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Analysis of Interfacial Transfer and Absorption Behavior of Drugs Following Dissolution from β-Cyclodextrin Complexes[1]

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The interfacial transfer and absorption behavior of drugs following dissolution from inclusion complexes was analyzed by the use of *in vitro* and *in situ* models (S-L_w-L_o and S-L_w-*in situ*). The experiments were carried out using 1 : 1 complexes of β-cyclodextrin (β-CyD) with some barbiturates and *p*-aminobenzoic acid esters as test compounds. Taking into account the dissolution equilibrium of complexes in the dissolution medium, the drug appearance in the organic phase of the S-L_w-L_o model and the amount of drug absorbed in the S-L_w-*in situ* model were analyzed in relation to time. The factors affecting the interfacial transfer and *in situ* absorption behavior of the complexes are discussed. The results indicate that the two experimental models are applicable to β-CyD complexes, and may be suitable for the evaluation of their oral bioavailability.

Keywords——model analysis; inclusion complex; β-cyclodextrin; barbiturate; *p*-aminobenzoic acid ester; stability constant; interfacial transfer; *in situ* perfusion behavior; dissolution; *in vitro*–*in situ* correlation

Recently, the pharmaceutical modification of poorly soluble drugs by means of inclusion complexation has been extensively studied to improve the dissolution and absorption characteristics of drug molecules.[2] In this respect, the development of simple and efficient experimental models that can mimic the *in vivo* absorption behavior of inclusion complexes is desirable.[3] We have previously described *in vitro* and *in situ* models (S-L_w-L_o and S-L_w-*in situ*) for the prediction of drug absorption following dissolution from compressed tablets.[4] Furthermore, *in vitro*, *in situ*, and *in vivo* correlations were examined in an attempt to find some reliable bioavailability parameters.[5] Takayama *et al.* reported that the S-L_w-L_o model is useful to evaluate the interfacial transfer of drug following dissolution from some coprecipitate systems.[6]

In the present paper, therefore, the two experimental models were applied to β-cyclodextrin (β-CyD) complexes to survey the validity of the proposed models. Among the various guest molecules studied so far,[7] some barbiturates and *p*-aminobenzoic acid esters were chosen because their solid complexes with β-CyD in 1 : 1 molar ratio were easily obtained.[8]

Experimental

Materials——Barbiturates used were the same as those described in a previous paper.[9] Pentyl *p*-aminobenzoate was prepared according to the reported method,[10] and recrystallized from ethanol–water. The other *p*-aminobenzoates were purchased from Nakarai Chemical Co., Ltd., and were recrystallized from ethanol– water. Pure β-CyD was kindly donated by Nihon Shokuhin Kako Co. All other materials and solvents were of analytical reagent grade, and deionized double-distilled water was used.

Solubility Study——Excess amounts of drug were added to aqueous solution containing various concentrations of β-CyD and the mixtures were shaken at $25 \pm 0.5\,^{\circ}\mathrm{C}$. After equilibration (approximately 5 d), an aliquot was centrifuged and pipetted through a cotton filter. A portion of the sample (0.5 ml) was diluted and analyzed spectrophotometrically. The stability constant (K_c) was calculated from the initial linear portion of the phase solubility diagrams according to the following equation.[11]

$$K_c = \frac{\text{slope}}{\text{intercept}\ (1 - \text{slope})} \tag{1}$$

Preparation of Complexes——On the basis of the results of the solubility study, the solid complexes were prepared by mixing appropriate amounts of β-CyD and drugs in water. Amounts were calculated from the descending curve of the B_s type[11] phase diagram (see Fig. 1). For example, 0.57 g propyl *p*-aminobenzoate and 5.4 g of β-CyD were added to 240 ml of water, and sealed in a flask, then the mixture was stirred with a magnetic stirrer at 25 °C for 5 d. The complex, which precipitated as a microcrystalline powder, was filtered and dried under a vacuum at 50 °C for 24 h.

S-L_w-L_o Experiment——This model[4] basically consists of a rotating disk dissolution apparatus with an organic phase. A disk of 1 cm diameter, an isotonic phosphate buffer (pH 7.4), and 1,2-dichloroethane–*n*-octanol (6 : 4)[12] were used as a solid phase (S), an aqueous phase (L_w), and an organic phase (L_o), respectively. The entire system was maintained at 25 °C using a circulation water-bath. The other experimental conditions were the same as described in a previous paper.[5] The drug concentrations in L_w and L_o were determined spectrophotometrically.

S-L_w-*In Situ* Experiment——This model[4] basically consists of a 60 cm loop of rabbit small intestine (male albino rabbits, 2.5—3.0 kg) *in situ*, a reciprocating pump, a constant temperature solution reservoir, and a rotating disk apparatus. A disk of 1 cm diameter and isotonic phosphate buffer (pH 7.4) were used as a solid phase (S) and an aqueous phase (L_w), respectively. The entire system was maintained at 37 °C using a circulation water-bath. The other experimental conditions and the methodology were the same as described in a previous paper.[5] The drug concentrations in L_w were determined spectrophotometrically.

Determination of Partition Coefficients——The partition coefficients (*PC*) were determined following the shaking of appropriate volumes of isotonic phosphate buffer (pH 7.4) containing drug (1×10^{-4} M) and of 1,2-dichloroethane–*n*-octanol (6 : 4) solution for 2 h at 25 °C. The *PC* was defined as the ratio of the equilibrium concentration in the organic phase to that in the aqueous phase. Correction was made for volume changes in both phases.

Results and Discussion

Analysis of Solubility Data

Figure 1 shows the B_s type phase solubility diagram obtained for the propyl *p*-aminobenzoate–β-CyD system, as an example; a microcrystalline complex was precipitated at higher β-CyD concentrations. The other drug–β-CyD systems also showed B_s type phase solubility diagrams. The stoichiometries of the complexes in the solid phase were analyzed on the basis of data in the plateau region of the solubility diagrams, and were estimated to be 1 : 1. This is consistent with the results obtained by isolation and analysis of the solid complexes. Thus, the 1 : 1 stability constants (K_c) were calculated from the initial linear portion of the solubility diagrams. The results of the solubility studies and *PC* of the drugs are summarized in Table I. In a series of *p*-aminobenzoates, the K_c values generally increased with increasing hydrophobicity of a guest molecule, as would be expected from the *PC* values. In the case of barbiturates, however, the importance of steric factors was apparent in these host-guest interactions.[8,9] The complexations in the solid state were confirmed by infrared spectroscopy, powder X-ray diffractometry, thermal analysis, and ^{13}C nuclear magnetic resonance spectroscopy,[13] and the results will be reported in detail elsewhere.

S-L_w-L_o Analysis

Prior to the analysis of β-CyD complexes, the interfacial transfer behavior of their guest molecules following dissolution was examined. Figure 2 shows typical S-L_w-L_o data for propyl *p*-aminobenzoate. Under these experimental conditions, the tablets maintained a constant shape, and the drug appearances in both L_w and L_o obeyed first-order kinetics. The solid lines indicate the theoretical curves of drug concentrations in L_w and L_o (C_w and C_o, respectively) estimated by the Runge–Kutta–Gill method as described previously.[4] The

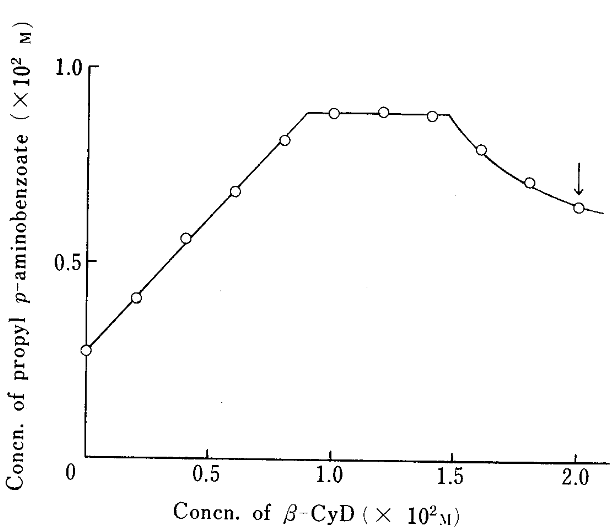

Fig. 1. Phase Solubility Diagram of Propyl *p*-Aminobenzoate–β-CyD System in Isotonic Phosphate Buffer (pH 7.4) at 25 °C

Each point is the mean of 3 experiments.
The arrow indicates the experimental conditions of the preparation of solid complex (see the text).

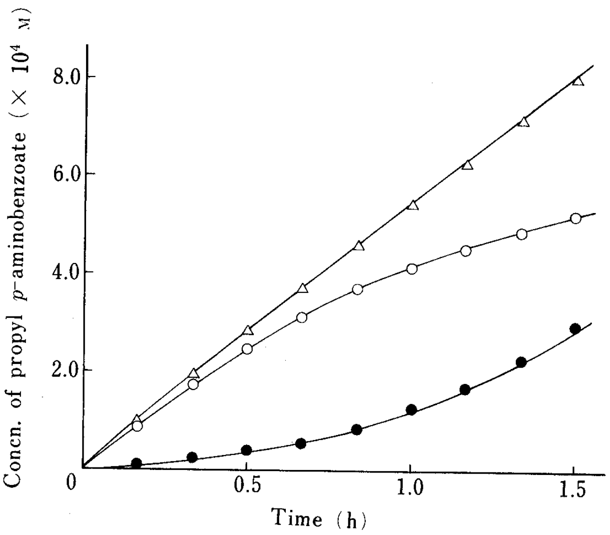

Fig. 2. Interfacial Transfer of Propyl *p*-Aminobenzoate Following Dissolution at 25 °C in the S-L_w-L_o Model

○, C_w; ●, C_o; △, C_w+C_o.
The solid line indicates the theoretical curve.[4]
Each point is the mean of 4 experiments.

TABLE I. Parameters for Inclusion Complexation of β-CyD with Drugs in Isotonic Phosphate Buffer (pH 7.4) at 25 °C

Compound	K_c[a] (M^{-1})	Molar[b] ratio	S_c[c] ($\times 10^3$ M)	PC[d]
1 Phenobarbital	433	1:1	7.48	50
2 Pentobarbital	170	1:1	4.68	24
3 Amobarbital	670	1:1	3.69	71
4 Allobarbital	470	1:1	4.08	128
5 Ethyl *p*-aminobenzoate	488	1:1	12.1	212
6 Propyl *p*-aminobenzoate	946	1:1	6.71	683
7 Butyl *p*-aminobenzoate	1618	1:1	3.05	1220
8 Pentyl *p*-aminobenzoate	2480	1:1	0.489	1600

a) Stability constant, determined from the initial linear portion of the phase solubility diagram.
b) Drug: β-CyD.
c) Solubility of complex, estimated from the descending curve of the phase solubility diagram.
d) Partition coefficient of drug.

parameters used for computation are listed in Table II. As can be seen in Fig. 2, the experimental values were well fitted to the theoretical curves. Similar results were obtained for other drugs studied. This indicates that such a treatment is valid for drugs as guest molecules in complexes.

When the S-L_w-L_o model is applied to β-CyD complex, however, the dissolution equilibrium of the complex in L_w must be taken into account, as illustrated in Fig. 3. In this scheme the assumption was made that after dissolution and dissociation of the complex in L_w, only the free form of the drug is able to transfer into L_o, depending upon the magnitude of PC. Furthermore, it has been demonstrated that the C_o value in S-L_w-L_o data is particularly useful to predict the initial absorption behavior drug following dissolution *in vivo*.[5] Based on the above considerations, the drug concentrations in L_o were measured during the S-L_w-L_o

Table II. Parameters for Dissolution and Interfacial Transfer of Drugs at 25 °C in the S-L_w-L_o Model

Compound	S_s[a] ($\times 10^3$ M)	k[b] ($\times 10\,\mathrm{h}^{-1}$)	k_f[c] ($\times 10\,\mathrm{h}^{-1}$)	k_b[d] ($\times 10^3\,\mathrm{h}^{-1}$)
1 Phenobarbital	8.03	0.947	7.56	15.3
2 Pentobarbital	5.68	1.53	2.85	11.9
3 Amobarbital	1.52	2.25	6.70	9.44
4 Allobarbital	1.82	2.23	5.88	4.59
5 Ethyl *p*-aminobenzoate	5.72	2.19	3.98	1.88
6 Propyl *p*-aminobenzoate	2.60	2.67	5.83	0.854
7 Butyl *p*-aminobenzoate	0.949	2.88	3.64	0.298
8 Pentyl *p*-aminobenzoate	0.183	4.08	8.77	0.547

a) Solubility in L_w.
b) Dissolution rate constant.
c) Rate constant for forward transfer.
d) Rate constant for backward transfer.

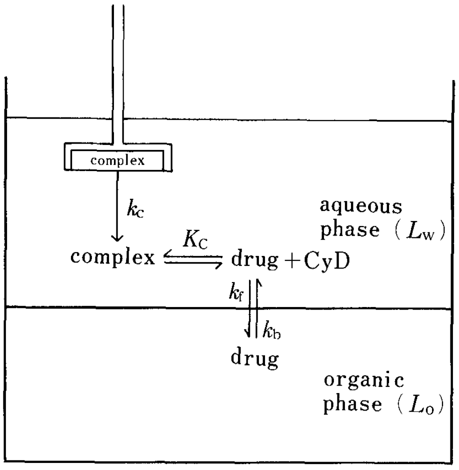

Fig. 3. Proposed Scheme for Interfacial Drug Transfer Following Dissolution from β-CyD Complex in the S-L_w-L_o Model

k_c, dissolution rate constant of the complex; K_c, stability constant of the complex; k_f, rate constant for forward transfer of drug; k_b, rate constant for backward transfer of drug.

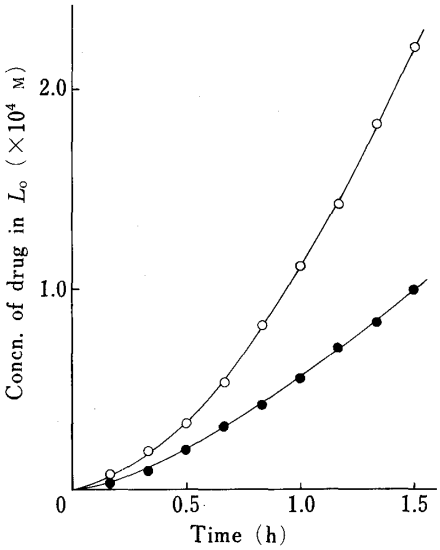

Fig. 4. Time Course of C_o Values Following Dissolution from β-CyD Complexes at 25 °C in the S-L_w-L_o Model

○, allobarbital; ●, propyl *p*-aminobenzoate. Each point is the mean of 4 experiments.

experiments. Unfortunately, reliable values of the dissolution rate constant of the complex and the free drug concentration in L_w could not be obtained because no sensitive assay is currently available for such low β-CyD concentrations. Figure 4 shows the changes in C_o values with time following the dissolution of β-CyD complexes. The curves exhibited the features of a consecutive end process in the interfacial transfer. The C_o profiles of other β-CyD complexes also showed characteristics those in Fig. 4. The change in C_o values of β-CyD complex affects various factors such as the extent of dissolution and dissociation of the complex in L_w along with the partition of free drug between L_w and L_o. Thus, an attempt was made to relate C_o to the parameters shown in Fig. 3 by means of multiple regression analysis, and the following result was obtained:

$$\log C_o^{t=1.5} = 0.267 \log S_c + 0.268 \log K_c - 0.405 \log PC - 3.07 \quad (2)$$

$$(n=8,\ r=0.934,\ s=0.128,\ F=9.04)$$

where $C_o^{t=1.5}$ is the drug appearance in L_o at 1.5 h after the start of dissolution. In this analysis, the apparent solubility of the β-CyD complex (S_c) and the partition coefficient of the drug were used as tentative measures of the dissolution characteristics of the complex and of interfacial transfer of the drug from L_w to L_o, respectively. Despite the simple treatment, quite a good multiple correlation coefficient was obtained with $p<0.05$. The partial correlation coefficients for $\log S_c$, $\log K_c$, and $\log PC$ were found to be 0.632, 0.290, and −0.693, respectively. Since the relative magnitudes of these coefficients are not significantly different, all three parameters may contribute substantially to the C_o^t values of β-CyD complexes.

S-L_w-In Situ Analysis

It has been suggested that the *S-L_w-in situ* model may more realistically mimic the physiological process *in vivo*.[5] When this model is applied to β-CyD complexes the assumption was also made that only the free form of drug is able to be absorbed from the small intestinal tract after dissolution and dissociation of the complex in L_w. On the basis of the above considerations, the $(C_w-C_i)^t$ value[5] was also used in this study as an absorption parameter in the *S-L_w-in situ* model. For the determination of $(C_w-C_i)^t$ values, the amount of the drug dissolved (C_w) in L_w was separately measured by a dissolution study (*S-L_w* system) using an equal volume of polyethylene tube instead of the rabbit intestinal tract, and then the drug concentration (C_i) in L_w for the *S-L_w-in situ* model was subtracted from the corresponding C_w value at that time.

Figure 5 shows a typical example of $(C_w-C_i)^t$ profiles for allobarbital and its β-CyD complex. The greater $(C_w-C_i)^t$ value observed for β-CyD complex may be due to the much easier dissociation of the complex along with the increased solubility of the drug in L_w, as expected from the magnitudes of the S_c and K_c values, respectively. In fact, a good correlation was obtained between $(C_w-C_i)^{t=1.5}$ and $1/K_c$ (dissociation constant) for β-CyD complexes, as shown in Fig. 6. For a full explanation of *S-L_w-in situ* data, however, various factors such as changes in physicochemical and pharmacokinetic properties of the drug, and interaction of the drug with biological substances should also be considered.[3]

It is interesting to note that the C_o^t curves showed characteristics similar to those of the $(C_w-C_i)^t$ profiles (*cf.* Figs. 3 and 5), indicating that C_o^t is pertinent parameter of the absorption behavior of a drug following dissolution. Figure 7 shows the relationships between

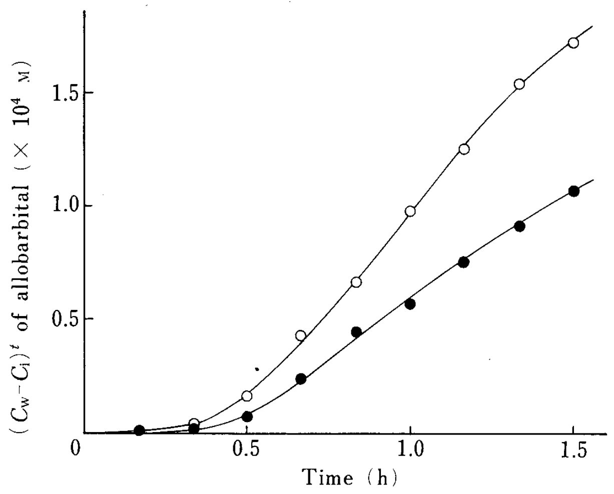

Fig. 5. The $(C_w-C_i)^t$ Profiles of Allobarbital and Its β-CyD Complex in the *S-L_w-in Situ* Model

●, allobarbital; ○, β-CyD complex.
Each point is the mean of 4 experiments.

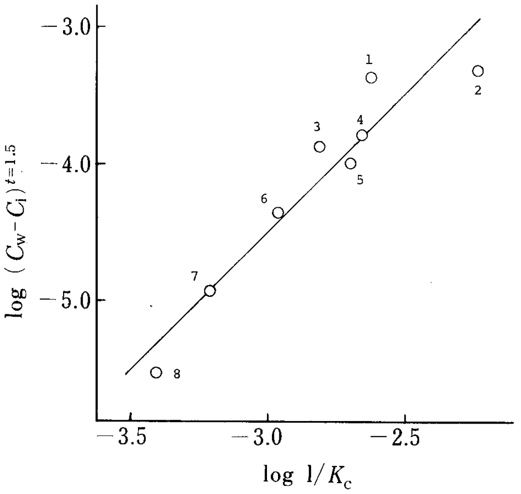

Fig. 6. Relationship between $\log(C_w-C_i)^{t=1.5}$ and $\log 1/K_c$ for β-CyD Complexes

The numbers refer to the compounds in Table I.

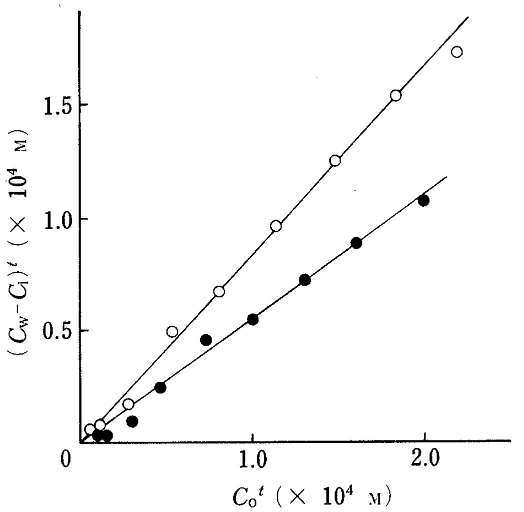

Fig. 7. Relationship between $(C_w - C_i)^t$ in the *S*-L_w-*in Situ* Model and C_o^t in the *S*-L_w-L_o Model for Allobarbital and Its β-CyD Complex

●, allobarbital; ○, β-CyD complex.
The plots show the value at every 10 min after the start of dissolution. Each point is the mean of 4 experiments.

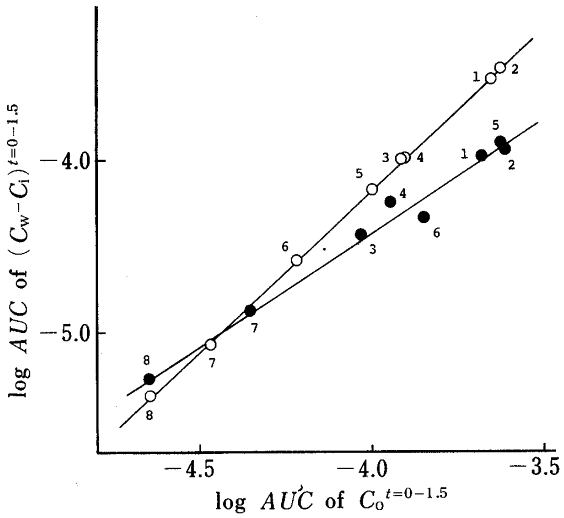

Fig. 8. Relationship between log AUC of $(C_w - C_i)^{t=0-1.5}$ in the *S*-L_w-*in Situ* Model and log AUC of $C_o^{t=0-1.5}$ in the *S*-L_w-L_o Model

●, drug alone; ○, β-CyD complex.
The numbers refer to the compounds in Table I. Each AUC value is the mean of 4 experiments.

C_o^t and $(C_w - C_i)^t$ of allobarbital and its β-CyD complex, where measurements were taken every 10 min up to 1.5 h after the start of dissolution. In both cases, an excellent correlation between *in vitro* and *in situ* absorption parameters was observed. As shown in Fig. 8, the areas under the curves (AUC) of C_o^t values were also correlated well with those of $(C_w - C_i)^t$ values for all the drugs and their β-CyD complexes studied, when both AUC values were determined up to 1.5 h after the start of dissolution.

From these results, it is concluded that the two experimental models used here are suitable to predict the initial absorption behavior of the β-CyD complexes as well as their guest molecules following dissolution. It is also suggested that the C_o^t and $(C_w - C_i)^t$ values may be useful parameters for the evaluation of oral bioavailability of β-CyD complexes. The advantage of the *S*-L_w-L_o model is that the C_o^t value is easily obtained by a simple procedure and reproducibility and accuracy (±3%) are not less than those of the *S*-L_w-*in situ* data.

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