MEASUREMENT OF BINDING CONSTANT OF CYCLODEXTRIN BY ULTRAFILTRATION

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We proposed a new measuring method of the binding constant K_B, by proving that the inclusion degree α in a host-guest reaction is equal to the rise ratio of rejection η in ultrafiltration. The K_B for nitrophenol (NP) and cyclodextrin (CD) system was measured. The data were compared with those of other investigators. The K_B measured by the proposed method decreased with increase in operating pressure, but a constant value of K_B was obtained below a certain pressure. The pressure was nearly 1 MPa for the NP-CD system. The constant value was the equilibrium binding constant K_B. With an increase in pH, the K_B became large because guest molecules were charged and the anion and hydrogen bonds between host and guest molecules became strong. With a decrease in the concentration ratio ε of guest molecules to host molecules, the inclusion degree α became large, but the binding constant K_B remained almost constant. After checking the validity of the new method by NP-CD system, the K_B value for optical isomers of amino acids, threonine and phenylalanine, were measured to investigate whether the optically selective inclusion of guest molecules by CD was possible or not. CD, which is an optically active D-form molecule, tended to include optical L-acid rather than D-acid under the present conditions. The optimum conditions were referred to resolute optical isomers.

Introduction

Cyclodextrins (CDs) are optically active materials. They interact with guest molecules selectively, and form inclusion complexes. By using the selective inclusion property, CDs have recently been used as means of separation for isomers or mixtures normally difficult to separate, for example, in liquid chromatography[2, 3, 10, 13, 14, 23], fixed membrane[12], liquid membrane[29] and selective titration[28] methods.

We proposed a new separation method by ultrafiltration using CD, carried out the separation of structural isomers nitrophenols (NPs) in accordance with the method, and confirmed the possibility of the separation[15, 16]. The binding constant K_B is an important property for not only host-guest chemistry but also separation engineering.

Measurement methods of the K_B can be classified as follows: a. liquid chromatography (LC)[7, 21, 23, 24], b. spectrophotometry[6, 8, 20, 26, 27], c. electro chemical method[9, 18, 19, 22], d. NMR[4, 11, 22, 25], e. microcalorimetry[5, 17], and f. solubility method[22, 26]. Among them, chromatographic methods have been reported widely. Uekama[11, 22-24, 27] measured the K_B for prostaglandin by NMR, LC, UV absorption, and solubility methods. Benzene, and its derivatives, especially nitrophenol (NP) and chiral compounds, have been used in many cases as inclusion reaction systems with CD. The K_B values for NP were measured by different methods and under different conditions, for example, different temperature and pH conditions. The values of K_B for NP reported were often very different from each other.

In this paper, we propose a new measuring method of K_B which was suggested from previous separation experiments[15, 16]. The K_B for NP was measured in accordance with the new method. The effects of pressure, pH and concentration on K_B were discussed and the values of K_B were compared with other works. To investigate the optical selectivity of CD, the K_B for optical isomers of amino acids, threonine and phenylalanine, were also measured.

1. Principle of Measurement

1. 1 Definition of binding constant

Host molecule cyclodextrin CD interacts equimolarly with guest molecule G and forms an inclusion complex CDG.

$$G + CD = CDG \tag{1}$$

The binding constant K_B is defined as an equilibrium constant in the inclusion reaction.

$$K_B = [CDG] / [G][CD] \tag{2}$$

The initial concentrations of guest and host molecules are set to be $[G]_0$ and $[CD]_0$, respectively. The binding constant K_B is given by the following equation,

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$$K_B = \alpha / \{[CD]_0 (1-\alpha)(1-\varepsilon\alpha)\} \quad (3)$$

where α is the inclusion degree based on guest molecules.

$$\alpha = [CDG] / [G]_0 \quad (4)$$

and

$$\varepsilon = [G]_0 / [CD]_0 \quad (5)$$

1. 2 The measuring method of inclusion degree

If the feed concentrations of guest and inclusion complex are $[G]_F$ and $[CDG]_F$, respectively, the feed concentrations are given as follows.

$$[G]_F = [G]_0 (1-\alpha) \quad (6)$$

$$[CDG]_F = [G]_0 \alpha \quad (7)$$

The total amount of guest molecules G permeated through an ultrafiltration (UF) membrane is given by the addition of molecules G included in CD, that is complex CDG, and bare molecules G not included in CD.

The rejections of G, CD and CDG are defined, as follows.

$$R_G = 1 - [G]_P / [G]_F \quad (8)$$

$$R_{CD} = 1 - [CD]_P / [CD]_F \quad (9)$$

and

$$R_{CDG} = 1 - [CDG]_P / [CDG]_F \quad (10)$$

The concentrations of G and CDG in permeate are given as follows.

$$[G]_P = (1-R_G)[G]_0 (1-\alpha) \quad (11)$$

$$[CDG]_P = (1-R_{CD})[G]_0 \alpha \quad (12)$$

It is assumed that rejection of inclusion complex CDG is equal to that of CD and each solution of inclusion reaction is so dilute that each component behaves independently for UF separation. The permeate fluxes of G and CDG are given as follows.

$$J_G = [G]_P J_V \quad (13)$$

$$J_{CDG} = [CDG]_P J_V \quad (14)$$

The total solute flux J_T of G is given by

$$J_T = J_G + J_{CDG} = J_V ([G]_P + [CDG]_P) \quad (15)$$

The concentration of total G permeated through the UF membrane (abbrev. as UF/m), $([G]_P)_{CD}$ is given by

$$([G]_P)_{CD} = J_T / J_V = [G]_0 \{(1-R_G)(1-\alpha) + (1-R_{CD})\alpha\} \quad (16)$$

$$= [G]_P + [CDG]_P \quad (17)$$

The concentration of total G at feed side $[G_T]_F$ is given by

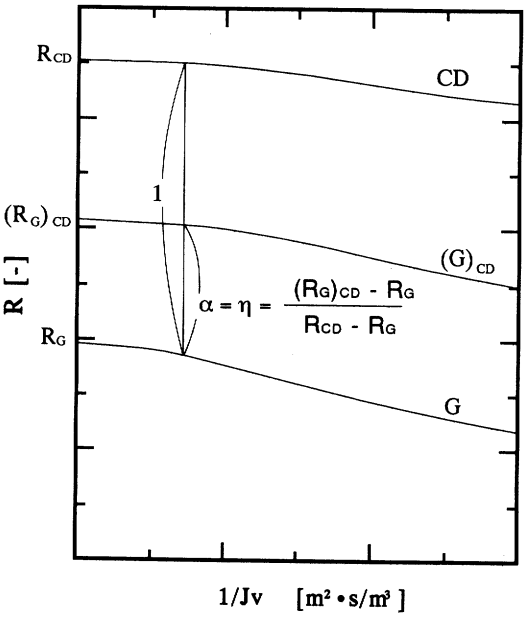

Fig. 1 Concept of the magnitude of η (rise ratio of rejection) or α (inclusion degree)

$$[G_T]_F = [G]_F + [CDG]_F = [G]_0 \quad (18)$$

The rejection of total G under CD is given by

$$(R_G)_{CD} = 1 - \{([G]_P)_{CD} / [G_T]_F\} = \alpha (R_{CD} - R_G) + R_G \quad (19)$$

The inclusion degree α is obtained from eq.(19), as follows.

$$\alpha = \{(R_G)_{CD} - R_G\} / (R_{CD} - R_G) \quad (20)$$

The right hand of Eq.(20) is equal to the rise ratio of rejection η at the UF separation experiment[15]. **Fig. 1** shows the magnitude of η (rise ratio of rejection) or α (inclusion degree), graphically. The binding constant K_B can be calculated from Eq.(3), setting $\alpha = \eta$ where η can be obtained from UF separation experiments.

2. Experimental

For the present work we used the partially improved experimental apparatus which was used for ultrafiltration (UF) experiment in our previous study[15]. In the host-guest reaction, α- and β-cyclodextrins (CDs) were used as host molecules. They were prepared by Ensuiko Sugar Refinery. As guest molecules, structural isomers, o-, m- and p-nitrophenols (NPs) and amino acid optical isomers, D- and L-threonine (Thrs) and D- and L- phenylalanine (Phes), were used. They were special grade and prepared by Wako Pure Pharmaceutical Co..

YM-1, PLBC and CA membranes were used as UF/m[15]. The pressure was maintained within the range of 1-5 MPa for CA membrane and 0.1 - 0.5 MPa for the other two membranes. The temperature was kept at 303 1K during the experiment. The concentration of host molecules

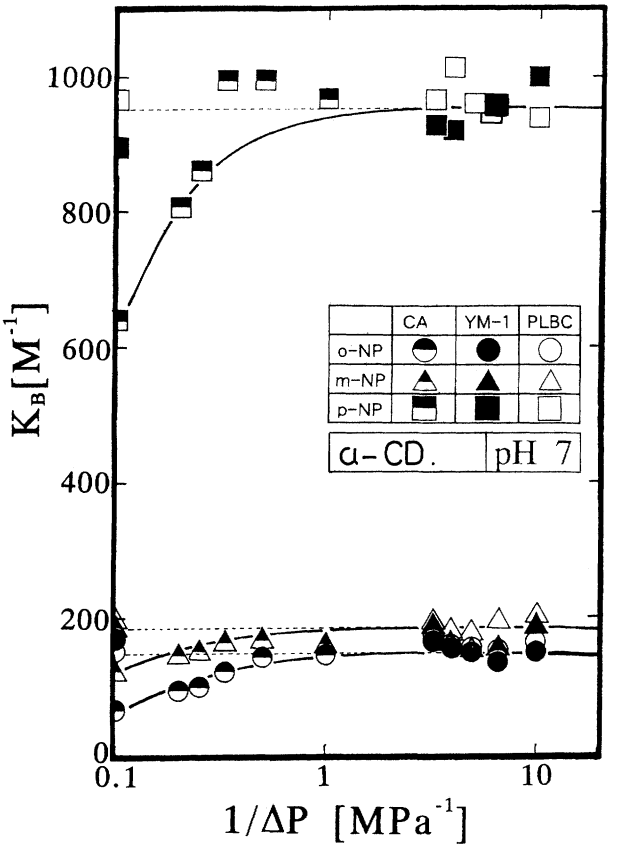

Fig. 2 Relationship between operation pressure and binding constant for nitrophenol-α-cyclodextrin system

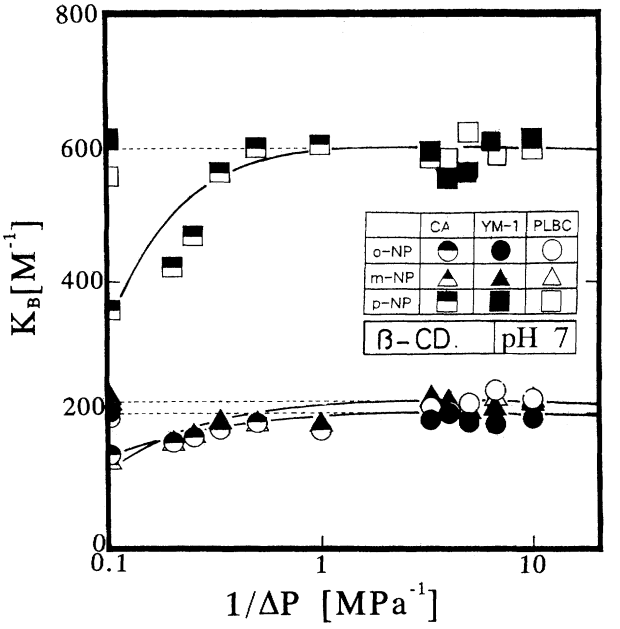

Fig. 3 Relationship between operation pressure and binding constant for nitrophenol-β-cyclodextrin system

Table 1. Membrane properties for ultrafiltration

Membrane			CA	YM-1	PLBC
Material			Cellulose Acetate	Polysaccharide	Regenerated Cellulose
Sources			present work	Amicon	Millipore
Cut off M. W.			above 10.000	1.000	3.000
Pressure [MPa]			1–5	0.1–0.3	0.1–0.3
Lp×10^5 [m/s·MPa]			0.458	1.17	3.10
CD	α-	p×10^9 [m/s]	0.58±0.06	0.75±0.08	11.9±1
		σ [–]	0.32±0.03	0.92±0.09	0.59±0.06
	β-	p×10^9 [–]	0.43±0.04	0.29±0.03	10.5±1
		σ [–]	0.39±0.04	0.97±0.1	0.67±0.07
NP	o	p×10^9 [m/s]	1.02±0.1	9.40±0.9	61.2±6
		σ [–]	0.16±0.02	0.10±0.10	0.05±0.01
	m-	p×10^9 [m/s]	1.26±0.1	9.44±0.9	41.0±4
		σ [–]	0.17±0.02	0.10±0.01	0.05±0.01
	p	p×10^9 [m/s]	1.16±0.1	9.08±0.9	52.0±5
		σ [–]	0.18±0.02	0.10±0.01	0.05±0.01

was kept constant at 5 mM, while that of guest molecules was varied from 2.5 to 10 mM. The pH was maintained between 3-11 by a buffer solution. The concentrations were analyzed by spectrophotometry for NPs solution, and liquid chromatography (LC) for optical isomers, Thrs and Phes. An optical column was used for LC, (TSKgel-80Tm, prepared by Toso Chemicals). The inclusion solution which was interacted with host and guest molecules for 24 hours was fed to the UF cell, where permeate flux and rejection were measured. In order to keep the feed concentration constant, permeate and retentate were totally refluxed. To prevent concentration polarization occuring the at membrane surface, the feed solution in the cell was stirred sufficiently.

3. Results and Discussion

3. 1 Effect of operating pressure on binding constant

Figs. 2 and **3** show the binding constant K_B calculated from eq. (3) for the NP-CD system by setting $\alpha = \eta$. The effects of pressure difference across the membrane ΔP on K_B of NPs at pH 7 for α-CD and for β-CD are represented in Fig. 2 and Fig. 3, respectively. The values of K_B at $1/\Delta P$ = 0.1 [MPa^{-1}] were calculated from reflected coefficients[15] σ. **Table 1** represents the properties of the membrane used in the present work. The K_B value becomes constant when $1/\Delta P$ is larger than a certain value, i.e., when ΔP is smaller than the value ΔP_B, and decreases when $1/\Delta P$ is smaller than $1/\Delta P_B$ for both the α-CD and β-CD systems.

Guest molecule NPs included in host molecule CDs rejected by UF/m are released by a large operating pressure from the CDs and permeate through UF/m. As the rejection of guest molecules is smaller than that of host molecules, the K_B becomes smaller when ΔP becomes larger than ΔP_B. The K_B depends on the host-guest inclusion system and experimental conditions but, is independent of the type of UF/m, CA, YM-1 and PLBC. The pressure difference ΔP_B is slightly different for inclusion systems but ΔP_B for NPs-CD systems can be estimated to be nearly 1 MPa. This value is similar to those of other researchers[1, 10]. The constant K_B obtained by this method gives true equilibrium binding constant. In the following the constant K_B will be analyzed.

3.2 Effect of pH on binding constant

Binding strength depends on the binding force of hydrophobic interaction and hydrogen bond between host and guest molecules[1, 10]. The effect of pH on the binding

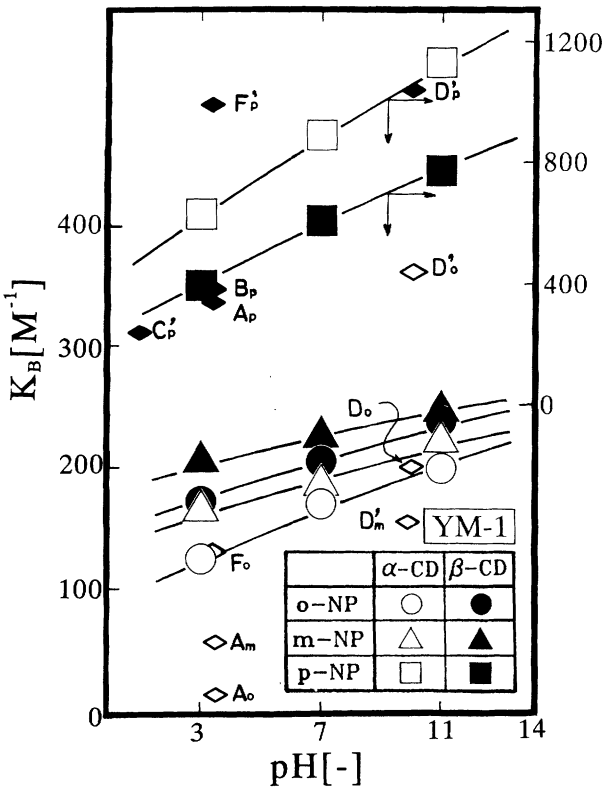

Fig. 4 Effect of pH on binding constant for nitrophenol cyclodextrin system. The characters represent the data described as symbols in the Table 2

Table 2. Binding constants measured by various methods for CD-NP system

Methods	Symbol	Host	Guest	pH	Temp [°C]	K_B [M^{-1}]	Liter.
Positron	A_o	α-CD	o-NP	3.5	22	8±4	
annihilation	A_m	α-CD	m-NP			54±12	9
	A_p	α-CD	p-NP			341±25	
UV absorption	B_p	α-CD	p-NP	3.5	25	385	6
	B_p	α-CD	p-NP	11.0		*3700	
Circular dichro	C'_p	β-CD	p-NP	1.0		244	20
Polarography	D_o	α-CD	o-NP	10.0	20	200	
	D_m	α-CD	m-NP			*500	19
	D_p	α-CD	p-NP			*2440	
	D'_o	β-CD	o-NP			357	
	D'_m	β-CD	m-NP			147	
	D'_p	β-CD	p-NP			1020	
^{1}H-NMR	E_p	α-CD	p-NP	11.0	23	*2700	4
Microcalorimetry	F_o	α-CD	o-NP	3.5	25	125	
	F_p	α-CD	p-NP			*5011	17
	F'_p	β-CD	p-NP			1000	

*scale over for Fig. 4

constant K_B for the NP-CD system is shown in **Fig. 4**. The values of K_B for o-NP and m- NP are read on the left axis and those for p-NP are read on the right axis. The K_B of p-NP is about 5 times larger than those of o- and m-NPs. As the K_B of p-NP for α-CD is larger than that for β-CD, the inclusion for p-NP-α-CD system is more stable structurally than that for p-NP - β-CD system. It was found that the inner diameter of the cavity of α-CD was so large that a benzene ring could just fit in[1, 10]. The inner diameter of the cavity of β-CD is larger than that of α-CD. p-NP might thus just fit in to the α-CD cavity. But o- and m-NPs fit in to β-CD better than in α-CD because of the steric hindrance

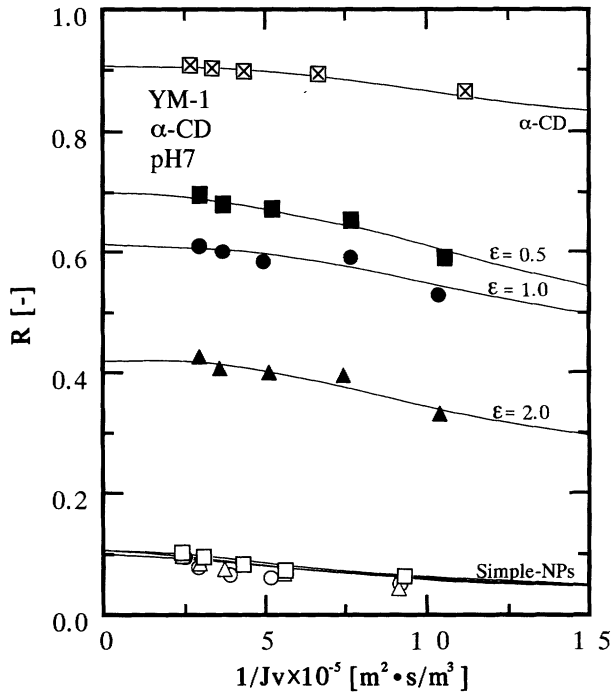

Fig. 5 Effect of concentration on rejection for p-nitrophenol-α-cyclodextrin system. The keys for simple-NPs, O, △, □ are the o-, m-, p-NPs, respectively

effect between o-NP or m-NP and CDs molecules. They are more structurally stable in β-CD than in α-CD[1, 10].

For all systems when pH is high, the K_B is large. This might be induced by dissociation of nitro group in NP to form nitrophenolate ion at high pH. The inclusion binding force between the nitrophenolate ion of guest molecules and secondary hydroxide group of CD becomes strong due to hydrogen bonding. The K_B for the NP-CD system which was investigated by others are listed in **Table 2**. The data include those measured by different methods and under different pH, and temperature. Their data were compared with the present data in Fig. 4. The characters A-F in the figure which label the keys ◇ or ◆ represent the data described as symbols in Table 2. The dash on the characters in Fig.4 and Table 2 represent the data for β-CD and the closed keys ◆ in the figure represent the data for p-NP. The values of K_B obtained by other researchers are often very different from each other, and go over the scale of Fig.4. These data are indicated by " * " in Table 2. It is found that the K_B obtained by the proposed method is appropriate. Therefore the assumptions used as principles of measurement are considered to be suitable for the present experimental conditions.

3. 3 Relationship between inclusion degree and binding constant with concentration

To investigate the effect of concentration of host and guest molecules on the K_B, three initial concentrations of guest molecules were used- 2.5 mM, 5 mM and 10 mM- while the concentration of host molecules was kept constant at 5 mM, i.e., the concentration ratio $\varepsilon = [G]_0/[CD]_0$ was either 0.5, 1, or 2. The effects of ε on rejection R for the p-NP-α-CD system and the m-NP-β-CD

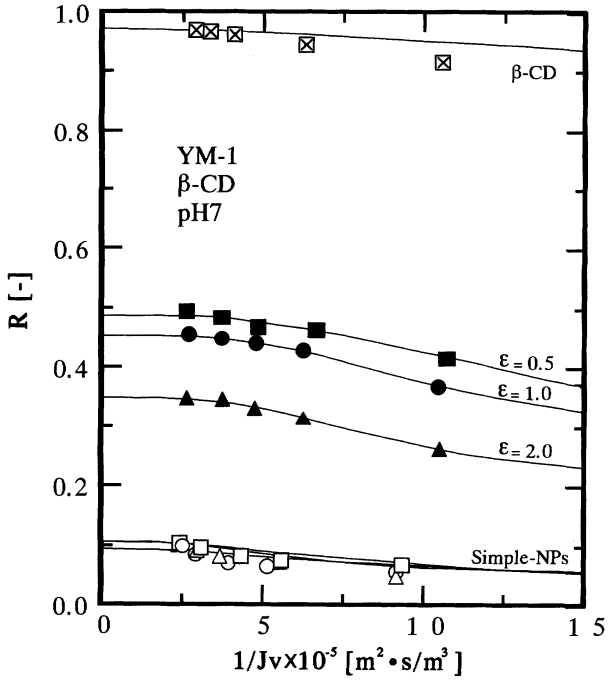

Fig. 6 Effect of concentration on rejection for m-nitrophenol-β-cyclodextrin system. The keys for simple-NPs, ○, △, □ are the o-, m-, p-NPs, respectively

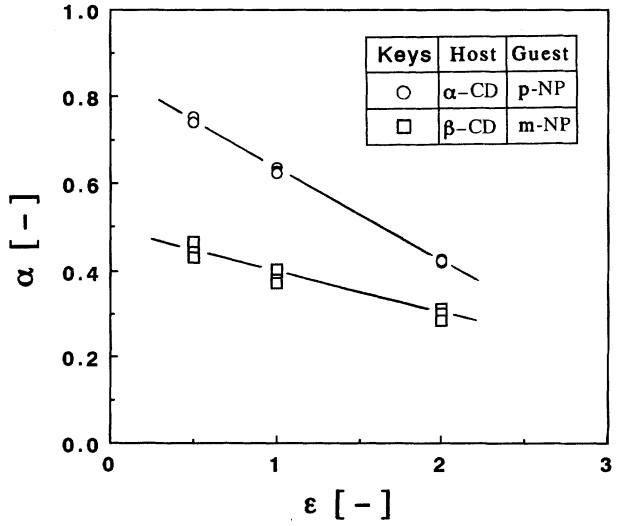

Fig. 7 Relationship between concentration ratio and inclusion degree for nitrophenol-cyclodextrin system

system are shown in **Fig. 5** and **Fig. 6**, respectively. When ε becomes small, the rejection of guest molecules increases for both systems, and the rise ratio of rejection $\eta = \{(R_G)_{CD}\text{-}R_G\}/\{R_{CD}\text{-}R_G\}$, i.e., the inclusion degree α, becomes large.

The effects of concentration on inclusion degree α and binding constant K_B are shown in **Fig. 7** and **Fig. 8**, respectively. The α decreases linearly while the K_B is nearly constant with increase of concentration. It is found that the binding constant K_B in inclusion reactions is not affected by concentration to the same extent as equilibrium constants in general reactions.

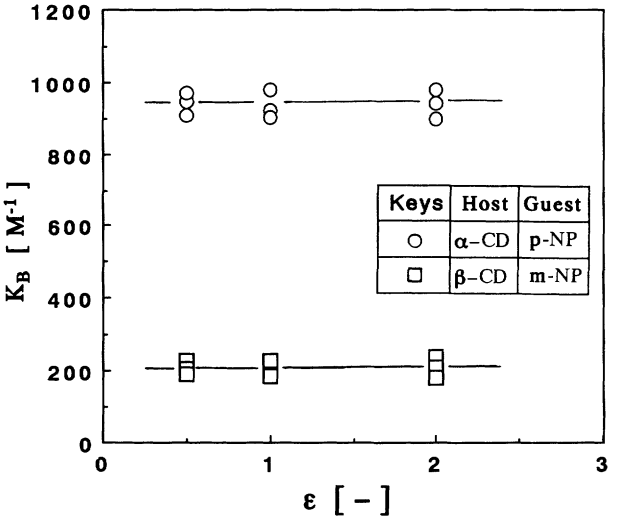

Fig. 8 Relationship between concentration ratio and binding constant for nitrophenol-cyclodextrin system

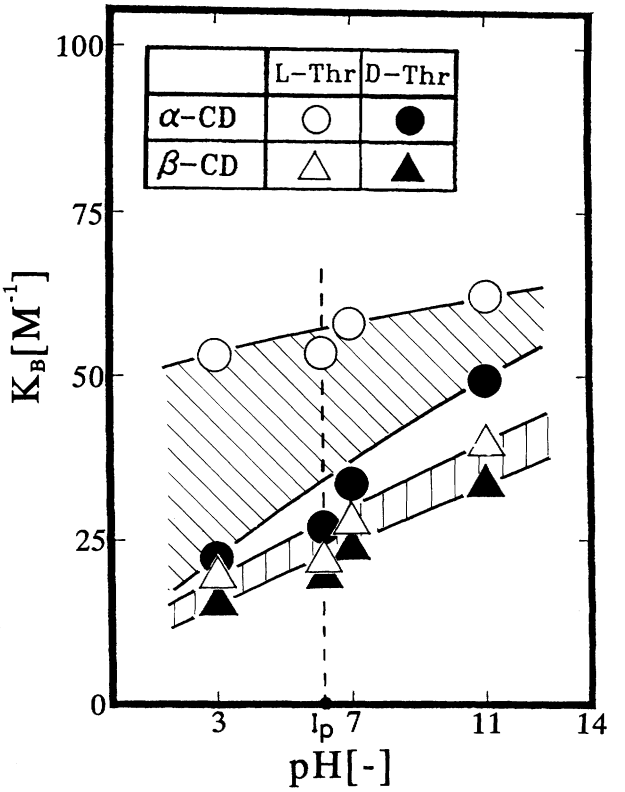

Fig. 9 Effect of pH on binding constant for threonine-cyclodextrin system

3.4 Binding constant of threonine

To investigate optical selectivity of CDs in inclusion reactions,which are optically active materials of the D-form, we measured the binding constant K_B of amino acid optical isomers by the new method. **Fig. 9** shows the effect of pH on K_B of threonine (Thr) isomers. As the diameter of Thr molecules is smaller than that of nitrophenol (NP), the K_B of Thr for α-CD is structurally larger than that for β-CD.

When the pH becomes high, the K_B becomes larger.

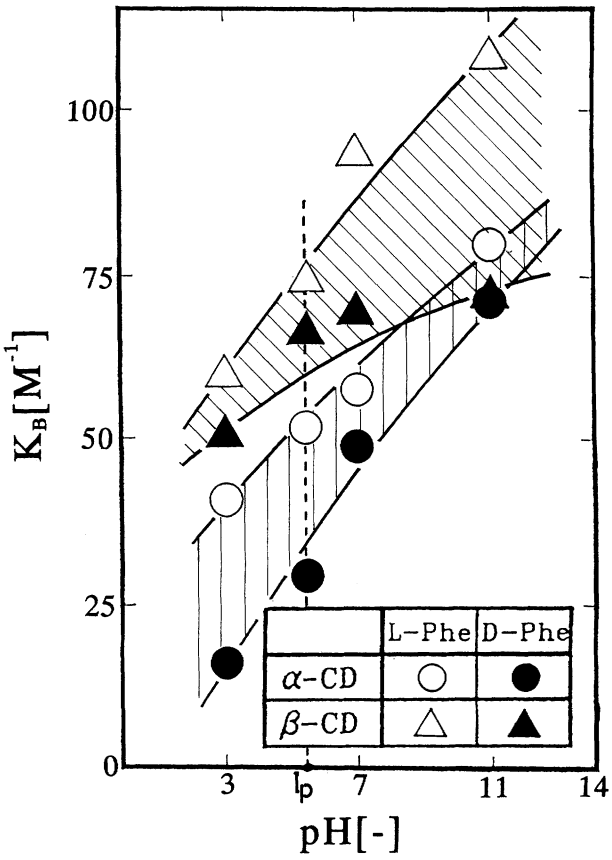

Fig. 10 Effect of pH on binding constant for phenylalanine-cyclodextrin system

At high pH, the guest molecule Thr, which is an amphoteric electrolyte, anionically is charged and, as in the case for NP, the binding force between host and guest molecules is increased through hydrogen bonding, K_B then becomes large.

Generally, the optically active D-form CD tends to include optically heterogeneous L-Thr rather than homogeneous D-Thr under the present conditions. The difference of K_B between L- and D-Thrs is larger at low pH than at high pH for α-CD and is nearly constant with pH for β-CD. The optical resolution of Thr by the present method must be carried out under conditions of low pH using α-CD, not β-CD. The isoelectric point (Ip) of Thr is pH 6.16. The K_B of Thr was not affected at the Ip. Previous studies of K_B for Thr-CDs system can not be found in the literature.

3. 5 Binding constant of phenylalanine

Fig.10 shows the effect of pH on the binding constant (K_B) of phenylalanine (Phe). The K_B becomes large with increase of pH for all Phe-CD systems. The K_B of Phe is a little larger than that of Thr. The K_B of Phe for β-CD is larger than that for α-CD, in contrast with that of Thr. The molecular weight of Phe, composed of benzene groups in the same case of NP, is larger than those of Thr and NP. The steric hindrance effect of o-NP or m-NP for CD is larger than that of p-NP. Therefore Phe is included more stable in β-CD than in α-CD.

The D-form optical isomer CD tends to include L-Phe rather than D-Phe in a similar manner as Thr. The difference of K_B between L- and D-Phes is larger at high pH. Optical resolution of Phe by using optically selective inclusion of CD must be carried out at high pH using β-CD, but not α-CD, in contrast with the case of Thr. K_B varies smoothly with pH at the isoelectric point Ip=5.48.

Lewis *et al.*[17] reported the K_B for a L-Phe-α-CD system to be 12600 M^{-1} at pH 7 and temperature 298.1 K. Cooper *et al.*[5] reported the K_B for a L-Phe-α-CD system and for a D-Phe-α-CD system to be 15.8 M^{-1} and 20.6 M^{-1} at pH 11 and temperature 298.1 K, respectively. But their reliabilities are not referred to.

Conclusion

By proving that the rise ratio of rejection η in ultrafiltration is equal to the inclusion degree α in a host-guest reaction, we proposed a new method of measurement of the binding constant K_B. At first, the K_B for the nitrophenol (NP) and cyclodextrin (CD) system were measured under conditions in which operating pressure was varied from 0.1 to 5 MPa, pH from 3 to 11 and concentration of NP from 2.5 to 10 mM. The results were compared with those of other investigators. After checking the validity of the proposed method using the NP-CD system, the K_B of optical isomer amino acids, threonine (Thr) and phenylalanine (Phe), were measured to investigate the optically selective inclusion of CD, and the following conclusions were obtained. 1.The K_B measured by the proposed method began decreasing at a high operating pressure, but a constant value of K_B was obtained below a certain pressure. The pressure was nearly 1 MPa for the NP-CD system. 2. K_B became large with an increase in pH because guest molecules were charged anionically and hydrogen bonding between host and guest molecules became strong. 3. The inclusion degree α became small with increase in the concentration ratio of guest to host molecules, but the binding constant K_B was nearly constant with concentration. 4. The K_B of Phe-CD system was larger than that of Thr-CD system. The K_B of Thr for α-CD was larger than for β-CD, while the K_B of Phe for β-CD was larger than for α-CD. 5. CD tended to include optical L-acid rather than D-acid under the present conditions.

As the proposed method was valid, CD inclusion supported equimolar reaction for the present systems because the principle of measuring method of K_B proposed is based on the assumption that the inclusion reaction of CD with guest molecules is equimolar.

Nomenclature

C	=	concentration	[mol/m^3]
$[CD]_0$	=	initial concentration of CD	[mol/m^3]
J	=	permeate flux	[mol/m^{2}s]
J_V	=	volume flux	[m^3/m^{2}s]
K_B	=	binding constant	[M^{-1}]
R	=	rejection = $1-C_P/C_F$	[-]
α	=	inclusion degree defined by Eq. (20)	[-]
ΔP	=	pressure difference across membrane	[Pa]
ε	=	initial concentration ratio of guest molecule to host molecule defined by $[G]_0/[CD]_0$	[-]

η	=	rise ratio of rejection defined by $\{(R_G)_{CD}-R_G\}/\{R_{CD}-R_G\}$	[-]

<subscripts>

0	:	initial
CD	:	cyclodextrin
CDG	:	inclusion complex
F	:	feed
G	:	guest molecule
p	:	permeate
T	:	total
[ ]	:	concentration

Literature cited

1) Armstrong,R.D.: *ACS Symp. Ser.(Am. Chem. Soc.) USA*, **342**, 272-279 (1987)
2) Armstrong,D.W. and W.DeMond: *J. Chromat. Sci.*, **22**, 411-415 (1984)
3) Armstrong,D.W., L.A.Spino and S.M.Han: *J. Chromatography*, **411**, 490 (1987)
4) Bergeron,R.J., M.A.Channing, G.J.Gibeily and D.M.Pillor: *J. Amer. Chem. Soc.*,**99** (15), 5146-5151 (1977)
5) Cooper,A. and D.D.MacNicol: J.C.S. Perkin II, 760-763 (1978)
6) Cramer,K. ,W.Saenger and Hi-Ch.Spatz: *J. Amer. Chem. Soc.*, **89**(1), 14-20 (1967)
7) Debowski,J. and D.Sybilska: *J. Chromat.*, **353**, 409-416 (1986)
8) Formoso,C.: *Biopolymers*, **13**, 909-917 (1974)
9) Hall.E.S. and H.J.Ache: *J.Phy.Chem.*,**83** (14),1805-1807 (1979)
10) Hinze,W.L.: *Separation and Purification Methods*, **10**, 159-237 (1981)
11) Hirayama,F., K.Uekama, H.Koinuma: *Chem. Pharm. Bull.*, **28**, (7) 1975-1980 (1980)
12) Ishihara,K., H.Shuzuki and K.Matui: *Nihon Kagakukaishi*, **446** (1987)
13) Kawaguchi,Y.: *Anal Chem.*, **55**, 852 (1983)
14) Komiyama,S.: *Kaigai Koubunshi Kenkyuu*, **32**, 18 (1986)
15) Kokugan,T., T.Takada and K.Hara: *Kagaku Kogaku Ronbunshu*, **20** (2) 162-169 (1994)
16) Kokugan,T., T.Takada and M.shimizu: *Kagaku Kogaku Ronbunshu*, **20** (1) 124- 127 (1994)
17) Lewis,E.A. and L.D.Hansen: J.C.S. Perkin II, 2081-2085 (1973)
18) Miyaji,T., Y.Kurono, K.Uekama and K.Ikeda: *Chem. Pharm. Bull.*, **24** (6), 1155-1159 (1976)
19) Osa,T., T.Matsue and M.Fujihiro: *Heterocycles*, **6** (11), 1833-1839 (1977)
20) Shimizu,H., A.Kaito and M.Hatano: *Bull. Chem. Soc. Jpn.*, **52** (9), 2678-2634 (1979)
21) Siegel,B., and R.Breslow: *J. American Chem. Soc.*, **97:23**, 6869-6870 (1975)
22) Uekama,K. and F.Hirayama; *Chem. Phram. Bull.*, **26** (4), 1195-1200 (1978)
23) Uekama,K., F.Hirayama, K.Ikeda and K.Inaba: *J. Pharm. Sci.*, **66** (5), 706-710 (1977)
24) Uekama,K., F.Hirayama and T.Irie: *Chem. Lett.*, 661-664 (1978)
25) Uekama,K., F.Hirayama and H.Koinuma: *Chemistry Letters*, 1393-1396 (1977)
26) Uekama,K., F.Hirayama M.Otagiri, Y.Okagiri and K.Ikeda: *Chem. Pharm. Bull.*, **26** (4), 1162-1167 (1978)
27) Uekama,K., F.Hirayama and S.Yamasaki: *Chemistry Letters*, 1389-1392 (1977)
28) Uemasu.I.: *Sekiyu Gakkaishi*, **34**, 371-374 (1991)
29) Yamazuchi,T. and M.Matuura: Japanese Patent, 61-50603 (1986)