ХИМИЯ: ИТОГИ И ПЕРСПЕКТИВЫ

# CHEMISTRY AND APPLICATIONS OF CYCLODEXTRIN COMPLEXES*

**© 2002 Noriaki Funasaki* ***

Noriaki Funasaki was born on November 27, 1941. Educational career: Master of Pharmacy from Kyoto University in 1965 and Ph.D.(Pharmacy) from Kyoto University in 1973. Ph.D. thesis: Surface chemical study on mixed monolayers of glycerides and cholesterol ester Research career: Research fellow, Faculty of Pharmaceutical Sciences, Kyoto University (1966-1973), Lecturer, Kyoto Pharmaceutical University (1973-1976), Associate Professor, Kyoto Pharmaceutical University (1976-1983), and Professor, Kyoto Pharmaceutical University (1983-Present). Teaching courses: Physical Chemistry and Advanced Physical Chemistry. Fields of research: (1) water permeability, dissolution, and collapse and formation of lipid monolayers, (2) micellar effects on chemical reactions, mixed micelles of fluorocarbon and hydrocarbon surfactants, molecular interactions in micelles, chromatographic estimation of self-associations, and micellization of bile salts, (3) structure and function of cyclodextrin inclusion complexes, (4) quantitative structure-activity relationship of drug, and (5) structure and function of hemoproteins. Publications: 130 original articles, eight review articles, six book chapters, and two patents.

## Introduction

Cyclodextrins (CDs) have homogeneous toroidal structures of different cavity sizes. Three of the most characterized CDs are $\alpha$-, $\beta$-, and $\gamma$-CDs, which contain six, seven, and eight glucose units, respectively. The toroidal structure has a hydrophilic surface resulting from the 2-, 3-, and 6-position hydroxyls, making CD water-soluble. Its cavity is composed of the methin hydrogens, giving it a hydrophobic character. As a consequence, CDs can include other hydrophobic molecules of appropriate dimensions inside their cavity. To a first approximation, the magnitude of binding constants correlates with the fit of the guest in the CD cavity. Therefore, CDs can give beneficial modifications of guest molecules not otherwise achievable: solubility enhancement, stabilization of labile guests, control of volatility and sublimation, and physical isolation of incompatible compounds. Because they are practically nontoxic, they are added into pharmaceuticals and foods [2]. Modified CDs are synthesized to enhance aqueous solubility, function, and guest specificity of native CDs. Some of them can exhibit high specificity and catalysis and chiral separations [1,2].

In some reviews and books, the data on the crystal [3,4] and solution structures [5,6] of CD complexes and the binding constants are summarized and several driving forces of CD complexation are suggested [1-8]. These forces include CD ring strain, van der Waals forces, hydrophobic interactions, and hydrogen bonds between CD and guest. Such driving forces of complexation, despite the many papers dedicated to this problem, have not yet been understood fully. Molecular-mechanical and molecular dynamic calculations have been applied to estimate the structures of complexes and have been compared with experimental data [9].

CD is one of the best investigated host molecules and provides a wealth of knowledge for supramolecular chemistry. Supramolecular chemistry has by now become a major field of chemistry. There are three major reasons for this: first, supramolecular chemistry requires a solid basis of synthetic methodologies of molecular chemistry for producing the building blocks of the supramolecular entities; second, the supramolecular entities are in principle of greater complexity and ability than molecular species, so that their study presents novel challenges; third, the development of supramolecular chemistry requires the availability of power-

** Эту статью профессор Фунасаки написал специально для читателей нашего журнала по предложению редколлегии*

*** Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan*

ful methods for the investigation of the structural, dynamic, and physicochemical features of supramolecular chemistry [8].

In this review, experimental methods for binding constant determination, data analysis of macroscopic and microscopic binding constants, solution structures of CD inclusion complexes, a molecular surface area approach for docking, and pharmaceutical applications of CDs will be presented in emphasis of our research.

## Experimental Methods for Binding Constant Determination

The binding constant is determined by spectroscopic methods (NMR, optical absorption, ESR, IR, circular dichroism, and fluorescence), thermodynamic methods (calorimetry, potentiometry, surface tension, solubility, partition coefficient, pKa, equilibrium dialysis, chromatography, and molar volume), measurements of transport properties (electric conductivity, diffusion constant, and reaction kinetics), measurements of colligative properties (vapor pressure and freezing point), and others (polarography, and refractometry) [7].

These methods utilize the difference in property between the free and bound species. For instance, no natural CDs change the surface tension of water. Their complexes with surface active substances can be assumed not to reduce the surface tension. Under these conditions the surface tension of a mixed CD and guest solution depends on the concentration of the free guest molecule alone [10-14]. This surface tension method is approximately applicable to weakly surface active CDs [11]. The electromotive force measurement also depends on the concentration of the free guest molecule alone [15,16]. The concentration of the free guest molecule is determined as a function of the concentration of CD or guest to estimate the binding constant.

The NMR chemical shift generally has different values for the free and bound species. The chemical shift is referred to internal or external standard. The chemical shift, $\delta$, of internal tetramethylammonium chloride (TMA), referred to external standard, changed linearly with increasing $\alpha$-CD concentration $C_2$. This linear change was ascribed to the change in volume magnetic susceptibility:

$$\delta = \delta_0 + 4\pi(\chi_2 - \chi_w)V_2C_2/3000 \quad (1)$$

Here $\chi_2$ and $\chi_w$ denote magnetic susceptibilities of $\alpha$-CD and water, and $V_2$ stands for the molar volume of $\alpha$-CD. This equation holds true for linear and cyclic oligosaccharides, oligoglycines, organic solvents, and sodium chloride [17]. The chemical shift corrected for this magnetic susceptibility change gives a valid binding constant [18,19]. The internal standard method does not require this correction, but needs inert compounds. Methanol and TMA are very good internal standards for cationic compounds and sodium methyl sulfate is a good internal standard for anionic compounds [18,19]. Water is a good internal standard, if temperature is kept constant [20].

To determine a binding constant from chemical shift data, one needs the value of chemical shift at full binding. Although this is a demerit for binding constant determination, it is a merit providing information about the structure of the complex [18-27].

## Data Analysis of Macroscopic and Microscopic Binding Constants

CD and guest may form complexes of 1:1, 1:2, 2:1, 2:2, and other ratios [7,10]. Here we deal with multiple equilibria for a system forming the 1:1 and 1:2 complexes simultaneously [15,22,23]. For instance, didecyldimethylammonium bromide (DDAB) has two binding sites for $\alpha$-CD (D). From a microscopic viewpoint these decyl groups of a DDAB molecule ($R_\alpha R_\beta$) are designated as $R_\alpha$ and $R_\beta$. Then we must take into consideration two 1:1 complexes, $R_\alpha DR_\beta$ and $R_\alpha R_\beta D$ separately, and a single 1:2 complex, $R_\alpha DR_\beta D$ (Fig.1). Four microscopic equilibrium constants are defined as follows: $k_{1\alpha} = [R_\alpha DR_\beta]/[R_\alpha R_\beta][D]$, $k_{1\beta} = [R_\alpha R_\beta D]/[R_\alpha R_\beta][D]$, $k_{2\alpha} = [R_\alpha DR_\beta D]/[R_\alpha R_\beta D][D]$, and $k_{2\beta} = [R_\alpha DR_\beta D]/[R_\alpha DR_\beta][D]$. The macroscopic 1:1 and 1:2 binding constants are connected with the microscopic constants as follows [7,15,22]:

$$K_1 = \{[R_\alpha DR_\beta] + [R_\alpha R_\beta D]\}/[R_\alpha R_\beta][D] = k_{1\alpha} + k_{1\beta} \quad (2)$$

$$K_2 = [R_\alpha DR_\beta D]/\{[R_\alpha R_\beta D] + [R_\alpha DR_\beta]\}[D] = k_{2\alpha}k_{2\beta}/(k_{2\alpha} + k_{2\beta}) \quad (3)$$

In Table 1 several $K_1$ and $K_2$ values for surfactants are summarized. For a single chain surfactant (for instance, dodecyltrimethylammnium bromide) the $K_1$ value for α-CD is close to that for β-CD. If two binding sites are equivalent and independent, we can expect $K_1 = 2k_{1\alpha}$ and $K_2 = k_{2\alpha}/ 2 = K_1/ 4$ from eq. 2 and 3. The $K_1$ value (8750 $M^{-1}$) for the sodium decyl sulfate and β-CD system may be used as $k_1$ and $k_2$ for the DDAB-α-CD system and the DDAB-β-CD system, respectively. Then, the $K_1$ values for the DDAB-α-CD system and the DDAB-β-CD system calculated from eq. 2 are both 17500 $M^{-1}$, which is close to the observed values. The $K_2$ value for the DDAB-α-CD system calculated from eq. 3 is 4375 $M^{-1}$, which is close to the observed value. Therefore, binding between DDAB and α-CD can be explained on the basis of the equivalent and independent model. The $K_2$ value for the DDAB-β-CD system calculated from eq. 3 is larger than the observed value. The first ligation of β-CD to DDAB is explained on the basis of the equivalent and independent model. The second ligation is inhibited by the first ligated β-CD, because β-CD is more bulky than α-CD. As shown in Table 1, the $K_1$ value for the DDAB-γ-CD system is much larger than that for the dodecyltrimethylammonium bromide-γ-CD system. This anomaly is explicable on the basis of the difference in structure between the complexes (Fig.1) [15].

Table 1. Binding Constants of Surfactants with α-, β-, and γ-CD at 298.2 K [15]

| CD | $K_1$ ($M^{-1}$) | $K_2$ ($M^{-1}$) |
|---|---|---|
| didecyldimethylammonium bromide | | |
| α-CD | 15000 | 6400 |
| β-CD | 15400 | 820 |
| γ-CD | 4290 | $1 \cdot 10^{-3}$ |
| dodecyltrimethylammonium bromide | | |
| α-CD | 17000 | 1000 |
| β-CD | 17000 | – |
| γ-CD | 110 | – |
| sodium decyl sulfate | | |
| β-CD | 8750 | 58 |
| diheptanoylphosphatidylcholine | | |
| α-CD | 550 | 8.6 |
| γ-CD | 748 | 1.9 |

Diheptanoylphosphatidylcholine (DHPC) has two heptanoyl chains at positions 1 and 2 of the glycerol moiety. Three rotamers (gauche+, gauche-, and trans), different dihedral angles of the glycerol moiety, are present and their populations can be determined from NMR vicinal coupling constants of the $H_XC2\text{-}C1H_AH_B$ spin system. DHPC forms the 1:1 and 1:2 complexes with α-CD, whereas it forms the 1:1 complexes with β- and γ-CDs [22,23]. The α-methylene protons of 1- and 2-heptanoyl chains have different chemical shifts and allow us to estimate preferential binding of these chains to CD, though there was no preference to α-CD. From vicinal coupling constants, we can estimate microscopic binding constants for the three rotamers of DHPC with CD. The trans rotamer has the highest affinity to α-CD among the three rotamers and has the lowest affinity to β-

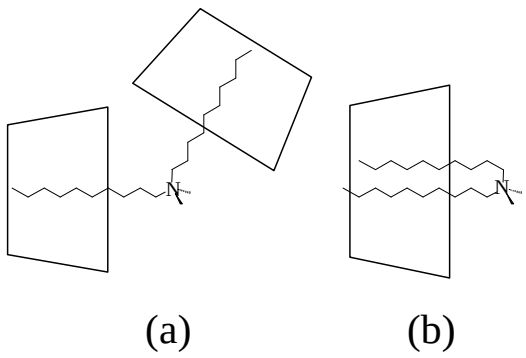


Fig.1. Proposed structures of (a) the 1:2 complex of DDAB and α- and β-CD and (b) the 1:1 complex of DDAB and γ-CD [15].

and γ-CDs among the three rotamers [22,23].

Oxyphenonium bromide (OB) has the phenyl and cyclohexyl groups for α-CD inclusion (Fig.2). Measurements of electromotive forces and NMR chemical shifts independently established that this system forms the 1:1 complex alone [16,24]. The proximity of the phenyl and cyclohexyl groups which both are chemically bound to the asymmetric carbon atom, will prevent OB from formation of the 1:2 complex with α-CD. This system simultaneously forms two 1:1 complexes, the phenyl-in and cyclohexyl-in complexes, and their ratio has been estimated from NMR data [24].

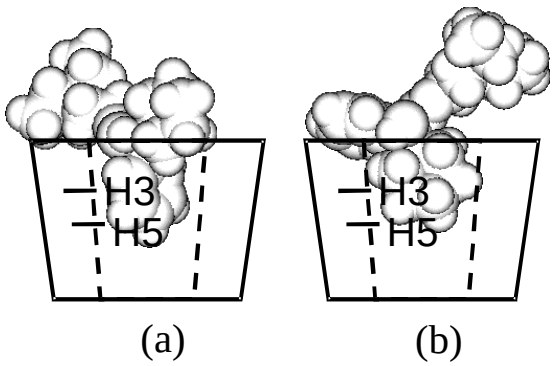


Fig.2. Structures of (a) the phenyl-in complex and (b) the cyclohexyl-in complex of OB with α-CD [24].

Single chain surfactants having alkyl chains shorter than the octyl group can form 1:1 complexes alone with α-CD, whereas single chain surfactants having alkyl chains longer than the decyl group can form 1:1 and 1:2 complexes [15]. For instance, dodecyl maltoside can form 1:1 and 1:2 complexes with α- and β-CDs and can form 1:1, 1:2, and 2:2 complexes with γ-CD [10]. Therefore, single long chain surfactants have multiple binding sites to CDs, and γ-CD can incorporate two surfactant molecules in its cavity.

The binding constant is closely related with the three-dimensional structure of the complex. Cooperativity and inhibition in multiple complexations have been analyzed on the basis of the structures of complexes [10,14].

**Solution Structures of CD Complexes**

When sodium benzenesulfonate (BS) is incorporated in an α-CD cavity, the chemical shifts of the CD protons depend on the geometry of the complex. The ring current effect of benzene on the chemical shift is well established theoretically [21]. This allows us to estimate the solution structure of the BS-α-CD complex from the observed chemical shift: the depth and orientation of BS in the cavity were determined rather accurately [25]. This ring current shift was employed to determine the solution structure of the propanetheline bromide (PB)-α-CD complex [20]. The proton chemical shift of DHPC bound to α-, β-, and γ-CDs was used to image the solution structures of their complexes [22,23]. The conformational change of OB induced by α-CD inclusion was estimated from the chemical shifts of the cyclohexyl protons on the basis of the ring current effect [24]. From the chemical shifts of the phenyl protons of OB bound to α-CD, the mole fraction of the phenyl-in complex was estimated to be 0.4 [24].

The vicinal coupling constant provides the molecular conformation. Analysis of the vicinal coupling constant for the $HC_\alpha$-$C_\beta H$ bond of propanesulfonate indicates that the internal rotation of this bond is hindered in the α-CD cavity [26]. The vicinal coupling constants of β-CD show large changes with incorporating DHPC. This change suggests that the macrocycle of β-CD is elliptically deformed by simultaneous incorporation of two heptanoyl chains in a β-CD cavity [23].

The cross-peak in the NOESY or ROESY spectrum becomes larger, as two protons approach more closely. This relationship can be used to estimate the solution structure. In crystals, the propyl groups of propanesulfonate and propanol are bound from different sides to the α-CD cavity. From analysis of the ROESY cross-peak volume (intensity), however, the propyl group is incorporated from the secondary alcohol side for these complexes in water Furthermore, the solution structures of the α-CD complexes with propanesulfonate and propanol were determined from the best correlation between the ROESY intensity and the inter-proton distance [26]. This method was applied to estimate the structures of the phenyl-in and cyclohexyl-in complexes between OB and α-CD and the populations of these complexes [24]. This

novel method for determination of the solution structure will be applicable to other supramolecules.

Molecular mechanical calculations (e.g., CVFF force fields) predict energetically stable structures in the presence and absence of water. Our experience suggests that hydration energy is not taken into consideration. This energy may be estimated from molecular surface area [27]. The solution structures of the complexes of PB-α-CD [20] and DHPC and β-CD [23], estimated by molecular mechanics calculations, reasonably agree with NMR data.

**Molecular Surface Area Approach for Docking**

Although the physical picture of hydrophobic interactions is still unclear, the magnitude of hydrophobicity or hydration free energy of a solute is empirically linear with its water-accessible surface area. This relationship is widely used to analyze aqueous solubility, water/oil partition coefficients, critical micelle concentrations, capacity factors in reversed phase HPLC, and biological activity of small molecules as well as the unfolding and binding of proteins [28].

The guest molecule accommodated in a CD cavity is normally oriented in the host in such a position as to achieve the maximum contact between the hydrophobic part of the guest and the hydrophobic CD cavity. The hydrophilic part of the guest molecule remains, as far as possible, at the outer face of the complex. This ensures maximum contact with both the solvent and the hydroxyl groups of the host. From this viewpoint, we developed method for calculating molecular surface areas of contact between host and guest [29]. This is a numerical method in which dots are developed evenly on an atom and the number of the dots is counted to calculate the atomic surface area. This atomic surface area is summed over all atoms forming a molecule to calculate the molecular surface area [28,29].

We have recently proposed that the decrease in hydrophobic (oleophilic) molecular surface area $\Delta A_o(HG)$ with the docking of host and guest plays an essential role in determining the stable structure of the complex and the binding constant. This decrease consists of the contributions of host (H), guest (G), and the complex (HG):

$$\Delta A_o(HG) = A_o(H) + A_o(G) - A_o(HG) = \Delta A_o(H) + \Delta A_o(G) \quad (4)$$

Here $\Delta A_o(H)$ denotes the decrease in hydrophobic area of host with the docking, and $\Delta A_o(G)$ are the corresponding values for guest. The decreased hydrophobic area, $\Delta A_o(H)$, of host consists of two terms $\Delta A_{oo}(H)$ and $\Delta A_{ow}(H)$. The first term denotes part of the hydrophobic host surface in complex in contact with hydrophobic groups of guest and the latter term stands for that in contact with hydrophobic groups of host. The first matching term promotes docking and the latter mismatching term inhibits it. Furthermore, we must consider the corresponding areas for the guest, $\Delta A_o(G)$, $\Delta A_{oo}(G)$, and $\Delta A_{ow}(G)$. Among these areas, the sum, $\Delta A_{oo}$, of $\Delta A_{oo}(H)$ and $\Delta A_{oo}(G)$ is the most important parameter and will be termed the matching hydrophobic area decrease. Similarly, $\Delta A_{ww}$ denotes the matching hydrophilic area decrease with contact between the hydrophilic areas of host and guest, and $\Delta A_{ow}$ denotes the mismatching area decrease with contact between the hydrophilic and hydrophobic areas of host and guest [29].

The crystal structure of the 1:1 complex of 4-tert-butylbenzyl alcohol and β-CD is available. This guest molecule was moved along the symmetry axis of β-CD and the $\Delta A_{oo}$ value was determined. The ΔAoo value exhibits the maximum at the crystal structure: this structure is stabilized by hydrophobic interactions. Therefore, from the maximal $\Delta A_{oo}$ value, we can predict a stable structure of the CD complex. For 11 systems including α-, β-, and γ-CDs and aliphatic and aromatic guests, the observed binding constants were correlated with the maximal $\Delta A_{oo}$ values as follows (Fig.3):

$$\text{Log } K_1 = 1.803 \Delta A_{oo} - 2.023 \quad (5)$$

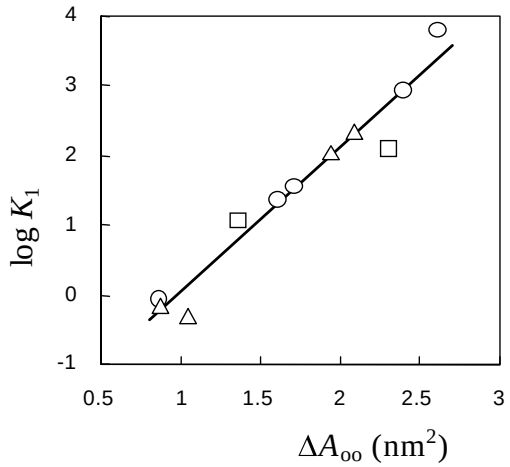


Fig.3. Correlation between binding constants and molecular surface areas for 11 guests with α- (circles), β- (triangles), and γ- (squares) CDs [29].

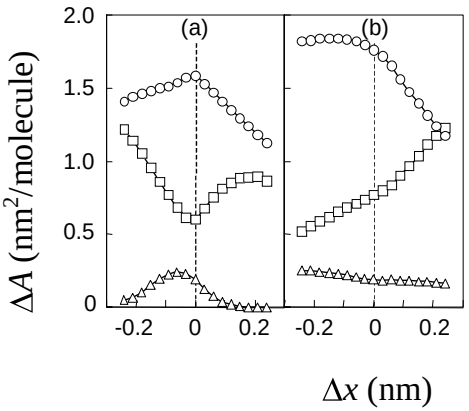


Fig.4. Surface area changes, $\Delta A_{oo}$ (circles), $\Delta A_{ww}$ (triangles), and $\Delta A_{ow}$ (squares), with docking of α-CD and propanol as a function of the penetration depth of the propyl group in an α-CD cavity for (a) the solution structure and (b) the crystal structure [26].

The solution structure of the BS-α-CD complex was determined by NMR and for this structure the $\Delta A_{oo}$ value had the maximum. Although the binding constants (9 to 18 $M^{-1}$), calculated from eq. 5, depended on the structures of complexes, they were close to an observed binding constant of 9.75 $M^{-1}$ [25]. The OB-α-CD system forms two 1:1 complexes (Fig.2). The ratio of the complexes calculated from two binding constants, estimated from eq. 5 with the $\Delta A_{oo}$ values calculated for the two complexes, was close to that obtained from NMR data [24]. The solution structure of the propanol-α-CD complex determined by NMR was distant from the crystal structure. Fig.4 shows the $\Delta A_{oo}$, $\Delta A_{ww}$, and $\Delta A_{ow}$ values as a function of the penetration depth of the propyl group in the α-CD cavity for the solution and crystal structures [26]. As Fig.4a shows that the solution structure ($\Delta x$ = 0) has the maximal $\Delta A_{oo}$ value, the maximal $\Delta A_{ww}$ value, and the minimal $\Delta A_{oo}$ value: the solution structure is stabilized by the hydrophobic and hydrophilic interactions. On the other hand, the crystal structure is stabilized by these interactions and hydrogen bonds [26].

**Pharmaceutical Applications of CDs**

As outlined in the Introduction Section, CDs have many industrial applications. Here we focus on a few applications of CDs in pharmaceutical industry.

PB is a bitter anticholinergic drug. As the PB concentration is increased, the aqueous PB solution tastes bitterer. The bitter intensity was evaluated as one of the six bitterness scores ranging from 0 = no bitter taste to 5 = extremely bitter taste. For instance, the 1.5 mM PB solution tastes very bitter (bitterness score of 4). Addition of α-, β- or γ-CD into this solution reduces the bitter score. The reason for this reduction is formation of PB-CD complexes, which taste little bitter. Generally, bitter compounds are hydrophobic. The hydrophobic xanthene ring of PB will exhibit a bitter taste. This ring is more or less incorporated in a CD cavity, so that the PB-CD complex exhibits little bitter taste. Therefore, we can assume that the bitter intensity of a mixed PB and CD solution is determined by the concentration of free PB [12]. The concentration of free PB can be estimated from the binding constant, determined by surface tension method. Thus we can predict the bitter taste intensity for the mixed PB and CD solution without any sensory test. Furthermore, the surface tension of this solution depends on the concentration of free PB alone. Therefore, the bitter taste of a mixed PB and CD solution is a function of surface tension alone, regardless of the concentrations of PB and CD and the kind of CD (Fig.5). This relationship enables us to predict the bitter taste intensity from the observed surface tension [12]. Similarly, the observed electromotive force, selectively responsible to drug, is used to predict the bitter taste intensity of a mixed drug and CD solution [16]. Furthermore, the opti-

cal absorption provides the binding constant and leads to another prediction of the bitter taste intensity of a mixed drug and CD solution [30].

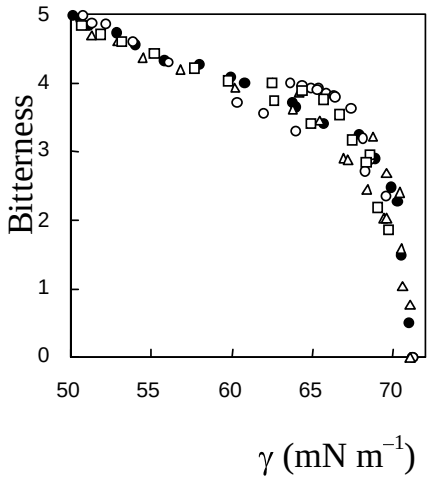


Fig.5. Relationship between the bitter taste intensity and the surface tension for mixed PB and CD solutions in the absence (solid circles) and presence of α- (open circles), β- (triangles), and γ-CD (squares) [12].

CDs at high concentrations have hemolytic activity, because they extract lipids from the erythrocyte membrane. However, they can suppress drug-induced hemolysis at low concentrations [11,13,14]. Complexes between CD and drug can be expected not to be hemolytic. Then one can assume that the hemolytic activity of a mixed drug and CD solution is determined by the concentration of free drug alone. This assumption holds true for many systems [11,13,14], though it does not hold for highly hemolytic CDs [11] and weakly binding CDs [13].

CDs can accelerate and inhibit chemical reactions [1]. Generally, when the reactive site is located near the catalytic group of CD, the reaction will be accelerated. On the other hand, when it is deeply incorporated in a CD cavity, the reaction will be inhibited. To understand the effect of CD on the reaction rate, we need to estimate the solution structure, binding constant, and stoichiometry of the CD-substrate complex. Most drugs lose bioactivity by hydrolysis and are protected from hydrolysis by CDs. Penicillin G is a labile antibiotics and reduces its activity by hydrolysis [31]. Penicillin G is stabilized by β- and γ-CD, though it is slightly catalyzed by α-CD [31]. PB is accelerated by α-CD, because its ester group is located near hydroxyl groups of α-CD [32]. The polarity of guest bound to CD may be estimated from the UV absorption maximum of the guest and has some correlation with the reactivity [30,32]. The effects of α-, β-, and γ-CD on the hydrolysis of PB and OB are analyzed on the basis of structures of complexes and stoichiometry in some detail [32].

## Conclusions

CD is one of the most useful host molecules. Its interactions with guests, such as binding constants, three-dimensional structures of complexes, and intermolecular forces, provide a wealth of knowledge for other supramolecules as well as industrial applications.

## References

1. Bender M.L., Komiyama M. Cyclodextrin Chemistry. Springer-Verlag: Berlin, 1978.
2. Szejtli J. Cyclodextrin Technology. Kluwer Academic Publishers: Dordrecht, The Netherlands, 1988. Chapters 2 and 3.
3. Saenger W., Jacob J., Gesseler K., Steiner T., Hoffmann D., Sanbe H., Koizumi K., Smith S., Takaha T. Chem. Rev. 1998. V.98. P.1787.
4. Harata K. Chem. Rev. 1998. V.98. P.1803.
5. Schneider H.J., Hacket F., Rudiger V., Ikeda H. Chem. Rev. 1998. V.98. P.1755.
6. Inoue Y. Ann. Rep. NMR Spectr. 1993. V.27. P.60.
7. Connors K.A. Chem. Rev. 1997. V.97. P.1325.
8. Schneider H.J., Yatsimirsky A.K. Principles and Methods in Supramolecular Chemistry. John Wiley and Sons: New York, 2000. Chapter E4.
9. Lipkowitz K.B. Chem. Rev. 1998. V.98. P.1829.
10. Funasaki N., Yodo H., Hada S., Neya S. Bull. Chem. Soc. Jpn. 1992. V.65. P.1323.
11. Funasaki N., Okuda T., Neya S. J. Pharm. Sci. 2001. V.90. P.1056.
12. Funasaki N., Uemura Y., Hada S., Neya, S. J. Phys. Chem. 1996. V.100. P.16298.
13. Funasaki N., Ohigashi M., Hada S., Neya S. Langmuir. 1999. V.15. P.594.
14. Funasaki N., Ohigashi M., Hada S., Neya S. Langmuir. 2000. V.16. P.383.

15. Funasaki N., Neya S. Langmuir. 2000. V.16. P.5343.
16. Funasaki N., Kawaguchi K., Ishikawa S., Hada S., Neya S., Katsu T. Anal. Chem. 1999. V.71. P.1733.
17. Funasaki N., Nomura M., Ishikawa S., Neya S. J. Phys. Chem. B. 2001. V.105. P.7361.
18. Funasaki N., Nomura M., Yamaguchi S., Ishikawa S., Neya S. Bull. Chem. Soc. Jpn. 2000. V.73. P.2727.
19. Funasaki N., Ishikawa S., Neya S. Bull. Chem. Soc. Jpn. 2002. V.75. P.719.
20. Hada S., Neya S., Funasaki N. J. Phys. Chem. B 1999. V.103. P.2574.
21. Bovey F.A. Nuclear Magnetic Resonance Spectroscopy. Academic Press: New York and London, 1969. Chapter 3 and Appendix C.
22. Ishikawa S., Funasaki N., Neya S. J. Phys. Chem. B 1998. V.102. P.2502.
23. Funasaki N., Ishikawa S., Neya S. Langmuir. 2002. V.18. P.1786.
24. Funasaki N., Yamaguchi H., Ishikawa S., Neya S. Bull. Chem. Soc. Jpn., in press.
25. Funasaki N., Yamaguchi H., Ishikawa S., Neya S. J. Phys. Chem. B. 2001. V.105. P.760.
26. Funasaki N., Ishikawa S., Neya S. J. Phys. Chem. B. 2002. V.106. P.6431.
27. Funasaki N., Yamaguchi H., Ishikawa S., Neya S. J. Phys. Chem. B. 2000. V.104. P.10412.
28. Ishikawa S., Hada S., Funasaki N. J. Phys. Chem. 1995. V.99. P.11508.
29. Ishikawa S., Hada S., Neya S., Funasaki N. J. Phys. Chem. B. 1999. V.103, P.1208.
30. Funasaki N., Kawaguchi K., Hada S., Neya S. J. Pharm. Sci. 1999. V.88. P.759.
31. Hada S., Neya S., Funasaki N. Chem. Pharm. Bull. 1997. V.45. P.577.
32. Funasaki N., Ishikawa S., Neya S. J. Pharm. Sci. 2001. V.90. P.740.

*Поступила в редакцию* 24 *декабря* 2002 *г.*