약제학회지(1988), 제 18권 제 1호
J. Kor. Pharm. Sci., Vol.18, No.1, 31-41(1988)

Studies on Optimization of Vehicle Composition for Percutaneous Absorption

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(Received February 8, 1988)

연고기제 조성의 최적화에 관한 연구

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Computer optimization technique was applied to obtain the optimum formula of o/w type ointment vehicle containing sodium lauryl sulfate and 1-methyl-2-pyrrolidinone (MP). In order to determine the feasibility of optimizing a vehicle composition with the aid of computer, the amounts of sodium lauryl sulfate (X_1), salicylic acid (X_2), and MP (X_3) were selected as the independent variables for the solubility and the absorption rates of salicylic acid (dependent variables). The experimental values of absorption rates agreed well with the calculation values obtained from the polynomial regression analysis, and the contour charts drawn by computer were useful in optimization process.

Percutaneous absorption of a drug can be improved by modifying the solubiliy of a particular drug in the vehicle, by altering the composition of the vehicle, or by modifying the chemical structure of the drug. Dempski, *et al.*,[1] Poulsen, *et al.*,[2] Inagi, *et al.*,[3] and Otagiri, *et al.*[4] demonstrated the effects of altering the vehicle composition on the release of dexamethasone, fluocinolone acetonide and its acetate ester, indomethacin and cyclodextrins, respectively. Ostrenga, *et al.*[5,6] showed that the flux equations describing the passive transport of two steroids across a membrane are useful in prediction of the optimal vehicle composition.

Solvents, other than water, such as dimethyl sulfoxide, dimethylformamide and azone, enhanced skin penetration[7-12] and Idson[13] reviewed the role of solvents in percutaneous absorption. N-methyl-2-pyrrolidinone enhanced the penetration flux of ibuprofen and flurbiprofen, over threefold, in an open cell *"in-vivo* mimic" design using human skin.[14] And Kurihara, *et al.*[15] reported the physicochemical study of percutaneous absorption enhancement by dimethyl sulfoxide.

Surfactants offer possibilities of improving topical vehicles and promoting a more thorough diffusion of the drug from the vehicle. Cooper[16] studied the effects of surfactants on the percutaneous absorption by using abdominal skin of human as a membrane and Scarpotdar, *et al.*[17] reported the effect of nonionic surfactants on the percutaneous absorption of lidocaine across hairless mouse skin.

Much of work on application of optimization techniques for the development of solid dosage forms, e.g. tablets or capsules, has been reported in the literature, but few articles reported on semisolid dosage forms, especially, ointment. Sheikh, *et al.*[18] studied the simplex method in optimization of a capsule formulation using the dissolution rate and compaction rate as the desired responses to

be optimized. Fonner, *et al.*[19] demonstrated the applicability of mathematical optimization techniques to pharmaceutical systems. Schwartz, *et al.*[20,21] utilized factorial design and computer techniques to optimize a formulation of tablets. And a computer optimization technique was applied to obtain the optimum formula of acrylic plaster vehicle containing ketoprofen[22] and the optimum solid dispersions which provide a high dissolution rate and high stability of flufenamic acid.[23]

In this paper, computer optimization technique was applied to obtain the formulation that maximizes the percutaneous absorption of salicylic acid from o/w type ointment containing sodium lauryl sulfate and a penetrating agent, 1-methyl-2-pyrrolidinone. Generally, the optimization techniques used in pharmaceutical field may be classified into two categories. The first is the simplex method[18] and the other is the method based on the statistically designed experiment as reported by Schwartz, *et al.*[20,21] The simplex procedure derives its name from the geometric figure that is moved along the response surface in search of the optimum. This optimization method approaches the optimum in stepwise fashion by moving away from low values of the response function rather than by moving in a line toward the maximum. Therefore, the simplex procedure can be likened to a search for a mountain summit on which one is blind-folded, feeling one's way a step at a time. On the other hand, the typical factorial design plus response surface analysis could be likened to developing a contour map of the mountain first and thereby determining where the summit lies. And there are two types in optimization problems. One is the constrained optimization problem that sacrifices one characteristic for another and the other is the unconstrained optimization problem that performs absolute optimization.

The work reported here was undertaken to determine the feasibility of optimizing a vehicle composition with the aid of computer. And the response surface methodology of the constrained optimization problem was applied to obtain the optimum composition of o/w type vehicle.

THEORY

A constrained optimization problem is one in which a function is optimized subject to restriction or limits placed on the controllable variables. Mathematically, the problem is to optimize

$$Y=\phi(x_i), \qquad i=1, 2, \cdots\cdots, n \qquad \text{Eq. 1}$$

such that

$$g_j(x_i)=\alpha_j, \qquad j=1, 2, \cdots\cdots, p\leq n \qquad \text{Eq. 2}$$

$$g_j(x_i)\geq\alpha_j, \qquad j=p+1, p+2, \cdots\cdots, m \qquad \text{Eq. 3}$$

Eq. 1 represents the function to be optimized and is generally referred to as the objective function. The function ϕ is called the response surface. Eqs. 2 and 3 are referred to as equality and inequality constraints for the specified constants, α_j. Only the greater than or equal to relationship is represented, since $h(x_i)\leq 0$ may be written as $g(x_i) = -h(x_i)\geq 0$. Thus, the constrained optimization problem involves locating levels of X_i that produce an optimal response in $\phi(x_i)$ such that the constraints of the problem are not violated.

Among the techniques available for solving the constrained optimization problem in its general form (Eqs. 1-3), the most versatile method appears to be the Lagrangian method[18]. This method contains one numerical example illustrating the process of optimization. The Lagrangian method of solving constrained optimization problems assumes that a mathematical relationship exists which relates the response variable to levels of the controllable variables. However, mechanisms in pharmaceutical product and process design problems are often so complicated that the formulation of an analytical mathematical model is out of the question. If an analytical model is impossible to derive, then empirical mathematical model may be developed by using multiple-regression techniques[24]. Thus, it is usually possible to fit a polynomial to the response surface which can be expected to give an adequate approximation of the response surface over the region of experimentation.

Then, experimental design is dependent on the number of variables involved in the study, but it is

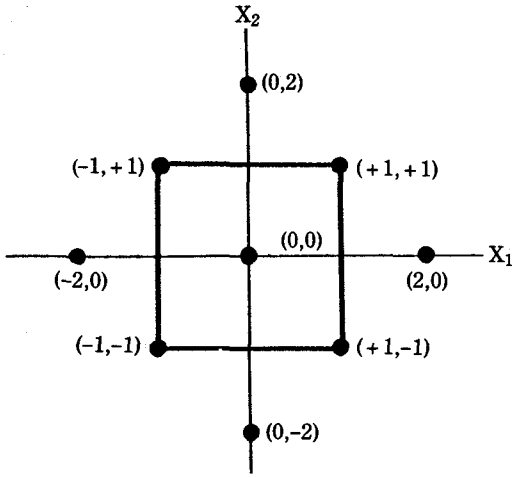

Figure 1a—Central composite design for k = 2.

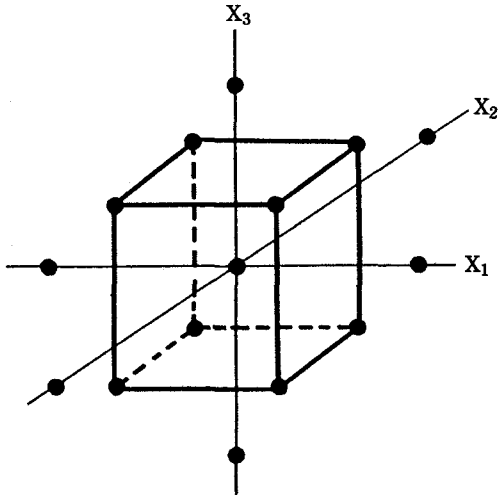

Figure 1b—Central composite design for k = 3.

not efficient to obtain data by performing experiments in all the points of the combinations of variables. Therefore, the central composite rotatable design[25] was used in this experiment and these designs consisted of a 2^k factorial or fractional factorial (coded to the usual 1 notation) augmented by 2k axial points and X_0 center point (0,0,0)[26]. Central composite rotatable designs for k = 2 and k = 3 are shown in Fig. 1a and 1b, and the modified factorial design shown in Table I, for the absorption test, requires a total of 15 experiments. In Table I, the first 8 experiments represent a factorial design for three factors at two levels. The

Table I—*Experimental Design for Three Factors.*

Formulation No.	Factor level in coded form		
	X_1	X_2	X_3
1	1	1	1
2	1	1	-1
3	1	-1	1
4	-1	1	1
5	-1	-1	1
6	-1	1	-1
7	1	-1	-1
8	-1	-1	-1
9	2	0	0
10	0	2	0
11	0	0	2
12	-2	0	0
13	0	-2	0
14	0	0	-2
15	0	0	0

Table II—*Experimental Design for Solubility Test.*

Formulation No.	Factor level in coded form	
	X_1	X_3
1	1	1
2	1	-1
3	-1	1
4	-1	-1
5	2	0
6	0	2
7	-2	0
8	0	-2
9	0	0

two levels here are represented as + 1 and -1. For the remainder of the study, three additional levels were selected; Zero represents the base level midway between the above mentioned levels and the positive and negative 2 values represent the extreme values. Table II for the solubility test of salicylic acid in o/w type ointment requires a total of 9 experiments, because it has two factors at two levels. Translations of experimental conditions to physical units for absorption and solubility tests

Table III—*Physical Amount and Statistical Code for Experiments.*

Factor	Factor level in coded form				
	-2	-1	0	1	2
X_1(Sodium lauryl sulfate, w/w%)	0.5	1.5	2.5	3.5	4.5
X_2(Salicylic acid, w/w%)	0.4	0.8	1.2	1.6	2.0
X_3(MP, v/w%)	0	2	4	6	8

are shown in Table III.

In this experiment, the work was performed with physical units and the resultant data were inputed into computer. The equation for computer analysis has the following form;

$$Y_i = a_0 + a_1X_1 + a_2X_2 + \cdots + a_{11}X_1^2 + \cdots a_{33}X_3^2 + \cdots + a_{12}X_1X_2 + \cdots a_{23}X_2X_3 \quad \text{Eq. 4}$$

where, Y_i = level of a given response (dependent variable)

a = regression coefficients for second-order-polynomial level

X_i = level of independent variable

It is obvious from the complexity of the equation that the measured responses are dependent on many factors and interactions between factors. Since no simple relationship exists for each of the evaluated properties, the equation as such are difficult to use for data analysis. However, response surface contours, the shape of the geometric surface generated by changes in a response produced by continuous variations in values of two interacting factors, may be generated from the above equation and plotted two dimensionally as contour charts. The analysis of the effect of many factors on a response is allowed from the preparation of a number of such charts.

EXPERIMENTAL

Materials

Salicylic acid (Hayashi Pure Chem., Japan), 1-methyl-2-pyrrolidinone (Sigma Chem., Co.), sodium lauryl sulfate (Katayama, Japan), white petrolatum (Fluka AG Chemicals, Switzerland), and cetyl alcohol (Wako Purechem., Japan) were used as received. Propylene glycol and stearyl alcohol were from Junsei Chem. Co., Japan. All other materials used were of reagent grade.

Test Animals

Male, Sprague-Dawley rats weighing between 180 and 240g were selected for this study.

Apparatus

Diffusion cell for absorption experiments was a specially designed acrylic cell described by Lee, *et al.*[27] The internal diameter and the height of cell body were 50 and 10 mm, respectively. The bottom of cell body was closed and the top was opened. And spectrophotometer (LKB, model 4050, U.K.), incubator (Napco, model 330, U.S.A.), water bath (Haake, type FS2 Germany), and personal computer (Hyosung, model PC-8000, Korea) were used.

Preparation of Ointments

All of the ointments used in this experiment were prepared according to Kim's formulation and method[27]. Salicylic acid was added to the warm base (75 °C) and the mixture was stirred until cooled in order to achieve homogeneity. 1-Methyl-2-pyrrolidinone, hereinafter referred as MP, was added to the base prior to incorporating with salicylic acid.

Solubility Test

Salicylic acid was passed through a 200-mesh sieve with shaking for five minutes and appropriate amounts, ranging from 0.5 to 6.0 w/v % with increment of 0.1, were incorporated into each of 9 ointment bases as written in Table II. Solubility of salicylic acid in each of 9 ointments was determined according to Bottari method[28]. That is, the ointments containing excess amount of salicylic acid were stored in a incubator 30 ± 0.1 °C for 3 days and they were examined on a 100 × microscope, whether or not suspended particles were visible.

Preparation of Skin Membranes

The membranes were full-thickness skin taken from the abdominal surface of the hairless rats. The rats were sacrificed by overdosing of ether and the hair was shaved with an electric clipper. A regular square section (55×55mm) of abdomi-

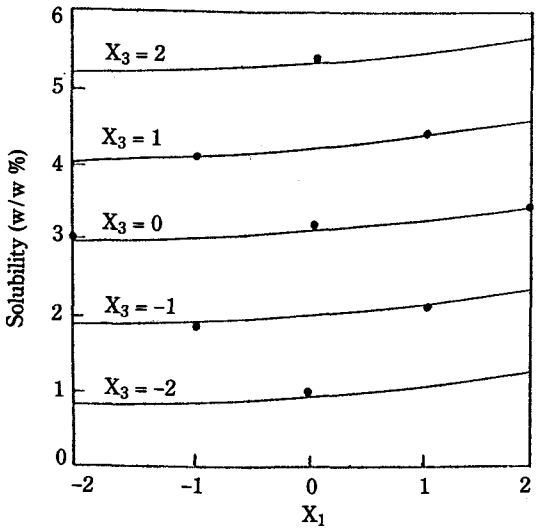

Figure 3—Solubility of salicylic acid as function of sodium lauryl sulfate (X_1) at various concentration of MP (X_3)
Key: — (solid line), computer generated plot; ● (points), experimental values

nal skin was excised from the animal with surgical scissors. The incised skin was not firmly attached to the viscera and lifted easily from the animal. Adhering fat and other visceral debris were removed carefully from the undersurface with tweezers.

***In Vitro* Absorption of Salicylic Acid from Ointments**

The donor part of diffusion cell was filled with an ointment and the excess was removed with a spatula to produce an even surface. The skin membrane was carefully set in place with stratum corneum facing the donor compartment and pressed on the ointments in order to keep up complete contact of the skin with the ointments.

The receiver compartment was a prewarmed 0.85 % saline solution and was always maintained at 37 °C and 150 rpm. The samples were withdrawn from the receiving phase at appropriate intervals and diluted with 0.1N HCl solution. The concentration of salicylic acid was determined spectrophotometrically at 297 nm. Controls consisted of the base without salicylic acid. Blank runs demonstrated the absence of diffusable substances which might interfere with the measurements. The experiment was stopped after 8 hr. Absorption rates were calculated from each of the experiments that was repeated five times and the mean value was taken.

Table IV—*Comparison of Solubilities of Salicylic Acid Obtained from Solubility Experiments and Optimum Polynomial Regression Equation.*

Formulation No.	Solubility (w/w %)	
	Experimental values	Calculation values
1	4.3	4.34
2	2.1	2.14
3	4.1	4.14
4	1.9	1.94
5	3.4	3.38
6	5.4	5.38
7	3.0	2.98
8	1.0	0.98
9	3.2	3.11

RESULTS AND DISCUSSION

Solubility of Salicylic Acid in Ointments

The results of solubility tests were shown in Fig. 3 and Table IV. The solubility of salicylic acid was increased with increasing the concentrations of MP and sodium lauryl sulfate in ointment bases prepared according to nine formulations in Table II. Moreover, it was known that MP was more effective than sodium lauryl sulfate on the increase of the solubility of salicylic acid, as shown in Fig. 3.

Among nine formulations listed in Table IV, the solubility of salicylic acid in ointments of formulation No. 6, which contained 8 v/w% MP was the highest as 5.4 w/w%. On the other hand, it was the lowest as 1.0 w/w% in ointments of formulation No. 8 which did not contain MP

Absorption of Salicylic Acid

The absorption of salicylic acid from 15 kinds of ointments which contained various concentrations of MP and sodium lauryl sulfate was examined *in vitro*. It was known that the amount of drug absorbed from ointments was approximately

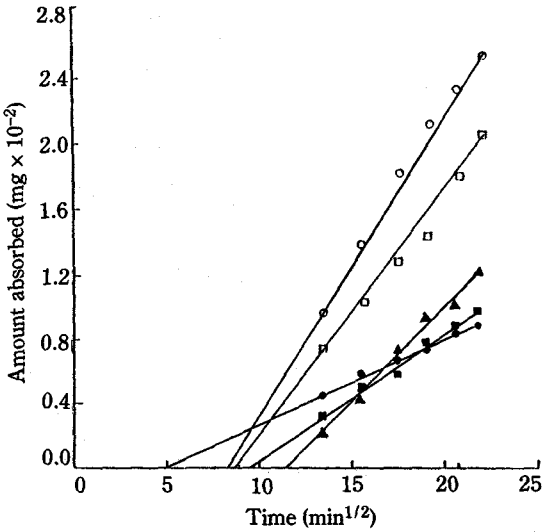

Figure 4—Absorption profile of salicylic acid abtained from some of 15 ointments through rat skin membrane. Key: ○, formulation No. 1; ▲, formulation No. 7; □, formulation No. 4; ●, formulation No. 13; ■ formulation No. 5

Table V—*Comparison of Absorption Rates Obtained from Absorption Experiments and Polynomial Regression Equation by a Computer.*

Formulation No.	Absorption rates ($mg/min^{1/2} \times 10^{-3}$)	
	Experimental values	Calculated values
1	1.774	1.790
2	1.901	2.012
3	0.697	0.770
4	1.437	1.352
5	0.907	0.821
6	1.630	1.700
7	1.008	1.006
8	1.169	1.183
9	1.379	1.302
10	2.200	2.159
11	0.943	0.999
12	0.985	1.042
13	0.606	0.623
14	1.657	1.582
15	1.488	1.464

Table VI—*Parameters for Optimum Regression Equation of Absorption Rate and Solubility of Salicylic Acid Determined by Multiple Regression Analysis.*

Coefficient	Solubility	Absorption Rate
a_0	3.111	1.464
a_1 (X_1)	0.1	0.065
a_2 (X_2)		0.384
a_3 (X_3)	1.1	-0.146
a_{11} (X_1^2)	0.017	-0.073
a_{22} (X_2^2)		-0.018
a_{33} (X_3^2)	0.017	-0.043
a_{12} (X_1X_2)		0.122
a_{13} (X_1X_3)	0.0	0.032
a_{23} (X_2X_3)		0.003
r	0.998	0.985
F		37

r: multiple correlation coefficient. F: level of significance

proportional to the square root of time[29] Thus, when the amounts of salicylic acid absorbed from ointments were plotted versus $t^{1/2}$, straight lines were obtained. Fig. 4 showed the absorption profiles of salicylic acid from some of 15 formulations in Table I.

The absorption rates were obtained from the slopes (absorption amount per time) of straight lines as shown in Fig. 4 and the values were calculated by a linear regression method using the personal computer. These values were listed in Table V.

In Table V, absorption rate of formulation 10 was the highest and formulation 13 was the lowest.

Regression Equation for the Prediction of Ointment Characteristics

In order to predict each characteristic of the model ointment formulation, the amounts of sodium lauryl sulfate (X_1), salicylic acid (X_2) and MP (X_3) were selected as the independent variables for the solubility test of salicylic acid in ointments. These variables are directly controllable

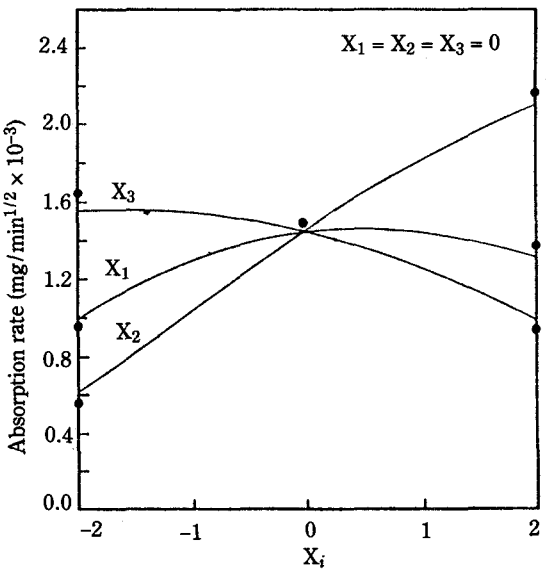

Figure 5—Composite plots for absorption rate as a function of each independent variable, X_i (i = 1,2,3).
Key: — (solid line), computer generated plot; ● (points), experimental values

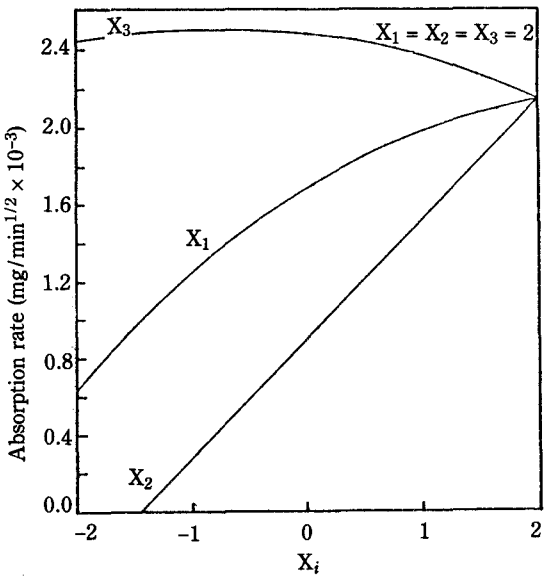

Figure 6—Composite plots for absorption rate as a function of each independent variable, X_i (i = 1,2,3).
Key: — (solid line), computer generated plot; ● (points), experimental values

factors. Eq. 4 in the theory of this paper was used for the prediction of absorption rates and the computerized regression analysis of the dependent variables was made with the aid of personal computer. The overall combinations of two or three independent variables were investigated at the point of statistical significance in order to obtain the optimum regression equations. Parameters of optimum regression equations obtained were summarized in Table VI. The correlation coefficient was used as the index of determination for selecting an optimum combination of various factors. High correlation coefficients were observed as shown in Table VI. Absorption rates of salicylic acid from each ointment of 15 formulations in Table I were obtained from the optimum regression equation using parameters in Table VI and listed in Table V. There were almost no differences between experimental values and calculation values except formulation No. 2. The solubility of salicylic acid obtained from the optimum regression equation was listed in Table IV and they also agreed well.

Graphical Approach

Two graphical techniques have been proven useful in the optimization procedure[20]. The first graphical procedure was a plot of a given response variable as a function of sodium lauryl sulfate (X_1) at various constant concentrations of MP (X_3). As shown in Fig. 3, computer generated plots were exactly fitted to experimental values. Figs. 5 and 6, composite partial derivative plots drawn from computer, showed composite plots for absorption rates as a function of each independent variable, X_i (i = 1,2,3), at once. That is, three of the single graph were superimposed by the computer, by keeping the abscissa in experimental units and the X axis in physical (coded) units. The values at the top of Figs. 5 and 6 represented the levels at which any other variables were being held constant during the partial derivative operation. In these figures, 0 and 2 levels were selected, respectively.

As shown in Fig. 5, it could be seen that the inflection points appeared on both of curves, X_1 and X_3, but it disappeared in X_1 curve of Fig. 6. From the results that these inflection points were moved or disappeared according to degree of constant levels, it could be known that there was an optimum formulation of ointments for the maximum

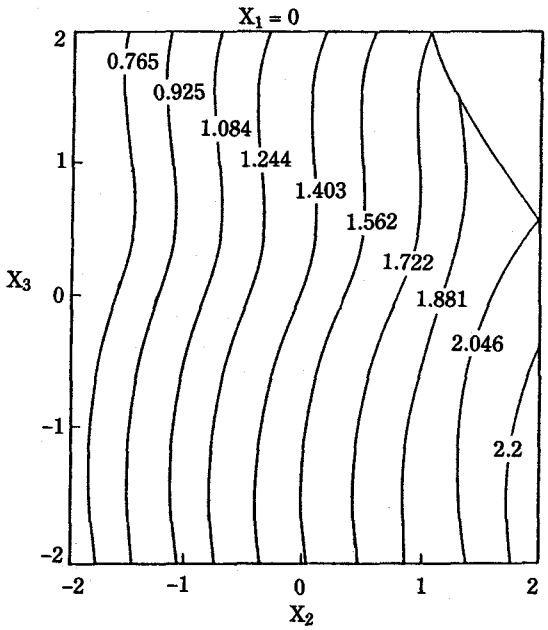

Figure 7—Contour plot of absorption rate as a function of salicylic acid and MP at a constant concentration of sodium lauryl sulfate ($X_1 = 0$).

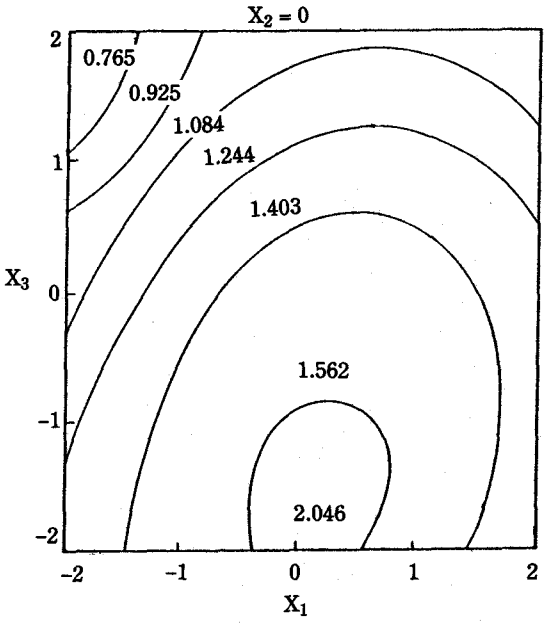

Figure 8—Contour plot of absorption rate as a function of sodium lauryl sulfate and MP at a constant concentration of salicylic acid ($X_2 = 0$).

absorption of salicylic acid.

The second type of plot useful in optimization was the contour plot drawn by computer. The shape, or contour, of the solid geometrical surface generated by changes in a response produced by continuous variations in values of two interacting factors was known as a response surface contour[24]. Response surface contours might be plotted two dimensionally as contour graphs. While only two interacting factors could be accommodated on a single contour graph, the preparation of a number of such graphs was allowed for the analysis of the effect of many factors on a response. Therefore, contour graph illustrated combination of the controllable variables producing the same response. A computer program for such purpose in this paper was writtein in basic language and contours were set by interpolation from X_1 and X_2 values obtained from the program output.

Three types of contour graphs were constructed for studying the interactions of factors contributing to the absorption rate of the drug in this study. The first type was the result of fixing sodium lauryl sulfate (X_1) Fig. 7 was a representative of the graphs of this type. The second type of contour graphs resulted from fixing salicylic acid (X_2) and plotting M.P. (X_3) versus sodium

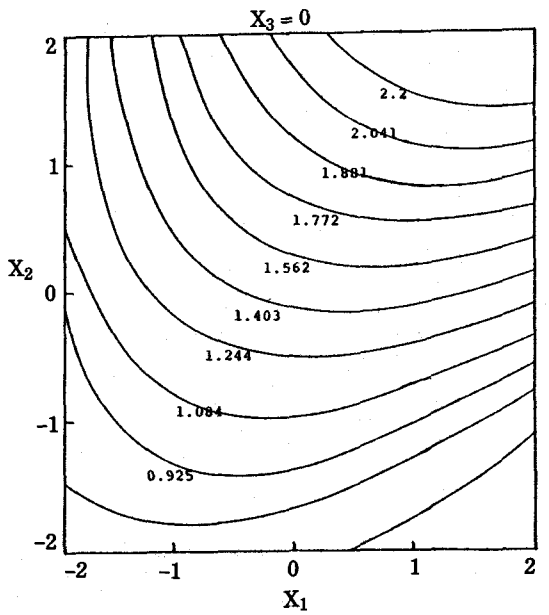

Figure 9—Contour plot of absorption rate as a function of sodium lauryl sulfate and salicylic acid at a constant concentration of MP ($X_3 = 0$).

Table VII—*Best Conditions for Optimum Formulation of O/ W Type Ointments.*

Variable	Physical level
X_1 (Sodium lauryl sulfate)	2
X_2 (Salicylic acid)	2
X_3 (MP)	-0.9

Table VIII—*Comparison of Experimental Value and Calculation Value at the Optimum Level.*

Response	Experimental value	Calculation value
Absorption rate $mg/min^{1/2} \times 10^{-3}$	2.471	2.515

lauryl sulfate (X_1). As shown in Fig. 8, the center area at the bottom of the figure was the point where the absorption rate was at maximum. Moving from this area along either X_3 or X_1 axis resulted in a decrease in absorption rate. The third type was Fig. 9 of fixing MP (X_3). The center area at the top of the figure showed the circle where the absorption rate was at a maximum. Similarly, absorption rate was decreased with moving from the center area along either X_1 or X_2 axis. Therefore, it could be known that the there was optimum values of X_1 and X_3 producing a maximum absorption rate.

Optimization

The polynomial regression equation in Table VI was structured as a constrained optimization problem for determining the best conditions for the absorption rate used as a constraint, and the formulation which gave the optimum value of each variable was obtained within the constant limits of values of X_i. As the constant limits of values of X_i, $-0.5 \leq X_1 \leq 2$, $1 \leq X_2 \leq 2$ and $-2 \leq X_3 \leq -0.5$ were selected from Figs. 5-8 and the absorption rates were calculated in the increment of 0.1 from the maximum value to the minimum value of each limit of X_i. The results were listed in Table VII.

According to the conditions for optimum formulation in Table VII, ointments was prepared again and absorption rate was determined. The experimental value of absorption rate and the calculation value obtained from polynomial regression analysis were summarized in Table VIII. As shown in Table VIII, experimental and calculation values agreed well.

SUMMARY

A computer optimization technique was applied to obtain the optimum formula of o/w type ointment vehicle containing sodium lauryl sulfate and MP. The *in vitro* absorption rate and the solubility of salicylic acid were determined as characteristics for deciding the optimum for formula of ointment vehicle and the following results were obtained: 1) Both of MP and sodium lauryl sulfate increased the solubility of salicylic acid but MP was more effective. 2) The experimental value of absorption rate obtained from ointments prepared according to optimum formulation agreed well with the calculation value obtained from polynomial regression analysis. Therefore, it was considered that optimum formula for any characteristics of semisolid dosage forms such as ointments could be obtained by application of the computer optimization technique described in this paper.

ACKNOWLEDGEMENT

This research work was supported by the research grant from the Ministry of Education, the Republic of Korea, 1987.

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