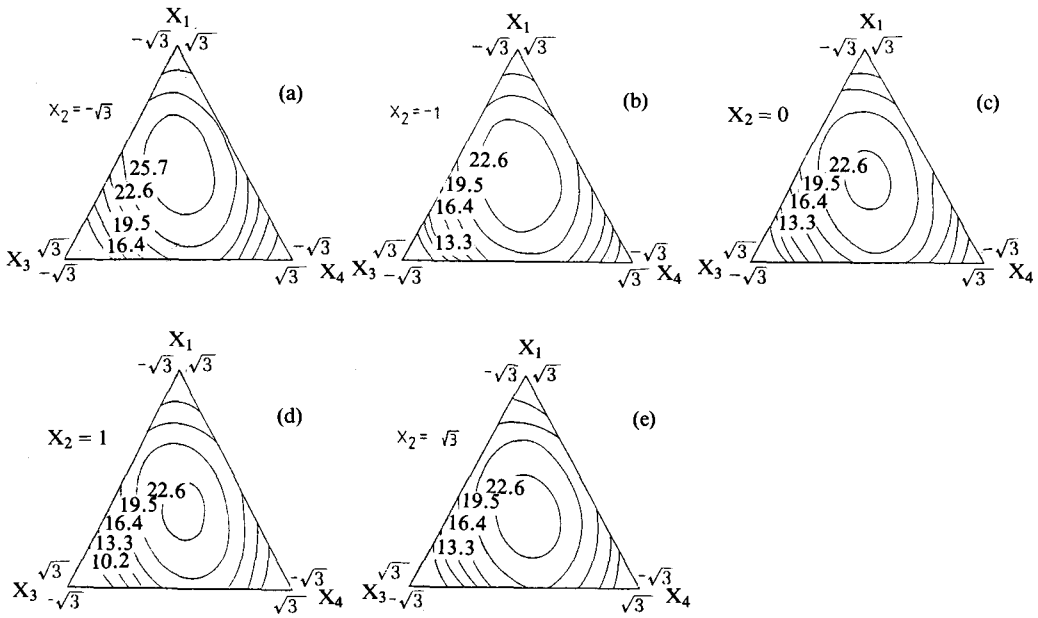


Fig. 4. Contour plots of absorption rate as a function of X_1, X_3, and X_4 at various, fixed level of X_2.

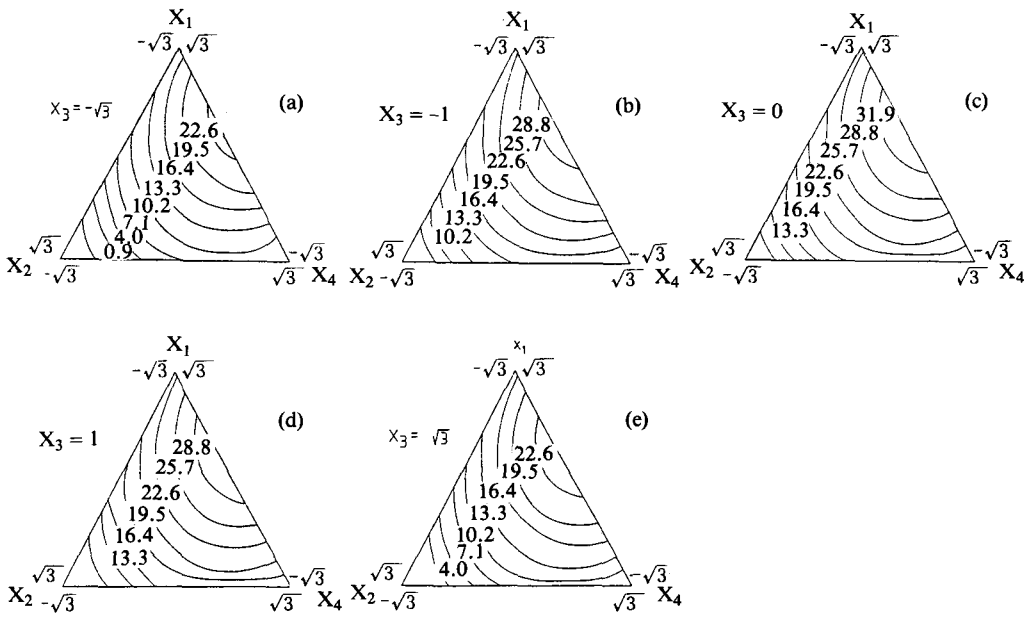


Fig. 5. Contour plots of absorption rate as a function of X_1, X_2, and X_4 at various, fixed level of X_3.

imum position was located in the region of X_1 values from 0 to 1, X_2 values from $-\sqrt{3}$ to -1.5, and X_4 values from -1 to +0.5.

Fig. 6 showed the contour graphs as a function of X_1, X_2 and X_3 at various, fixed levels of X_4 (stearyl alcohol). Among five contour graphs of Fig. 6, the maximum position was observed in the graph (c) drawn at the value of $X_4 = 0$. From the graph(c) of Fig. 6, the maximum values were 31.9 or more and the region was surrounded by X_1 values of $-0.5 \sim +0.5$, and X_2 of $-\sqrt{3} \sim 1.5$, and X_3 of $-0.5 \sim +0.5$.

The second graphical procedure was a plot of a given response variable as a function of one of the independent variables, while the others were held as a constant level. This type was called a partial deri-

vative graph drawn from computer. The responses were functions of the four independent variables and could be represented by Eq. 4 with the full relationship given by Eq. 1.

$$Y = f(X_1, X_2, X_3, X_4) \quad \text{(Eq. 4)}$$

Therefore, the relationship between the absorption rate and any one variable, e.g., X_1, might be viewed as a partial derivative of Y with respect to X_1 while

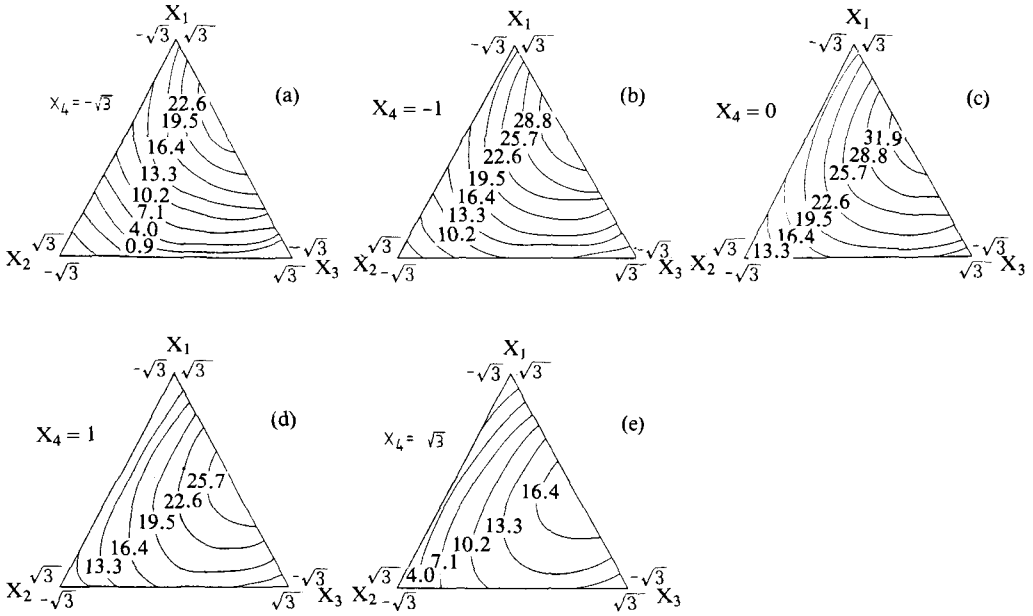


Fig. 6. Contour plots of absorption rate as a function of X_1, X_2, and X_3 at various, fixed level of X_4.

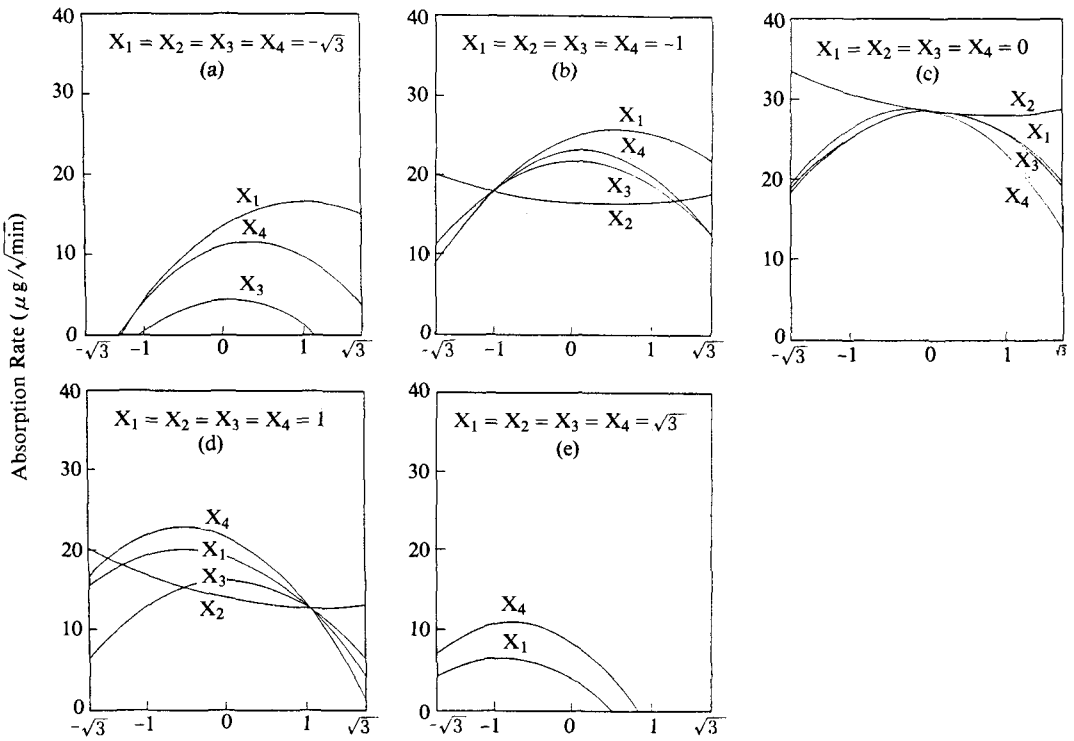


Fig. 7. Composite plots for absorption rate as a function of each independent variable, X_i(i = 1, 2, 3, and 4).

holding all other X's constant.

$$\frac{\partial Y}{\partial X_1}\Big)_{X_2X_3X_4} = f'(X_1, X_2, X_3, X_4) \quad \text{(Eq. 5)}$$

However, the value of the partial derivative was still a function of all the independent variables, because Eq. 1 was a second-order polynomial equation with all possible cross-terms. Accordingly, the full partial derivative with respect to X_1 was illustrated in Eq. 6;

$$\frac{\partial Y}{\partial X_1}\Big)_{X_1X_3X_4} = a_1 + 2a_{11}X_1 + a_{12}X_2 + a_{13}X_3 + a_{14}X_4 \quad \text{(Eq. 6)} ;$$

Also, since a change in value would change the graph significantly, the values at which X_2, X_3, and X_4 were held constant were very important.

Fig. 7 showed composite partial derivative plots for the *in vitro* absorption rates as a function of each of independent variables X_i (i = 1, 2, 3, and 4). Each of the three or four curves in each of Fig. 7 (a-e) represented one of the independent variables and the values noted at the top of each of the five graphs indicated the level at which any variable was being held constant during the partial derivative operation. For example, (b), (c), and (d) of figure 7 represented the superimposing of four curves drawn from computer for each of the four independent variables at the constant level of the top. But, in (a) and (e) of figure 7, two or three curves only were superimposed by computer, because the points of X_2 or X_3 were located out of the range of the graphs.

As shown in Fig. 7, the inflection points appeared at various values for all of the graphs. From these results that these inflection points were moved according to degree of the constant level, it could be known that there was an optimum formulation of ointment for the maximum absorption rate of griseofulvin through skin membrane.

Optimization of vehicle composition

The second-order polynomial regression equation was structured as an optimum regression model for determining the optimum values of independent variables for the *in vitro* maximum absorption rate of griseofulvin. From the results of Figs. 3-7, $0 < X_1 < 1$, $-\sqrt{3} < X_2 < -1$, $-0.5 < X_3 < 0.5$, and $-1 < X_4 < 0.5$ were selected as the constant limits of values of X_i and the absorption rates were calculated by the increment of 0.1 from minimum value to maximum value for each of constant limits of X_i. Within the constant limits of values of X_i, the optimum formulation was obtained from the computer and listed in Table VII. According to the conditions of optimum formulation listed in Table VII, hydrophilic ointment was prepared again and absorption rate was determined in the same manner as the previous. The experimental value by *in vitro* absorption experiment and the prediction value obtained from computer for absorption rates were summarized in Table VIII and they agreed well each other.

CONCLUSION

A computer optimization technique was applied to obtain the optimum formula of hydrophilic ointment giving the *in vitro* maximum absorption rate through skin membrane. The *in vitro* absorption rate was used as a characteristics for deciding the optimum formula of ointment vehicle and the amounts of sodium lauryl sulfate (X_1), white petrolatum (X_2), propylene glycol (X_3), and stearyl alcohol (X_4) were selected as the independent variables. And the following results were obtained from both of partial derivative and contour graphs, and a second-order polynomial regression equation;

1) The values of independent variables for optimum formulation were $X_1 = 0.3$, $X_2 = -1.7$, $X_3 = 0$, and $X_4 = -0.3$, respectively.
2) Formulation of hydrophilic ointment containing in KP IV was very similar to the optimum formula obtained from this study, but the amount of white petrolatum containing in the optimum formula was less than that in the official compendiums. It was considered that the degree of affinity between white petrolatum and griseofulvin was an important factor.

Table VII. The optimum conditions for formulation of hydrophilic ointment

Variable	Code level	Physical amount(w/w%)
X_1(Sodium lauryl sulfate)	0.3	1.65
X_2(White petrolatum)	-1.7	16.5
X_3(Propylene glycol)	0	12.0
X_4(Stearyl alcohol)	-0.3	19.6

Table VIII. Comparison of experimental and prediction values at the optimum level

Response	Experimental value	Prediction value
Absorption Rate ($\mu g/\sqrt{min}$)	33.9912	33.8733

3) According to the conditions of optimum formula obtained, hydrophilic ointment was prepared again and tested. The results agreed very well. Therefore, it was believed that optimum formula for semi-solid dosage forms could be obtained from the application of optimization technique used in this study.

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LITERATURE CITED

1. Barry, B.W.: *Dermatology formulation.* Dekker, New York, p. 3 (1983).
2. Crank, J.: *The mathematics of diffusion.* 2nd ed., Clarendon press, Oxford. p. 2 (1975).
3. Katz, M. and Shaikhi, Z.I.: Percutaneous corticosteroid absorption correlated to partition coefficient. *J. Phzrm. Sci.* **54,** 591 (1965).
4. Higuchi, T.: Physical chemical analysis of percutaneous absorption process from creams and ointments. *J. Soc. Cosmet. Chem.* **11,** 85 (1969).
5. Dempski, R.E., Portnoff, J.B. and Wase, A.W.: *In vitro* release and *in vivo* penetration studies of a topical steroid from nonaquevehicles. *J. Pharm. Sci.* **58,** 579 (1969).
6. Poulsen, B.J., Young, E., Coquilla, V. and Katz, M.: Effect of topical vehicle composition on the *in vitro* release of fluocinolone acetonide and its acetate ester. *J. Pharm. Sci.* **57,** 928 (1968).
7. Whitworth, C.W.: Effect of various liquids on the diffusion of salicyclic acid from ointment bases. *J. Pharm. Sci.* **57,** 1540 (1968).
8. Akhter, S.A. and Barry, B.W.: Absorption through human skin of ibuprofen and flurbiprofen; Effect of dose variation, deposited drug films, occulusion and the penetration enhancer N-methyl-2-pyrrolidinone. *J. Pharm. Sci.* **37,** 27 (1985).
9. Berstrom, T.K., Flynn, G.L. and Higuchi, W.I.: Phisico- chemical study of percutaneous absorption enhancement by dimethyl sulfoxide; Kinetic and thermodynamic determinates of dimethyl sulfoxide mediated mass transfer of alkanols. *J. Pharm. Sci.* **75,** 479 (1986).
10. Coldman, M.F., Poulsen, B.J. and Higuchi, T.: Enhancement of percutaneous absorption by the use of volatile; Nonvolatile systems as vehicle. *J. Pharm. Sci.* **58,** 1098 (1969).
11. Kaiho, F., Nomura, H., Makabe, E. and Kato, Y.: Percutaneous absorption of indomethacin from mixture of fatty alcohol and propylene glycol (FAPG Bases) through rat skin; Effects of fatty acid added to FAPG Base. *Chem. Pharm. Bull.* **35,** 2928 (1987).
12. Seki, T., Sugibayashi, K. and Morimoto, Y.: Effects of solvents on the permeation of nicardipine hydrochloride through the hairless rat skin. *Chem. Pharm. Bull.* **35,** 3054 (1987).
13. Flynn, G.L. and Roseman, T.R.: Membrane diffusion II; Influence of physical adsorption on molecular flux through heterogeneous dimethyl-polysiloxane barrier. *J. Pharm. Sci.* **60,** 1788 (1971).
14. Gsginella, T.S., Welling, P.G. and Bass, P.: Nicotine base permeation through silicon elastomers; Comparison of dimethyl polysiloxane and trifluoropropyl methyl-polysiloxane systems. *J. Pharm. Sci.* **63,** 1849 (1974).
15. Washitake, M., Takashima, Y., Tanaka, S., Anmo, T. and Tanaka, I.: Drug permeation through egg shell membrane. *Chem. Pharm. Bull.* **28,** 2855 (1980).
16. Durrheim, H., Flynn, G.L., Higuchi, W.I. and Behl, G.R.: Permeation of hairless mouse skin I: Experimental methods and comparison with human epidermal permeation by a alkanols. *J. Pharm. Sci.* **69,** 781 (1980).
17. Flynn, G.L., Durrheim, H. and Higuchi, W.I.: Permeation of Hairless mouse skin II; Membrane sectioning techniques and influence on alkanol permeabilities. *J. Pharm. Sci.* **70,** 52 (1981).
18. Bronaugh, R.S. and Stewart, R.F.: Methods for *in vitro* percutaneous absorption studies III; Hydrophobic compound. *J. Pharm. Sci.* **73,** 1255 (1984).
19. Foreman, M.I., Clanachan, I. and Kelly, I.P.: Diffusion barrier in skin a new methods of comparison. *Brit. J. Dermatol.* **108,** 1549 (1983).
20. Guy, R.H., Hadgraft, J. and Maibach. H.I.: A pharmacokinetic model for percutaneous absorption. *Inter. J. Pharmaceutics* **11,** 119 (1982).
21. Ozawa, Y., Yamahira, T. and Nadai, T.: A new technique for *in vivo* percutaneous absorption. *Chem. Pharm. Bull.* **35,** 2114 (1987).
22. Bronaugh, R.L. and Stewart, R.F.: Methods for *in vitro* percutaneous absorption studies VI; Preparation of the barrier layer. *J. Pharm. Sci.* **75,** 487 (1986).

23. Tojo, K., Chiang. C.C. and Chien, Y.W.: Influence of donor solution upon skin permeation of drug. *J. Chem. Engin. Japan* **19,** 153 (1986).
24. Tojo, K., Valia, K.H. and Chien, Y.W. and: Skin permeation of drugs-diffusion and partitioning. *J. Chem. Engin. Japan* **18,** 174 (1985).
25. Bronaugh, R.L. and Stewart, R.F.: Methods for *in vitro* percutaneous absorption studies IV; The flow-through diffusion cell. *J. Pharm. Sci.* **74,** 64 (1985).
26. Scheuplein, R.J.: Mechanism of percutaneous adsorption I; Routes of penetration and the influence of solubility. *J. Invest. Dermatol.* **45,** 334 (1965).
27. Scheuplein, R.J.: Mechanism of percutaneous adsorption II; Transient diffusion and the relative importance of various routes of skin penetration. *J. Invest. Dermatol.* **48,** 79 (1967).
28. Idson, B.: Percutaneous absorption. *J. Pharm. Sci.* **64,** 901 (1975).
29. Katz, M. and Poulsen, B.J.: Corticoid, vehicle and skin interaction in percutaneous absorption. *J. Soc. Cosmet. Chem.* **23,** 565 (1972).
30. Forner, D.E., Buck, J.R. and Banker, G.S.: Mathematical optimization techniques in drug product design and process analysis. *J. Pharm. Sci.* **59,** 1587 (1970).
31. Schwarz, J.B., Flsmholz, J.R. and Press, R.H.: Computer optimization of pharmaceutical formulation I; General procedure. *J. Pharm. Sci.* **62,** 1165 (1973).
32. Akitoski, Y., Takayama, K., Machida, Y. and Nagai, T.: Computer optimization of the formulation of acrylic plaster. *Chem. Pharm. Bull.* **33,** 4536 (1985).
33. Sheikh, E., Ghani, M. and Jones, R.E.: Simplex search in optimization of capsule formulation. *J. Pharm. Sci.* **69,** 1135 (1980).
34. Schwarz, J.B.: Optimization techniques in product formulation. *J. Soc. Cosmet. Chem.* **32,** 1536 (1985).
35. Takayama, K., Nambu, N. and Nagai, T.: Computer optimization of formulation of flufenamic acid/polyvinylpolypyrrolidone/methyl cellulose solid dispersions. *Chem. Pharm. Bull.* **31,** 4496 (1983).
36. Takayama, K., Imaizumi, H., Nambu, N. and Nagai, T.: Mathematical optimization of formulation of indomethacin/Polyvinylpolypyrrolidone/methyl cellose solid dispersions by the sequential uncontrained minimization technique. *Chem. Pharm. Bull.* **33,** 292 (1985).
37. Korea Pharmacopoeia IV, p. 919 (1982).
38. United States Pharmacopoeia XXI, p. 775 (1985).
39. Japan Pharmacopoeia XI, D-474 (1986).
40. Brook, R.J. and Arnold, G.C. ed.: *Applied regression analysis and experimental design* (Statistics, textbooks and monographs) vol. 62, Marcel, Dekker, Inc., New York. 1985.
41. D.C. Montgomery ed.: *Design and analysis of experiments.* John Wiley & Sons. New York. 1976.
42. Higuchi, T.: Rate of reiease of medicaments from ointment bases containing drugs in suspension. *J. Pharm. Sci.* **50,** 874 (1961).
43. Zia, H., Proveaux, W.J., O'Donnell, J.P. and Ma, J.K.: Chromatographic analysis of griseofulvin and metabolites in biological fluids. *J. Chromat.* **181,** 77 (1980).
44. Hackett, L.P. and Dusci, L.J.: Determination of griseofulvin in human serum using high-performance liquid chromatography. *J. Chromat.* **155,** 206 (1978).
45. Townley, E. and Rodon, P.: High performance liquid chromatographic analysis of griseofulvin in drug substance and solid dosage forms: Separation of impurities and metabolites. *J. Pharm. Sci.* **69,** 523 (1980).
46. Nation, R.L., Peug, G.W., Smith, V. and Chiou, W.L.: Simple, rapid and micro high-pressure liquid chromatographic determination of plasma griseofulvin levels. *J. Pharm. Sci.* **67,** 805 (1973).
47. Chowhan, E.T. and Prichard, R.: Release of corticoids from oleaginous ointment bases containing drug in suspension. *J. Pharm. Sci.* **64,** 754 (1975).
48. Bottari, F., Di Colo, G., Nannipieri, E., Saettone, M.F. and Serafini, M.F.: Influence of drug concentration on *in vitro* release of salicylic acid from ointment bases. *J. Pharm. Sci.* **63,** 1779 (1981).
49. Myers, Roy E.: *Microcomputer graphics.* Addison-Wesley publishing Company, Inc. 1983.