Novel Approach for Determination of Correlation between *in Vivo* and *in Vitro* Dissolution Using the Optimization Technique[1)]

Kuniaki ISHII,* Yoko SAITOU, Ryota YAMADA, Shigeru ITAI, and Masami NEMOTO

Pharmaceutics Laboratory, Pharmaceutical Research Laboratories, Taisho Pharmaceutical Co., Ltd., No. 403, Yoshino-cho 1-chome, Ohmiya-shi, Saitama 330, Japan. Received January 19, 1996; accepted April 10, 1996

A new approach to determination of good correlations between *in vivo* and *in vitro* dissolution was studied using the optimization technique. Ibuprofen, which exhibits dissolution rate-limiting absorption, was used as a model drug. Ibuprofen capsules of two different release types were prepared, and their *in vivo* dissolution profiles were obtained from measurements of plasma concentration following oral administration of the capsules to beagle dogs by the mathematical deconvolution method using solution data of oral administration as a weight function. For the dissolution test to correspond to the *in vivo* dissolution profiles, the test was carried out at 12 levels (9 different sets of conditions) and results were analyzed with the optimization technique to deal with two factors. The first-order rate constant (k_d) and the dissolution time at 50% (t_{50}%) of the *in vivo* dissolution were selected for use as the response variables. Regression analysis was performed to describe the *in vitro* dissolution characteristics as functions of the pH of dissolution medium and paddle rotation speed in the paddle method. The *in vivo/in vitro* correlation obtained from the k_d was better than that obtained from the t_{50}%. The optimum conditions for dissolution testing corresponding to the *in vivo* k_d were determined to be a pH 6.6 for the dissolution medium and a 56 rpm paddle rotation rate. The experimental data obtained by dissolution testing was well fit by the predicted curve derived from *in vivo* and *in vitro* dissolution profiles. This dissolution test is applicable to the formulations containing ibuprofen of particle size within the experimental range.

Key words *in vivo* dissolution; *in vitro* dissolution; optimization technique; *in vivo/in vitro* correlation

Dissolution characteristics of formulations have been considered important in the evaluation of drug absorption for the development of oral solid dosage forms. In particular, the particle size of drug on dissolution from formulation is a dominant factor in the initial studies of oral solid dosage form. If a dissolution test reflecting *in vivo* dissolution in the gastrointestinal tract could be obtained, drug absorption could be accurately predicted. Therefore, numerous attempts have been made to determine the correlation between *in vivo* performance and *in vitro* dissolution.[2)]

However, it is not easy to determine the *in vivo/in vitro* correlation for dissolution, since dissolution tests designed for adaptation to the *in vivo* performance must be carried out under a great number of conditions. For this reason, few approaches have been used for finding *in vivo/in vitro* correlation, even though thorough study of correlation has been made.[3)]

In the present study, therefore, the optimization technique was applied to determine the *in vivo/in vitro* correlation for dissolution of oral solid dosage forms containing drugs of different particle size, in order to reduce the number of experimental conditions. In the pharmaceutical field, the optimization technique has been proven to be a useful approach for modeling of the *in vivo* performance of suppositories[4)] and selecting pharmaceutical formulations.[5)] In this optimization study, the method reported by Takayama *et al.*[5)] was used to elucidate *in vitro* dissolution behavior for capsules containing ibuprofen of two different particle sizes. The *in vivo* dissolution profiles were obtained from plasma concentrations of ibuprofen following oral administration of capsules to beagle dogs using the mathematical deconvolution method.[6)] In general, the dissolution profiles of capsules follow the first-order kinetics or Wagner's dissolution model.[7)] The model is based on the log–normal density function, and dissolution time at 50% dissolution (t_{50}%) is closely related to the mean of the log–normal density function. Therefore, the first-order rate constant (k_d) or t_{50}% is widely used as an index of dissolution for pharmaceutical formulations.[7)] For the determination of *in vivo/in vitro* correlation, these indexes have been used as important response variables for comparison of dissolution behavior. The *in vivo* k_d and the *in vivo* t_{50}% were obtained from *in vivo* dissolution profiles. Using them as the response variables, regression analysis was performed to describe the *in vitro* dissolution characteristics as a function of the pH of the dissolution medium and the paddle rotation speed in the paddle method. A contour graph was used to estimate the meaning of the regression equation. The optimum condition for the dissolution test corresponding to *in vivo* dissolution performance was determined from the equations obtained by regression analysis.

Materials and Methods

Materials Ibuprofen was purchased from Boots Co., Ltd., and was recrystallized to obtain particles of two sizes. All other chemicals were of reagent grade or the Pharmacopoeia of Japan (JP) grade.

Measurement of Particle Size The particle size was measured using a laser diffraction method (Maicrotrac FRA, Nikkiso Co., Ltd.). The median diameters of the particles were 16 and 149 μm.

Preparation of Capsules Capsule A and capsule B contained ibuprofen particles with median diameters of 16 and 149 μm, respectively.

Ibuprofen (80 g), lactose (70 g), and hydroxypropylcellose (10 g) were mixed, the mixture was kneaded with water (15 ml) and the wet granule was dried at 50 °C. Then, the granule (150 mg) containing 80 mg of ibuprofen was hand-filled in size 3 hard capsule.

Preparation of Ibuprofen Solution Ibuprofen (80 mg) was dissolved in 0.1 M NaOH solution (20 ml). The pH of the ibuprofen solution was 7.5.

***In Vivo* Absorption Study** Three beagle dogs (11—12 kg, 2 years old) were used at intervals of longer than 7 d. They were fasted for 16 h before drug administration. Blood samples (2.5 ml) were taken at 15, 30, 45,

* To whom correspondence should be addressed.

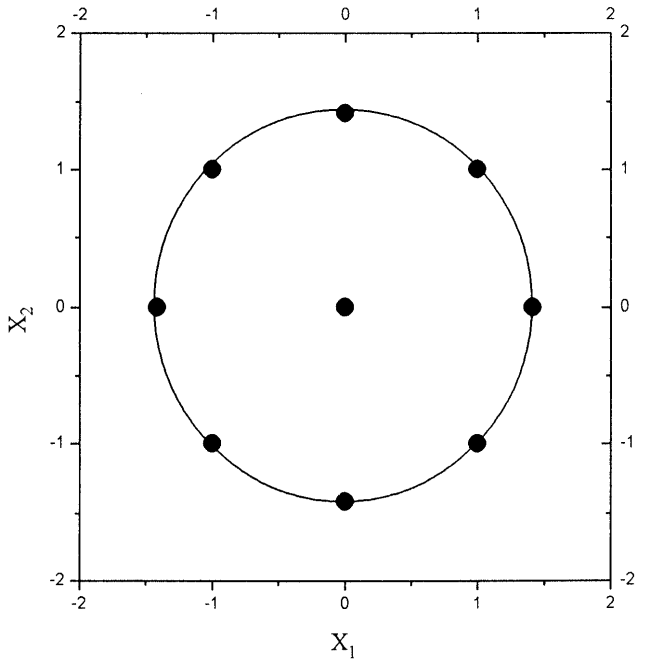

Fig. 1. Central Composite Experimental Design for Two Factors

60, 90, 120, 150, 180, 210, 240, 300, 360, 480 and 1440 min after the oral administration. For administration of the drug solution, blood samples were also taken at 5, 10 and 20 min. The samples were centrifuged (3000 rev./min, 10 min) and plasma was stored in a refrigerator until assayed. A 0.5 ml portion of plasma was added to 0.5 ml 1 M HCl, and the plasma was then extracted with 5 ml of cyclohexane containing 6 μg/ml of flurbiprofen as an internal standard. After centrifugation (3000 rev./min, 10 min), the organic phase (4 ml) was transferred to a new tube, and the solvent was evaporated. The residue was dissolved in 100 μl of acetonitrile and assayed by HPLC. The operating conditions for HPLC were as follows: detector, UV absorption spectra; detector wavelength, 220 nm; column temperature, 50 °C; flow rate, 1 ml/min; mobile phase, purified water, acetonitrile, and phosphoric acid (42 : 58 : 0.1).

Experimental Design A total of 12 levels (9 dissolution conditions) based on the central composite experimental design for two factors graphically illustrated in Fig. 1 were tested. There are more experimental conditions for the box-wilson method than for the simplex method, however, the former was used in this study because it is more reliable. To show variations in the dissolution test, the test in the condition of central level in the experimental design was repeated four times. The experiments listed in Table 1 in coded form were transformed to physical units as summarized in Table 2. The pH of dissolution medium (X_1) and paddle rotation rate (X_2) were selected as the factors for two-dimensional composite experimental design, as shown in Table 1. Other conditions of the dissolution test were kept constant throughout experiments.

***In Vitro* Dissolution Test** The procedure and apparatus described in dissolution test No. 2 (paddle method) in JP XII were used. The pH of the dissolution medium and the paddle rotation rate in the dissolution tests met the conditions specified in the experimental design. The capsule was kept at the bottom of the dissolution flask by means of a sinker. The concentration of ibuprofen was determined by the UV absorption method. At appropriate intervals, approximately 10 ml aliquots of the solution were withdrawn for testing, and then returned to the dissolution medium immediately after determination of UV absorption.

Kinetic Analysis The *in vivo* dissolution profiles based on total amount of drug absorbed were obtained from plasma concentrations of ibuprofen following oral administration to beagle dogs using the mathematical deconvolution method.[6] The kinetic rate of *in vivo* solution data was used as the weight function. The parameters of dissolution were then calculated from the dissolution profiles using non-linear least-squares regression analysis. The *in vivo* k_d and the *in vivo* t_{50}% were obtained from first order equation and the cumulative probability density function of log-normal distribution.

Table 1. Experimental Design for Two Factors

Test number	Factor level	
	X_1	X_2
1	1	1
2	1	−1
3	−1	1
4	−1	−1
5	$\sqrt{2}$	0
6	$-\sqrt{2}$	0
7	0	$\sqrt{2}$
8	0	$-\sqrt{2}$
9	0	0
10	0	0
11	0	0
12	0	0

Table 2. Levels of Factor in Physical Units

Factor	Factor level in coded form				
	$-\sqrt{2}$	−1	0	1	$\sqrt{2}$
X_1: pH of dissolution medium	4.6	5.0	6.0	7.0	7.4
X_2: paddle rotation rate (rpm)	40	50	75	100	110

Regression Analysis In the optimization study a computer program, written by Takayama *et al.*[5] was used for multiple regression analysis.

Results and Discussion

Determination of *in Vivo* Dissolution Index Figure 2 shows the plasma concentration data for ibuprofen after a single oral administration of the formulation containing 80 mg ibuprofen to beagle dogs. Ibuprofen in solution was rapidly absorbed compared with that in capsules A and B. This rapid absorption appears to be due to the nature of the process of dissolution of solid dosage form like capsules. Moreover, statistical analysis for the time to reach the maximum plasma concentration (T_{max}) between capsules A and B was carried out using *t*-test. Consequently, T_{max} for capsule A was significantly less than that for capsule B ($p < 0.1$). Therefore, the *in vitro* dissolution corresponding to the plasma concentration in a biobatch of the same subjects can be obtained. Figure 3 shows the *in vivo* dissolution profiles of ibuprofen from capsules A and B following oral administration to beagle dogs obtained by the mathematical deconvolution method. Though the first point for capsule A was about 80%, the simulated plasma concentration curve obtained by mathematical convolution method using the rate obtained from *in vivo* dissolution profile was consistent with *in vivo* data. The *in vivo* dissolution rate of ibuprofen from capsule A was faster than that from capsule B, and appeared to depend on the available surface area related to particle size of ibuprofen. If the drug absorption for a formulation can be predicted by the dissolution test, the test will be useful for the study of formulation and minor changes in it, and for quality control. Therefore, an approach using conditions of the dissolution test corresponding to these *in vivo* dissolution profiles was examined using the optimization technique. Then, to obtain a suitable index for the *in vivo* dissolution of ibuprofen from capsules,

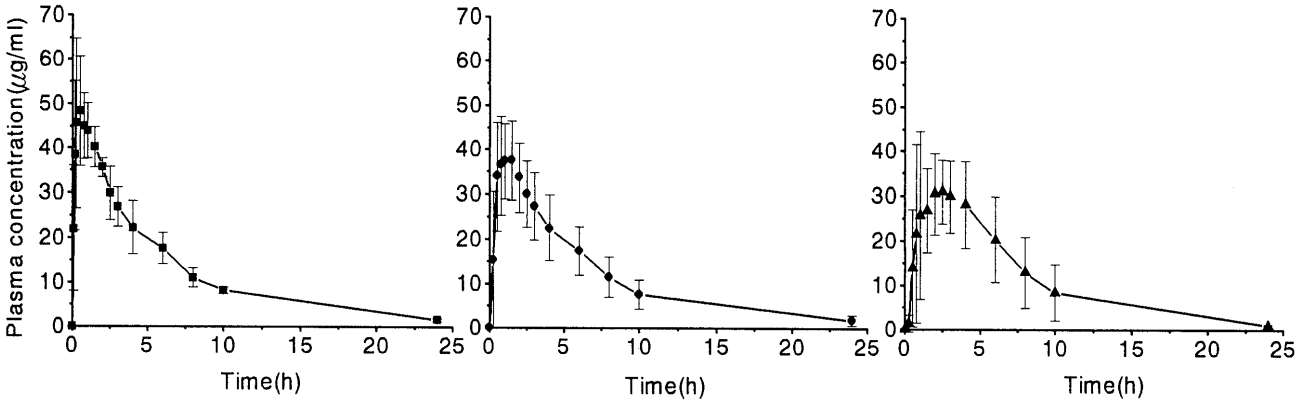

Fig. 2. Plasma Concentrations of Ibuprofen Following Oral Administration of Formulations Containing 80 mg of Ibuprofen to Beagle Dogs
Key: ■, drug in solution; ●, capsule A; ▲, capsule B.

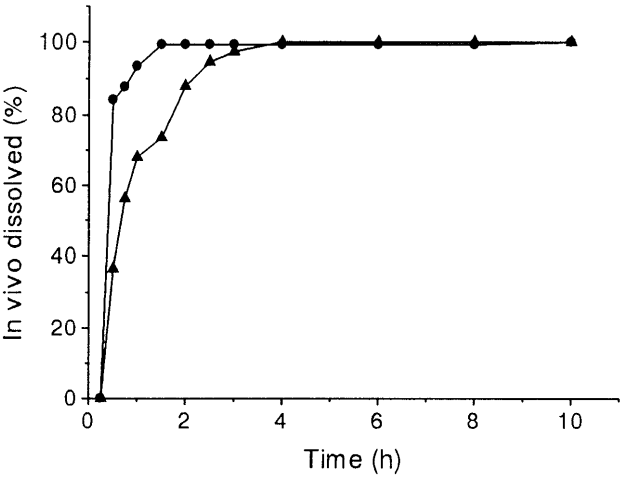

Fig. 3. Profiles of *in Vivo* Release from Capsules Containing 80 mg Ibuprofen for Three Beagle Dogs
Key: ●, capsule A; ▲, capsule B.

first-order kinetics and Wagner's dissolution model were applied to the *in vivo* dissolution profiles.

The *in vivo* k_d of capsules A and B obtained from first-order kinetics were 4.7 and 2.2 h^{-1}, and the *in vivo* t_{50}% obtained from Wagner's dissolution model were 0.17 and 0.60 h, respectively.

Regression Analysis in the Optimization Study To elucidate the *in vitro* dissolution characteristics of capsules A and B, a dissolution test was carried out using the JP paddle method. The *in vitro* dissolution parameters of first-order kinetics and Wagner's dissolution model of dissolution profiles for 12 conditions of dissolution testing are summarized in Tables 3 and 4, respectively.

The following second-order polynomial equation was used to predict each response variable:

$$Y=b_0+b_1X_1+b_2X_2+b_3X_1^2+b_4X_2^2+b_5X_1X_2 \quad (1)$$

where Y is the response variable, b_i is the regression coefficient and X_i is the value of the independent variable.

The optimum regression equations obtained are summarized in Table 5. The best combination of factors for the prediction of each response was selected from among $31(2^5-1)$ kinds of regression equations. Therefore, the optimum regression equation may be obtained by determining the overall combination of factors at the point of statistical significance.[8] With respect to the results of determination of *in vitro* k_d and in vitro t_{50}%, values of r were satisfactory and the regression equations were significant with high F_0 values, although the dissolution test in the condition of central level deviated slightly. Thus each response variable could be accurately predicted using second-order polynomial equations. However, the values of r and F_0 for analytical results for the *in vitro* k_d were a little higher than those for the *in vitro* t_{50}%. The physical significance of the regression equations was then illustrated using contour graphs.

Table 3. Experimental k_d Values of Response Variables for First-Order Kinetics

Test number	Capsule A (h^{-1})	Capsule B (h^{-1})
1	10.08	8.40
2	2.70	2.94
3	7.86	0.84
4	4.74	0.72
5	4.02	8.22
6	3.18	0.90
7	13.62	6.30
8	4.62	0.90
9	6.96	1.44
10	5.76	2.28
11	5.04	2.22
12	7.44	1.92

Table 4. Experimental t_{50}% Values of Response Variables for Wagner's Dissolution Model

Test number	Capsule A (h)	Capsule B (h)
1	0.106	0.093
2	0.287	0.178
3	0.134	0.776
4	0.179	0.999
5	0.218	0.120
6	0.245	0.855
7	0.088	0.124
8	0.188	0.667
9	0.135	0.293
10	0.167	0.212
11	0.180	0.234
12	0.127	0.271

Table 5. Optimum Regression Equation for Each Dissolution by Multiple Regression Analysis

Y	b_0	b_1	$Y=b_0+b_1\cdot X_1+b_2\cdot X_2+b_3\cdot X_1^2+b_4\cdot X_2^2+b_5\cdot X_1\cdot X_2$ b_2	b_3	b_4	b_5	$s^{a)}$	$r^{b)}$	$F_o^{c)}$
The k_d for first-order kinetics									
Capsule A	6.3192	—	1.0746	−1.3716	1.4004	2.9052	0.7998	0.978918	40.198**
Capsule B	1.8878	2.5086	1.6530	1.0650	0.5976	1.3362	0.6420	0.986176	42.506**
The t_{50}% for Wagner's dissolution model									
Capsule A	0.14481	—	−0.04605	0.03942	—	−0.03426	0.00044	0.924023	15.576**
Capsule B	0.25265	−0.31792	−0.13448	0.13480	0.089137	—	0.00169	0.968698	26.648**

a) Standard deviation of residual. *b*) Multiple correlation coefficient. *c*) Observed F_o value, ** $p<0.01$.

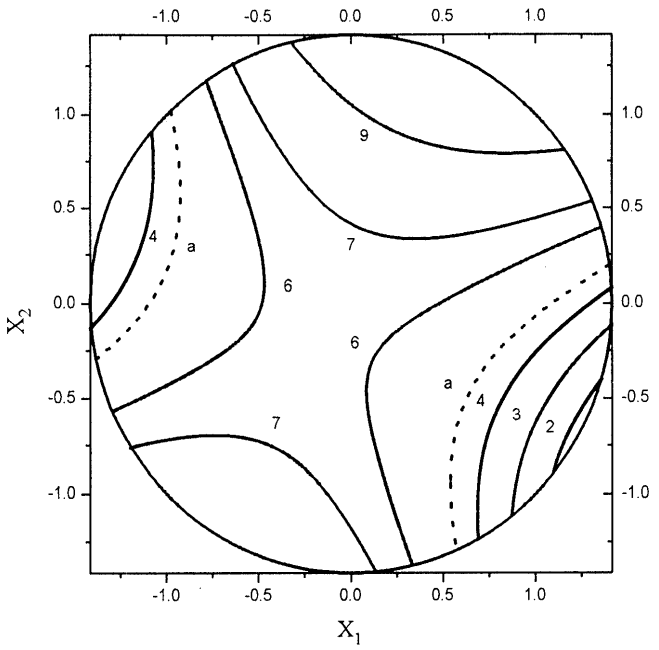

Fig. 4. Contour Graph of k_d as a Function of X_1 (pH of Dissolution Medium) and X_2 (Paddle Rotation Rate) for Capsule A

Key: a, *in vivo* k_d.

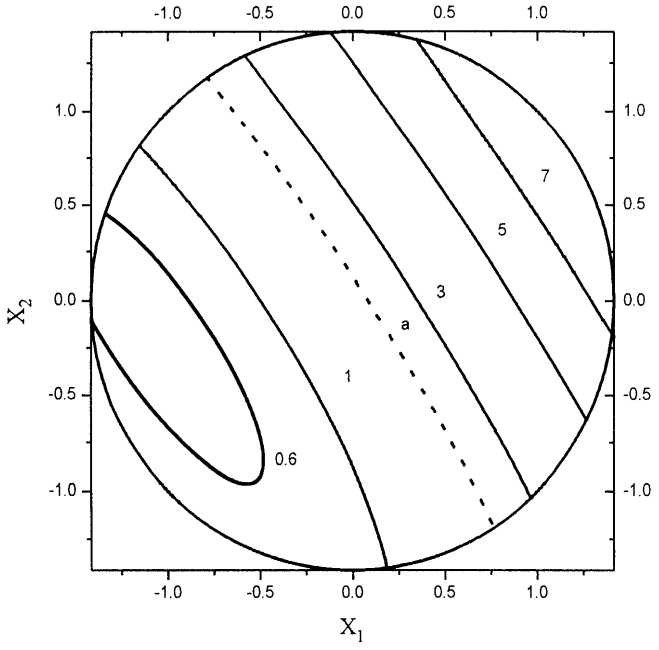

Fig. 5. Contour Graph of k_d as a Function of X_1 (pH of Dissolution Medium) and X_2 (Paddle Rotation Rate) for Capsule B

Key: a, *in vivo* k_d.

Graphical Approach with Contour Curves The contour graph is quite useful to elucidate the significance of a regression equation. Figures 4 and 5 show the contour curves as a function of X_1 (pH of the dissolution medium) and X_2 (the paddle rotation speed) for the *in vitro* k_d of capsules A and B predicted from *in vitro* dissolution data. For capsule A, as both X_1 and X_2 increased, the *in vitro* k_d increased at high paddle rotation rates. This apparently occurred because the solubility of ibuprofen increased as the X_1 increased, since ibuprofen is an acidic drug.[9] In contrast, at slow paddle rotation rates, as X_1 increased, the *in vitro* k_d decreased; the main factor affecting this decrease appeared to be the solubility of the capsule shell made of gelatin, since the solubility of gelatin in acidic solution is higher than that in basic solution.

In contrast, for capsule B, as X_1 and X_2 increased, the *in vitro* k_d increased (Fig. 5). The results obtained using these methods agreed with the finding predicted from the physicochemical properties of the drug.

Figures 6 and 7 show the contour curves for *in vitro* t_{50}% of capsules A and B. For capsule A, *in vitro* t_{50}% decreased with increase in both X_1 and X_2. In this case, it appeared that the rate-limiting factor for t_{50}% was not the dissolution of drug in the capsule, but breakage of the

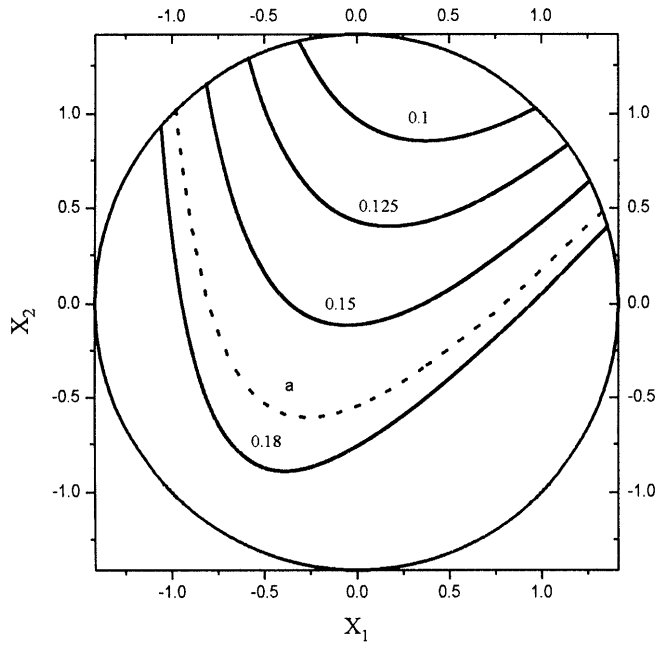

Fig. 6. Contour Graph of t_{50}% as a Function of X_1 (pH of Dissolution Medium) and X_2 (Paddle Rotation Rate) for Capsule A

Key: a, *in vivo* t_{50}%.

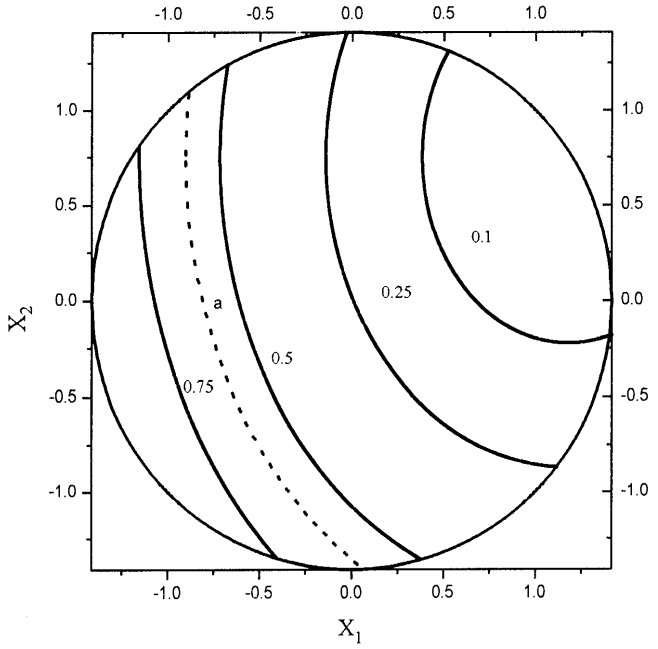

Fig. 7. Contour Graph of t_{50}% as a Function of X_1 (pH of Dissolution Medium) and X_2 (Paddle Rotation Rate) for Capsule B

Key: a, *in vivo* t_{50}%.

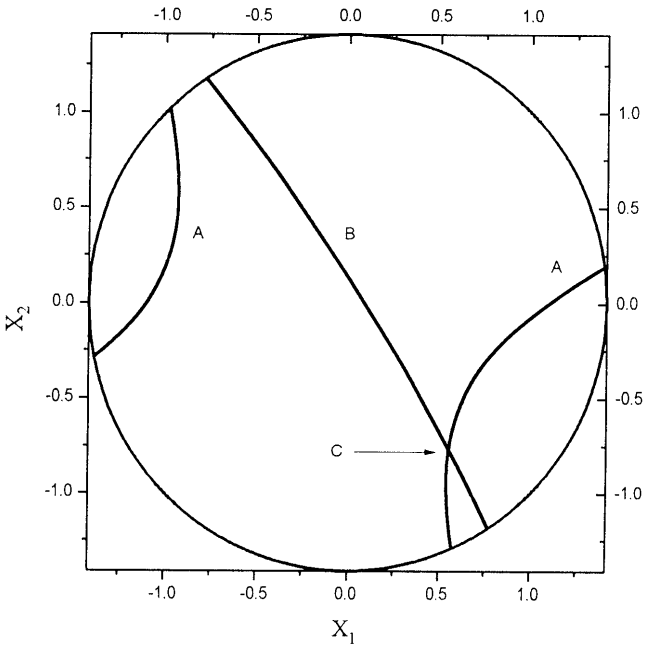

Fig. 8. Relationship between k_d Values Corresponding to *in Vivo* Dissolution for Capsules A and B

Key: A, capsule A; B, capsule B; C, point of agreement between capsules A and B in k_d corresponding to *in vivo* dissolution.

capsule shell made of gelatin, since the *in vitro* t_{50}% was very small within the range tested.

For capsule B, at slow paddle rotation rate, *in vitro* t_{50}% decreased with increase in X_1 (Fig. 7). However, the effect of the paddle rotation rate was not large, and at a high rate, the effect was very small.

Determination of Conditions for Dissolution Testing Corresponding to *in Vivo* Dissolution The *in vivo* k_d of capsules A and B was substituted into the equations obtained by regression analysis. Then, the optimum condition for dissolution testing corresponding to *in vivo* dissolution performance was determined from the above regression equations for both capsules, as shown in Figs. 8 and 9. The point of agreement in the equation for the two capsules (C in Figs. 8 and 9) specifies the conditions of dissolution testing yielding the highest *in vivo/in vitro* correlation.

Using these results, the conditions for dissolution testing corresponding to *in vivo* k_d were found to include a dissolution medium of pH 6.6 and a rate of paddle rotation of 56 rpm. Ibuprofen is insoluble in acidic solution, since ibuprofen is acidic, its pK_a being 4.59.[9] However, ibuprofen may be soluble in the gastrointestinal tract above about pH 4.5. Moreover, Aoyagi *et al.*[10] reported that the JP XII paddle method at 30 rpm provided the best *in vivo/in vitro* correlation for indomethacin capsules for humans. In addition, Katori *et al.*[11] found that a good *in vitro/in vivo* correlation for controlled release acetaminophen tablets was obtained at a paddle speed of 10 rpm for humans. However, the motility of the gastrointestinal tract is higher in dogs than in humans.[12] Therefore, the rate of paddle rotation corresponding to motility in the gastrointestinal tract of the dog should be higher than that for humans. The results obtained using our method thus appear to be reasonable. On the other hand, the conditions of dissolution testing corresponding to the *in vivo* t_{50}% obtained from the equation were pH 5.2 for dissolution medium and a rotation rate of 78 rpm.

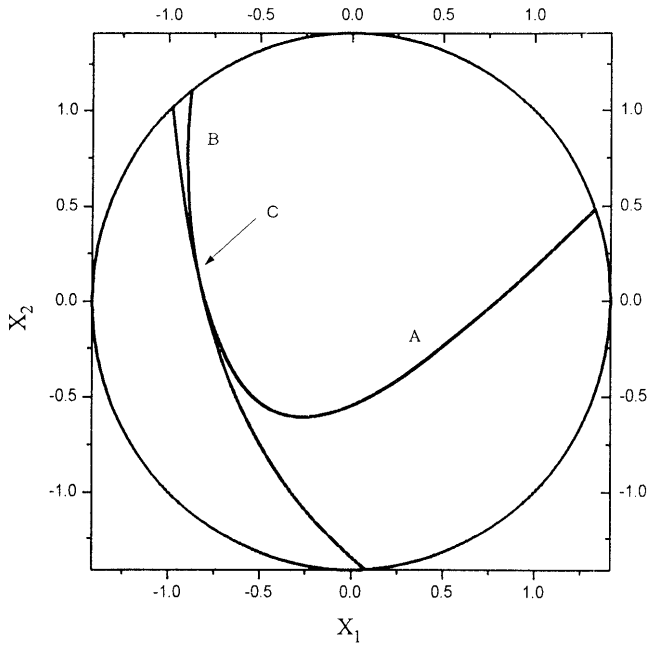

Fig. 9. Relationship between t_{50}% Values Corresponding to *in Vivo* Dissolution for Capsules A and B

Key: A, capsule A; B, capsule B; C, point of agreement between capsules A and B in t_{50}% corresponding to *in vivo* dissolution.

The paddle rotation rate obtained from *in vitro* t_{50}% was higher than that obtained from *in vitro* k_d. In contrast, Table 6 shows that the pH obtained from *in vitro* t_{50}% was lower than that from *in vitro* k_d.

***In Vivo/in Vitro* Correlation** First, the dissolution tests for capsules A and B were carried out under the conditions (dissolution medium: pH 6.6, paddle rotation rate: 56 rpm)

Table 6. Conditions of Dissolution Test for Good *in Vivo/in Vitro* Correlation

	pH of dissolution medium	Paddle rotation rate (rpm)
k_d (first-order kinetics)	6.6	56
t_{50}% (Wagner's dissolution model)	5.2	78

Table 7. Experimental and Predicted Values for the *in Vivo/in Vitro* Correlation

	Predicted		Experimental	
	Capsule A	Capsule B	Capsule A	Capsule B
k_d (first-order kinetics)	4.7	2.2	4.8	2.5
t_{50}% (Wagner's dissolution model)	0.17	0.60	0.20	0.75

obtained from the optimum *in vitro* k_d corresponding to *in vivo* k_d, and the results were compared to the predicted values. A good agreement was found between the *in vitro* k_d obtained from the *in vitro* experimental data and the *in vitro* k_d predicted from the regression equations (*in vivo* k_d), as shown in Table 7. The dissolution tests for capsules A and B were carried out under the conditions (dissolution medium: pH 5.2, paddle rotation rate: 78 rpm) obtained from the optimum *in vitro* t_{50}%. The t_{50}% values obtained from experimental data were also well-correlated with the *in vivo* t_{50}%. However, the *in vivo/in vitro* correlation of k_d obtained from first-order kinetics was better than that of t_{50}% obtained from Wagner's dissolution model. This finding appears to be because the values of r and F_0 for analytical results for the *in vitro* k_d were slightly higher than those for *in vitro* t_{50}%. Consequently, k_d is useful as a dissolution index for determination of *in vivo/in vitro* correlation for these ibuprofen capsules.

Conclusion

The optimization technique was used to determine the *in vivo/in vitro* correlation for capsules containing ibuprofen of two different particle sizes as an oral solid dosage form. Experimental results obtained from dissolution testing with good correlations for *in vivo* and *in vitro* dissolution agreed well with the predicted values. Therefore, this dissolution test is applicable to formulations containing ibuprofen with particle sizes in the experimental range, though it is not necessarily a common dissolution test for all ibuprofen formulations. Furthermore, in the case of different subjects, the prediction obtained from the *in vitro* dissolution may deviate a little from the *in vivo* plasma concentration. However, this condition obtained by the optimization technique method is useful for the primitive study of formulations, since the *in vitro* dissolution corresponding to the plasma concentration in a biobatch of the same subjects can be obtained.

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References and Notes

1) A part of this work was presented at the 115th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, March 1995, Abstract of Papers, p. 74.
2) Langenbucher F., Mysicka J., *Br. J. Clin. Pharm.*, **19**, 151S—162S (1985); Kottke M. K., Rhodes C. T., *Drug Dev. Ind. Pharm.*, **17**, 1157—1176 (1991); Stavochansky S., Doluisio J. T., Martin A., Martin C., Cabana B., Dighe S., Loper A., *J. Pharm. Sci.*, **69**, 1307—1310 (1980).
3) Möller H., Langenbucher F., *Pharm. Ind.*, **44**, 1065—1071 (1982); Bradbrook I. D., John V. A., Morrison P. J., Rogers H. J., Spector R. G., *Br. J. Clin. Pharmac.*, **19**, 163S—169S (1985).
4) Oribe A., Takahashi M., Shirakura O., Ishimaru S., Goto S., Takayama K., Nagai T., Abstracts of Papers, The 112th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, March 1992, p. 27.
5) Takayama K., Nambu N., Nagai T., *Chem. Pharm. Bull.*, **31**, 4496—4507 (1983); Takayama K., Nagai T., *ibid.*, **37**, 160—167 (1989); *idem*, *Int. J. Pharm.*, **74**, 115—126 (1991).
6) Iga K., Ogawa Y., Yashiki T., Shimamoto T., *J. Pharmacokinet. Biopharm.*, **14**, 213—225 (1986); Yamaoka K., Tanigawara Y., "A Primer on Pharmacokinetics Using a Microcomputer," Nankodo, Tokyo, 1983.
7) Wagner J. G., Nelson E., *J. Pharm. Sci.*, **53**, 1392—1403 (1964).
8) Haga T., Takeuchi H., Okuno T., *Quality J.S.Q.C.*, **6**, 35—40 (1976).
9) Millar F. C., Corrigan O. I., *Int. J. Pharmaceut.*, **92**, 97—104 (1993).
10) Aoyagi N., Ogata H., Kaniwa N., Ejima A., *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **23**, 529—534 (1985).
11) Katori N., Aoyagi N., Terao T., *Pharm. Res.*, **12**, 237—243 (1995).
12) Aoyagi N., Ogata H., Kaniwa N., Ejima A., Nakata H., Tsutsumi J., Fujita T., Amada I., *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **23**, 578—584 (1985).