Analysis of the Phase Solubility Diagram of a Phenacetin/Competitor/β-Cyclodextrin Ternary System, Involving Competitive Inclusion Complexation

Naomi ONO, Fumitoshi HIRAYAMA, Hidetoshi ARIMA, and Kaneto UEKAMA*

Faculty of Pharmaceutical Sciences, Kumamoto University, 5–1 Oe-honmachi, Kumamoto 862–0973, Japan.
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The competitive inclusion complexations in the ternary phenacetin/competitors/β-cyclodextrin (β-CyD) systems were investigated by the solubility method, where *m*-bromobenzoic acid (*m*-BBA) and *o*-toluic acid (*o*-TA) were used as competitors. The solubility changes of the drug and competitors as a function of β-CyD concentration in the ternary systems were formulated using their stability constants and intrinsic solubilities. The decrease in solubility of phenacetin by the addition of competitors could be quantitatively simulated by the formulation, when both drug and competitor give A_L type solubility diagrams. On the other hand, when one of the guests gives a B_S type solubility diagram, its solubility change was clearly reflected in that of the another guest, *i.e.*, phenacetin gave an A_L type solubility diagram in the binary phenacetin/β-CyD system and *o*-TA gave a B_S type diagram in the binary *o*-TA/β-CyD system, but in the ternary phenacetin/*o*-TA/β-CyD system, a new plateau region appeared in the original A_L type diagram of phenacetin. This was explained by the solubilization theory of Higuchi and Connors. The solubility analysis of the ternary drug/competitor/CyD systems may be particularly useful for determination of the stability constant of a drug whose physicochemical and spectroscopic analyses are difficult, because they can be calculated by monitoring the solubility change of a competitor, without monitoring that of a drug. Furthermore, the present results suggest that attention should be paid to the type of the phase solubility diagram, as well as the magnitude of the stability constant and the solubility of the complex, for a rational formulation design of CyD complexes.

Key words cyclodextrin; inclusion complex; competitive inclusion; solubility method; stability constant; ternary system

Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of usually six, seven or eight glucose units, and are capable of forming inclusion complexes with various guest molecules in aqueous solution and in solid state.[1—4] Since this molecular encapsulation leads to changes in physicochemical and biological properties of guest molecules, CyDs are successfully utilized as a drug carrier for improvements in aqueous solubility, chemical and physical stability and bioavailability, *etc*. of drugs in pharmaceutical fields.[5—7] However, the degree of changes in drug properties is dependent on environmental conditions such as concentrations of the guest and host molecules, dilution, presence of competing agents, and temperature, *etc*., because CyD complexes are always in equilibrium with each component, *i.e.*, free guest and host molecules, in solution. For example, the CyD-augmented aqueous solubility of itraconazole decreased when second components are added, due to the competitive inclusion.[8] Therefore, it is important to estimate how the competitive inclusion affects the property change of drugs when drugs are formulated as CyD complexes. In a previous paper,[9] we reported that the permeation profile of drugs through an artificial membrane in the drug/competing agent/β-CyD ternary system can be quantitatively simulated using the permeation rate constants of drugs and the stability constants of complexes with drugs and competing agents. In this paper, we investigated the solubility changes of phenacetin (employed as a model drug) in drug/competitor/β-CyD ternary systems, *i.e.*, the change in phase solubility diagram of drug/β-CyD system by the addition of competing agents.

Experimental

Materials β-CyD was supplied from Japan Maize Co. (Tokyo, Japan). The following chemicals were used after recrystallization from methanol–water: phenacetin (Sigma-Aldrich, U.S.A.), *o*-toluic acid (*o*-TA, Wako Pure Chemicals Co., Osaka, Japan) and *m*-bromobenzoic acid (*m*-BBA, Nacalai Tesque, Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Solubility Measurements Solubility study was carried out according to the method of Higuchi and Connors.[10] The screw-capped vials containing phenacetin (25 mg) and *o*-TA (15 mg) or *m*-BBA (50 mg) in excess amounts in aqueous β-CyD solutions (3.0 ml of phosphate buffer) at various concentrations were shaken at 37 °C. The pH of the solution was adjusted to pH 3.5 for the *o*-TA system and pH 4.5 for the *m*-BBA system, because the addition of the former and latter guest molecules changed the pH of the solutions to about 3.5 and 4.5, respectively. We confirmed that the pH was unchanged after the solubility equilibrium. After equilibrium was attained (about 5 d), the solution was centrifuged at 2000 rpm for 5 min and the supernatant was filtered through a membrane filter (ADVANTEC DISMIC-13CP, Toyo-Rochi, Tokyo, Japan), and analyzed for phenacetin, *o*-TA and *m*-BBA by high-performance liquid chromatography under the following conditions: a Hitachi 655A-11 pump, a 635-A UV detector and a Hitachi D-2500 Chromato-integrator (Tokyo, Japan), a Yamamura AM-303-S-5 ODS column (5 μm, 4.6 mm×250 mm, Kyoto, Japan), a mobile phase of acetonitrile/0.15 M phosphoric acid (1 : 1 v/v), a flow rate of 1.0 ml/min, and a detection of 240 nm.

Theoretical

1. The Binary Drug/CyD or Competitor/CyD System: The initial straight line of A_L or B_S type phase solubility diagrams can be described as Eqs. 1 and 2 for the drug/CyD system and the competitor/CyD system, respectively. These equations are the same as that used for determination of the ordinary 1 : 1 stability constant of CyD complexes from the initial straight line portion of phase solubility diagrams, $K_C = \text{slope}/S_0(1-\text{slope})$.[10]

$$[\text{Drug}]_t = \frac{K_1 S_1}{1 + K_1 S_1}[\text{CyD}]_t + S_1 \quad (1)$$

* To whom correspondence should be addressed. e-mail: uekama@gpo.kumamoto-u.ac.jp

$$[CA]_t = \frac{K_2 S_2}{1 + K_2 S_2}[CyD]_t + S_2 \quad (2)$$

In Eqs. 1 and 2, $[Drug]_t$, $[CyD]_t$ and $[CA]_t$ represent total concentrations of a drug, CyD and a competitor, respectively, S_1 and S_2 are intrinsic solubilities of a drug and a competitor, respectively, and K_1 and K_2 are 1 : 1 stability constants of a drug/CyD complex and a competitor/CyD complex, as defined by Eqs. 3 and 4, respectively:

$$K_1 = \frac{[\text{Drug/CyD complex}]}{[Drug]_f [CyD]_f} \quad (3)$$

$$K_2 = \frac{[\text{CA/CyD complex}]}{[CA]_f [CyD]_f} \quad (4)$$

where [Drug/CyD complex] and [CA/CyD complex] are concentrations of drug/CyD and competitor/CyD complexes, respectively, and $[Drug]_f$, $[CA]_f$ and $[CyD]_f$ are concentrations of free drug, competitor and CyD, respectively.

2. The Ternary Drug/Competitor/CyD System: The solubility- and inclusion-equilibria for the ternary system can be described as shown in Chart 1, where the interaction between a drug and a competitor is assumed to be negligible. The total concentrations of drug, competitor and CyD are represented by Eqs. 5, 6 and 7.

$$[Drug]_t = S_1 + [\text{Drug/CyD complex}] = S_1 + K_1 S_1 [CyD]_f \quad (5)$$

$$[CA]_t = S_2 + [\text{CA/CyD complex}] = S_2 + K_2 S_2 [CyD]_f \quad (6)$$

$$[CyD]_t = [CyD]_f + [\text{Drug/CyD complex}] + [\text{CA/CyD complex}] = [CyD]_f (1 + K_1 S_1 + K_2 S_2) \quad (7)$$

The combination of Eqs. 5 and 7 gave Eq. 8 and that of Eqs. 6 and 7 gave Eq. 9. Equations 8 and 9 represent the solubility changes of a drug and a competitor as a function of CyD concentration in the ternary system.

$$[Drug]_t = \frac{K_1 S_1}{1 + K_1 S_1 + K_2 S_2}[CyD]_t + S_1 \quad (8)$$

$$[CA]_t = \frac{K_2 S_2}{1 + K_1 S_1 + K_2 S_2}[CyD]_t + S_2 \quad (9)$$

3. The Multiple Drug/Competitors/CyD System Consisting of n Components: The solubility changes of a drug and an i-th competitor as a function of CyD concentration can be expressed by Eqs. 10 and 11:

$$[Drug]_t = \frac{K_1 S_1}{1 + \Sigma K_i S_i}[CyD]_t + S_1 \quad \text{where } i = 1-(n-1) \quad (10)$$

$$[CA_{i+1}]_t = \frac{K_{i+1} S_{i+1}}{1 + \Sigma K_i S_i}[CyD]_t + S_{i+1} \quad \text{where } i = 1-(n-1) \quad (11)$$

where $[CA_{i+1}]_t$, S_{i+1} and K_{i+1} are the solubility of the $(i+1)$th competitor in the presence of CyD, the intrinsic solubility of the $(i+1)$th competitor in the absence of CyD, and the stability constant with the $(i+1)$th competitor.

Equations 1, 2, 8—11 indicate that the solubilities of a drug and competitors increased linearly as a function of CyD concentration. The stability constant of one complex can be calculated from the slope of the initial straight line portion in the multi-component system, if the stability constants of other complexes are known. In the ternary drug/competitor/CyD system, for example, the stability constant of a drug can be calculated by monitoring the solubility change of a competitor, without monitoring that of a drug, and by analyzing the initial linear solubility-increase of a competitor according to Eq. 9. Similarly, the stability constant of a competitor can be determined by monitoring the solubility change of a drug in the ternary system.

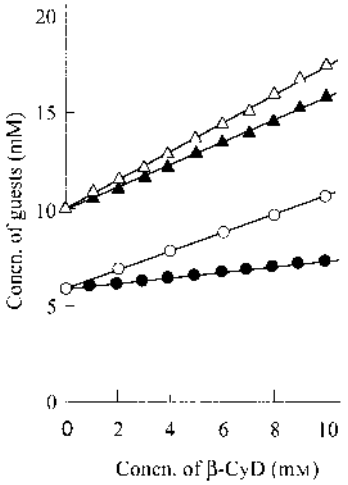

Fig. 1. Phase Solubility Diagrams of Phenacetin and *m*-BBA with *β*-CyD in Phosphate Buffer (pH 4.5) at 37 °C

(○), phenacetin in phenacetin/*β*-CyD system; (●), phenacetin in phenacetin/*m*-BBA/*β*-CyD system; (△), *m*-BBA in *m*-BBA/*β*-CyD system; (▲), *m*-BBA in phenacetin/*m*-BBA/*β*-CyD system. The solid lines are calculated using Eqs. 8 and 9.

Results and Discussion

Figure 1 shows the phase solubility diagrams of binary phenacetin/*β*-CyD, binary *m*-BBA/*β*-CyD and ternary phenacetin/*m*-BBA/*β*-CyD systems in phosphate buffer (pH 4.5). Both phenacetin and *m*-BBA are known to form inclusion complexes with *β*-CyD in a molar ratio of 1 : 1 in aqueous solution.[9,11] The guest molecules showed typical A_L type phase solubility diagrams in that the solubility of the guests increased linearly with CyD concentrations under the experimental conditions. The stability constants (K_C) of the phenacetin/*β*-CyD complex and the *m*-BBA/*β*-CyD complex were calculated from these straight lines according to the equation $K_C = \text{slope}/S_0(1-\text{slope})$, where the intrinsic solubilities of phenacetin and *m*-BBA were $5.51(\pm 0.04)\times 10^{-3}$ M and $1.019(\pm 0.011)\times 10^{-2}$ M, respectively. The K_C values were $182(\pm 3)\ \text{M}^{-1}$ and $312(\pm 5)\ \text{M}^{-1}$ for the former and latter complexes, respectively, as shown in Table 1. In the case of the ternary phenacetin/*m*-BBA/*β*-CyD system, the slopes of the solubility diagrams decreased, when compared with those of the corresponding binary systems. The decreasing degree of slopes was greater for phenacetin than *m*-BBA, because the stability constant of phenacetin is smaller than that of *m*-BBA, and thus the former complex was greatly affected by the competitive inclusion. The intrinsic solubility of each guest was not changed by the addition of the second guest, indicating a negligible interaction between phenacetin and *m*-BBA. The K_1 value of the phenacetin complex was calculated to be $186(\pm 5)\ \text{M}^{-1}$ from the solubility change of *m*-BBA in the ternary system (▲ in Fig. 1), with using Eq. 9. This value was in good agreement with the stability constant ($K_C = 182\ \text{M}^{-1}$) determined from the data of the binary system

Table 1. Solubilities and Stability Constants of Phenacetin/CyD, *m*-BBA/CyD and *o*-TA/CyD Complexes, Determined from Data of Binary and Ternary Systems

System	Solubility (M)		Stability constant (M^{-1})	
	S_1 ($\times 10^3$)	S_2 ($\times 10^2$)	K_1	K_2
Phenacetin/β-CyD	5.51±0.04	—	182±3	—
m-BBA/β-CyD	—	1.019±0.011	—	312±5
o-TA/β-CyD	—	1.863±0.020	—	109±3
Phenacetin/*m*-BBA/β-CyD	5.53±0.08	1.021±0.032	186±5	316±4
Phenacetin/*o*-TA/β-CyD	5.54±0.07	1.868±0.035	185±4	105±4

S_1, solubility of phenacetin; S_2, solubilities of *m*-BBA and *o*-TA; K_1, stability constant of phenacetin/CyD complex; K_2, stability constants of *m*-BBA/CyD and *o*-TA/CyD complexes.

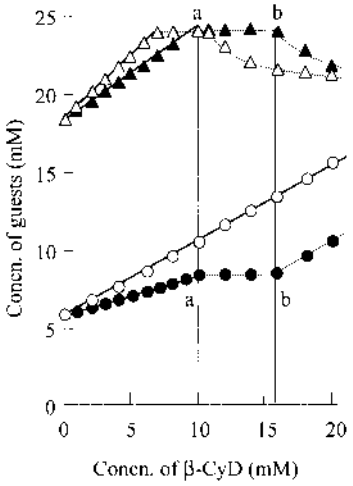

Fig. 2. Phase Solubility Diagrams of Phenacetin and *o*-TA with β-CyD in Isotonic Phosphate Buffer (pH 3.5) at 37 °C

(○), phenacetin in phenacetin/β-CyD system; (●), phenacetin in phenacetin/*o*-TA/β-CyD system; (△), *o*-TA in *o*-TA/β-CyD system; (▲), *o*-TA in phenacetin/*o*-TA/β-CyD system. The solid lines are calculated using Eqs. 8 and 9.

(○ in Fig.1) with using Eq. 1 (see Table 1). In the same way, the K_2 value of the *m*-BBA complex was calculated to be 316(±4) M^{-1}, using the data of the solubility change of phenacetin (● in Fig. 1). This value coincided with that determined from the data of the binary system (△ in Fig.1) with using Eq. 2. The apparent solubilities of phenacetin and *m*-BBA in the presence of β-CyD fell well on the simulated line drawn according to Eqs. 8 and 9, supporting the equilibria of Chart 1A.

Figure 2 shows the phase solubility diagrams of binary phenacetin/β-CyD, binary *o*-TA/β-CyD and ternary phenacetin/*o*-TA/β-CyD systems in phosphate buffer (pH 3.5). Although the pH of the *o*-TA systems (pH 3.5) was different from that (pH 4.5) of the *m*-BBA systems, phenacetin showed no change in its intrinsic solubility between the pHs, because phenacetin is a neutral compound. *o*-TA is known to form the inclusion complex with β-CyD in a molar ratio of 1 : 1 in aqueous solution,[9] but it showed a typical B_S type phase solubility diagram. Therefore, the solubility- and inclusion-equilibria for the ternary system can be described as Chart 1B, where one of the complexes precipitates as a solid complex at higher CyD concentrations, *i.e.*, a combination of A_L and B_S type solubility diagrams. The intrinsic solubility of each guest (*i.e.*, in the absence of CyDs) was not changed

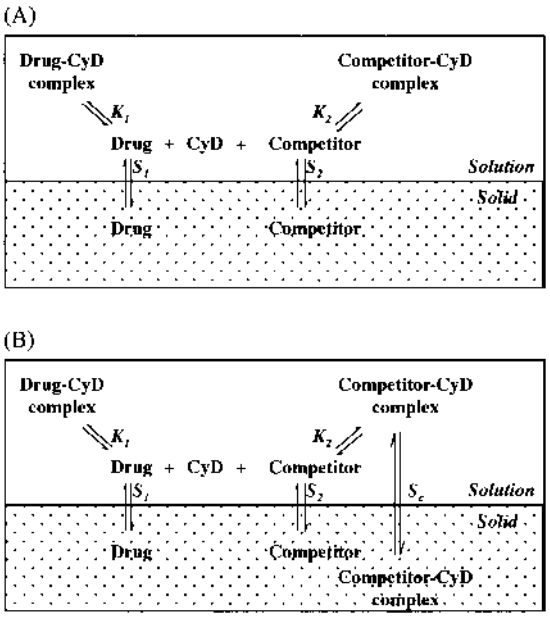

Chart 1. Schematic Representation for Solubility- and Inclusion-Equilibria of Drug/Competitor/CyD Ternary System

(A), formation of soluble competitor/CyD complex; (B), formation of less soluble competitor/CyD complex. K_1 and K_2, stability constants of drug/CyD and competitor/CyD complexes, respectively; S_1, S_2 and S_C, solubilities of drug, competitor and complex, respectively.

by the addition of the another guest molecules, indicating a negligible interaction between phenacetin and *o*-TA. The stability constants of the phenacetin/β-CyD complex and the *o*-TA/β-CyD complex were calculated by using the data of the initial straight line portions of the diagrams, in the same way as described above. The stability constants were as follows: K_1=185(±4) M^{-1} for the phenacetin complex determined from the ternary phenacetin/*o*-TA/β-CyD system (▲ in Fig. 2), K_C=109(±3) M^{-1} for the *o*-TA complex determined from the binary *o*-TA/β-CyD system (△ in Fig. 2) and K_2= 105(±4) M^{-1} for the *o*-TA complex determined from the ternary phenacetin/*o*-TA/β-CyD system (● in Fig. 2). The K_1 value of 185 M^{-1} was in good agreement with the stability constants (182 and 186 M^{-1}) of the phenacetin/β-CyD complex determined from Fig. 1 (see Table 1). The K_1 value (105 M^{-1}) of the *o*-TA/β-CyD complex coincided with the K_C value (109 M^{-1}) determined from the binary system. The observed solubility changes of phenacetin and *o*-TA in the initial straight line portion were quantitatively reproduced by using Eqs. 8 and 9, supporting the equilibria of Chart 1B.

o-TA showed B_S type solubility diagrams in both binary and ternary systems, but the plateau region of the ternary system shifted to higher β-CyD concentrations, compared with the binary system. This is because larger amounts of β-CyD are necessary for the *o*-TA/β-CyD complex to reach its saturated solubility (about 2.3×10^{-2} M when expressed in *o*-TA concentration, Fig. 2), because the slope of the initial straight lines was smaller for the ternary system than the binary system. The analysis of the length of plateau regions by the method of Higuchi and Connors indicated a formation of 1 : 1 *o*-TA/β-CyD complex. This length slightly increased in the ternary system, suggesting no formations of phenacetin/*o*-TA/β-CyD complex and (*o*-TA)$_2$/β-CyD complex, *etc*. After the plateau regions, the solubility of *o*-TA de-

creased in both systems, because the remaining *o*-TA in solution is converted to the solid *o*-TA/β-CyD complex (see Chart 1B), although it was difficult to analyze quantitatively the descending curvature.

It is of interest to note that a plateau region appeared in the solubility diagram of the ternary phenacetin system, in spite of the fact that phenacetin gave originally the A_L type diagram with β-CyD, *i.e.*, a linear solubility increase, under the host concentration range. The plateau region of the ternary phenacetin system appeared in the same concentration range of β-CyD as that of the ternary *o*-TA system, and the length was exactly the same between the phenacetin and *o*-TA systems. After the plateau region, the solubility of phenacetin increased linearly with the slope (slope=0.51) similar with that (slope=0.52) in the absence of *o*-TA. These phenomena can be explained as follows: the *o*-TA/β-CyD complex begins to precipitate when β-CyD concentration reaches the point a (see Fig. 2). Between the points a and b, the added β-CyD is consumed for the conversion of the remaining solid *o*-TA to its β-CyD solid complex. Therefore, the concentration of β-CyD is constant between the point a and b, and thus there was no increase in the phenacetin solubility, *i.e.*, a plateau region. After the plateau, the added β-CyD is exclusively utilized for a formation of the phenacetin/β-CyD complex, thus the solubility of phenacetin increases with the slope similar as that in the absence of the competitor.

The present data indicated that the solubility change of CyD complexes can be estimated by using the stability constants of the complexes and intrinsic solubilities of the additives in formulations, when all components give A_L type phase solubility diagrams. However, when one or several components in formulations exhibit B_S type diagrams with CyD, they may affect not only the solubilization of CyD but also the type of solubility diagram. Therefore, attention should be paid to the type of the phase solubility diagram, as well as the magnitude of the stability constant and the solubility of the complex, for rational formulation design of CyD complexes. Furthermore, the solubility analysis of the ternary drug/competitor/CyD systems may be useful for determination of the stability constant of a drug whose physicochemical and spectroscopic analyses are difficult, because they can be calculated by monitoring the solubility change of a competitor, without monitoring that of the drug.

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