Inclusion Complex Formation of γ-Cyclodextrin. One Host-Two Guest Complexation with Water-soluble Dyes in Ground State

Hidefumi Hirai,* Naoki Toshima, and Satoshi Uenoyama
Department of Industrial Chemistry, Faculty of Engineering, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113
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β-Cyclodextrin forms the 1:1 inclusion complex with Crystal Violet, Methylene Blue, Methyl Orange, or Congo Red in water. On the other hand, γ-cyclodextrin forms the 1:2 complex with Crystal Violet, Methylene Blue, or Methyl Orange, while the 1:1 complex is given with Congo Red. The one host-two guest complexations are shown on the bases of the changes of the electronic spectra, dependence of induced circular dichroisms on the concentrations of dyes, and the continuous variation methods with induced circular dichroism. The dissociation constants for the 1:2 complexes are determined with the improved Benesi-Hildebrand equation. Alternation between the 1:1 and 1:2 complexation is discussed in terms of the cavity size of cyclodextrin and the aggregation character of dye.

Cyclodextrin, to be denoted here as CyD, forms the inclusion complex with a large number of organic or inorganic molecules and anions in an aqueous solution.[1,2] The internal diameters of hydrophobic cavities of α-CyD (cyclohexaamylose), β-CyD (cycloheptaamylose), and γ-CyD (cyclooctaamylose) are 4.5, 7.0, and 8.5 Å, respectively, and the depths of the cavities are given by the same value of 6.7—7.0 Å.[1] The inclusion complex of CyD has been vigorously investigated for a potential model of the enzyme in biomimetic chemistry[1,2] and also for the pharmaceutical application.[3] The stability of the inclusion complex depends mainly on the size and hydrophobicity of the CyD cavity. Since the cavity diameter of γ-CyD is nearly twice of that of α-CyD, γ-CyD seems to be able to include a large molecule. However, the stability of the complex of γ-CyD remains equivocal since the cavity diameter of γ-CyD is much larger than that of α- or β-CyD in spite of their common depths.

Recently, attentions have been paid for inclusion of two guest molecules in a cavity of CyD[4-14] from the viewpoint of conformational regulation of two molecules or as a model of CyD-catalyzed bimolecular reactions.[15,16] Ueno *et al.*[4] have shown the complexation of two molecules with one γ-CyD by enhancement of the excimer fluorescence. The enhancement gave the evidence for the inclusion of two molecules in the excited state, and gave the suggestion for the inclusion of two molecules in the ground state. We have separately communicated that a γ-CyD molecule forms the 1:2 complex in the ground state with three kinds of water-soluble dye molecules.[5] The present paper is a full paper of the previous letter. We would like to present here not only the detailed data but also the analytical methods supporting the 1:2 complexation.

Experimental

Materials. α- and β-CyD (Nakarai Chemicals) and dyes are used as received. γ-CyD used is also commercially available and its purity is more than 98%.

Sample Preparation. The aqueous solution of CyD or dyes are prepared by using the distilled water and phosphate buffer (pH=7.0, ion strength, μ=0.1). Stirring for 2 d at room temperature is performed for preparation of the aqueous solution of dye for the aggregated dye to come to equilibrium with its monomer form. The concentrations mainly used are 2.5×10^{-3} and 5×10^{-5} mol dm^{-3} for CyD and dyes, respectively.

Spectral Measurement. Electronic absorption spectra were measured by using a Hitachi 340 automatic spectrophotometer. Spectra of circular dichroism were obtained at 20°C with a JASCO J-20 automatic spectropolarimeter. The magnitude of circular dichroism induced by inclusion of a dye with CyD is presented by Eq. 1.

$$\theta_{tr} = \frac{c \cdot \Delta E_c}{x} \quad (1)$$

Here, θ_{tr}, ΔE_c, and x are the molar ellipticity of the complex, the circular dichroism measured, and the concentration of the complex, respectively. The term c is the constant. In the present paper, however, the initial concentration of dye is used instead of the concentration of the compelx, x, since x is unknown. Consequently, the apparent molar elipticity, θ, is used as the measure of the induced circular dichroism instead of θ_{tr}.

Determination of Dissociation Constant, K_d, for CyD–Dye Complex with Visible Spectral Measurement. The absorption spectra of dyes change with the concentration of CyD. For the 1:1 complex, the Benesi-Hildebrand equation can be applied for determination of K_d by plotting $(ab/\Delta E)$ against a, where a, b, and ΔE are initial concentrations of CyD and the dye, and difference of absorbance in the visible spectra, respectively. For the 2:1 complex, K_d must be determined from the intercept of the ordinate of the straight line obtained by a method of least squares for the plot of $(ab^2/\Delta E)$ against a. The details for providing the expression are shown in Appendix A.

Spectral Change Depending on the Concentration of Dyes. Induced circular dichroism changes with the concentration of dyes at the constant concentration of CyD. The measured magnitude of induced circular dichroism was plotted against the concentration of a dye under the conditions that the concentration of CyD is much larger than that of the dye. For the system of the 1:1 complexation, the straight line obtained passes through the point of the origin. For the 2:1 complex, on the other hand, no linear relationship exists between the concentration of dye and the magnitude of the induced circular dichroism as shown in Appendix B. Therefore, the linearities have been examined for four dyes by plotting ΔE_c of induced circular dichroism against the

concentration of the dye (5×10^{-6}—5×10^{-5} mol dm^{-3}) in order to determine the molar ratio of the inclusion complex.

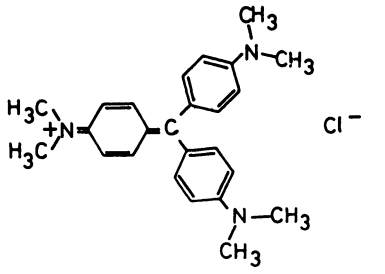
Continuous Variation Method. Continuous variation method was also applied to determine the molar ratio of the inclusion complex by assuming the equilibrium 2 as described in Appendix C.

$$n\text{·Dye} + \text{CyD} \rightleftharpoons \text{Complex} \quad (2)$$

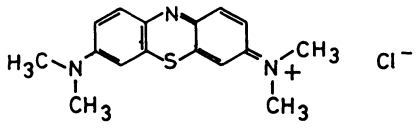
The kinds of aqueous solutions of CyD and the dye at various molar ratio were prepared with keeping the total concentration constant at 6×10^{-4} mol dm^{-3}. The apparent ellipticities, ΔE_c's, of the mixed solutions at different ratio were measured with a spectropolarimeter with a 1 mm-cell. The molar ratio with the maximum ellipticity indicates the ratio of the inclusion complex.

Results

Electronic Spectra and Induced Circular Dichroism in the Presence of CyD. Inclusions of Crystal Violet (**1**, CV), Methylene Blue (**2**, MB), Methyl Orange (**3**, MO), and Congo Red (**4**, CR) with CyDs have been examined in the aqueous solutions by the electronic spectra and the circular dichroisms. Generally, the absorption band of the guest molecule shifts by inclusion with CyD and the new circular dichroism is induced by the inclusion. Results are shown in Figs. 1—4.



CRYSTAL VIOLET (1)



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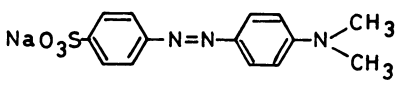
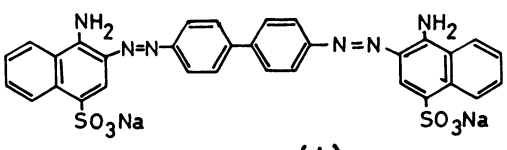


METHYL ORANGE (3)



CONGO RED (4)

The aqueous solution of CV at the concentration of 5×10^{-5} mol dm^{-3} had the absorption peak at 580 nm and no circular dichroism was observed. When γ-CyD were introduced to this solution at the concentration of 6.4×10^{-3} mol dm^{-3}, the absorption maximum shifted to 548 nm and the new circular dichroism appeared. Continuous change of the concentration of CyD indicated that the original absorption peak at 580 nm disappeared and the new peak at 548 nm appeared with increase of the concentration of CyD, as shown in Fig. 1a. The Silimar changes in the electronic spectra were observed in the case of MB and MO in the presence of γ-CyD (Figs. 2a and 3a). On the other hand, the small shift of the absorption band of CR was observed with addition of γ-CyD while the large circular dichroism were induced in this case (Fig. 4a).

When β-CyD was used as an additive instead of γ-CyD, the wavelength of the maximum absorption of CV shifted slightly to the longer wavelength (red shift by 5 nm) although γ-CyD provided large blue shift (32 nm). Similar small shift of the maximum wavelength as well as increase of the absorption coefficient was observed in the case of MB, MO, and CR with β-CyD as shown in Figs. 2b, 3b, and 4b, respectively. In these cases the circular dichroisms were also induced with the different shape from those with γ-CyD.

Addition of α-CyD resulted in no significant change in the electronic spectra of CV, MB, and CR and no circular dichroism were induced as well. In the case of MO, however, wavelength of the maximum absorption shifted from 462 nm to 454 nm with addition of α-CyD and the circular dichroism was induced. These effects of α-CyD on the spectra of MO is similar to those of β-CyD.

Change of Induced Circular Dichroism with Concentration of Dye. The magnitude of the induced circular dichroism increased with the concentration of the dye in the presence of large excess of CyD, so long as the circular dichroism was induced by addition of CyD. The apparent ellipticity of the solution was plotted against the concentration of the dye in the presence of 2.5×10^{-3} mol dm^{-3} of CyD as shown in Figs. 5 and 6. The linear relationships were clear in the cases of CR-γ-CyD (in Fig. 5) and MO-β-CyD (in Fig. 6), but the nonlinearly increasing curves were obtained in the cases of CV-γ-CyD, MB-γ-CyD, and MO-γCyD (Fig. 5).

Continuous Variation Method. Apparent ellipticity of the mixed solution of the dye and CyD was plotted against the molar fraction of the dye in the solution keeping the total concentration of the dye and CyD to be constant at 6.0×10^{-4} mol dm^{-3}. Here the fraction f is defined as molar ratio of dye to the sum of dye and CyD. In the solution of CR and γ-CyD, where

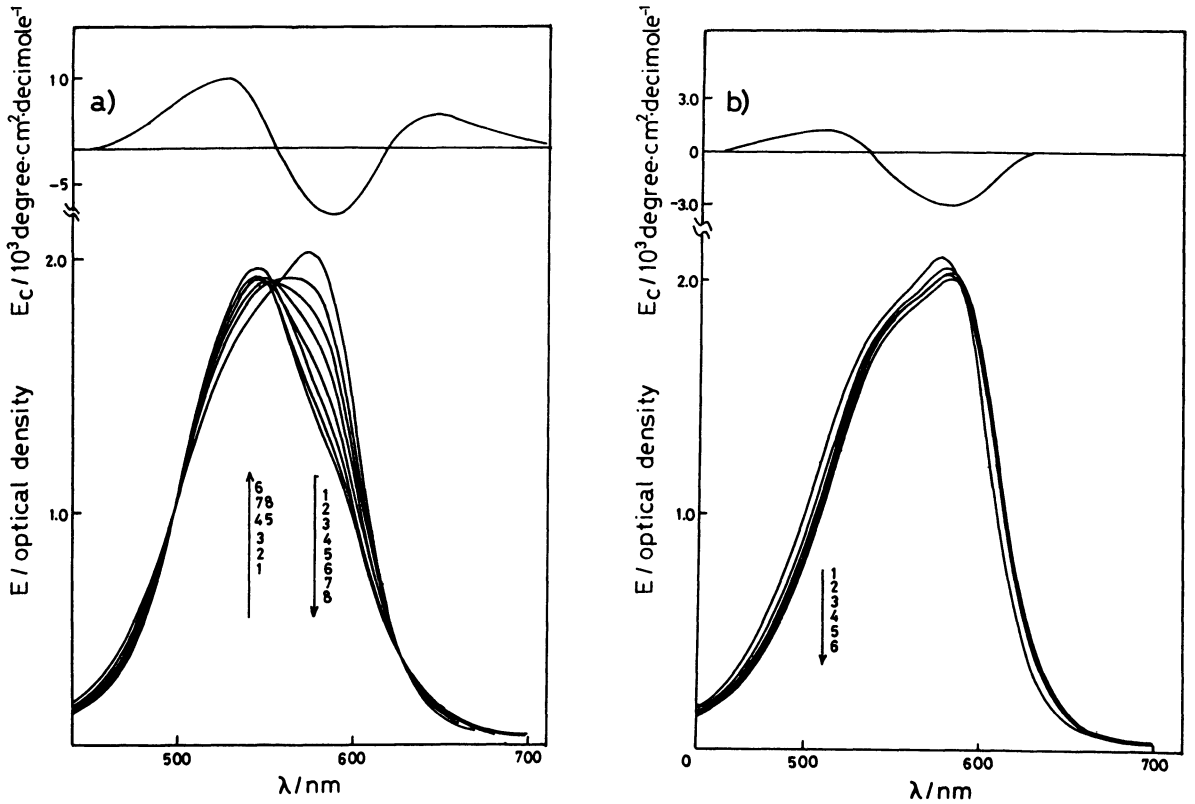


Fig. 1. Induced circular dichroism and absorption spectra of Crystal Violet (5×10^{-5} mol dm^{-3}) at various concentrations of γ-cyclodextrin (a: ICD, [γ-CyD]=2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.1; 3, 0.2; 4, 0.4; 5, 0.8; 6, 1.6; 7, 3.2; 8, 6.4×10^{-3} mol dm^{-3}) and β-cyclodextrin (b: ICD, [β-CyD]=2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.25; 3, 0.5; 4, 1.6; 5, 2.0; 6, 4.0×10^{-3} mol dm^{-3}).

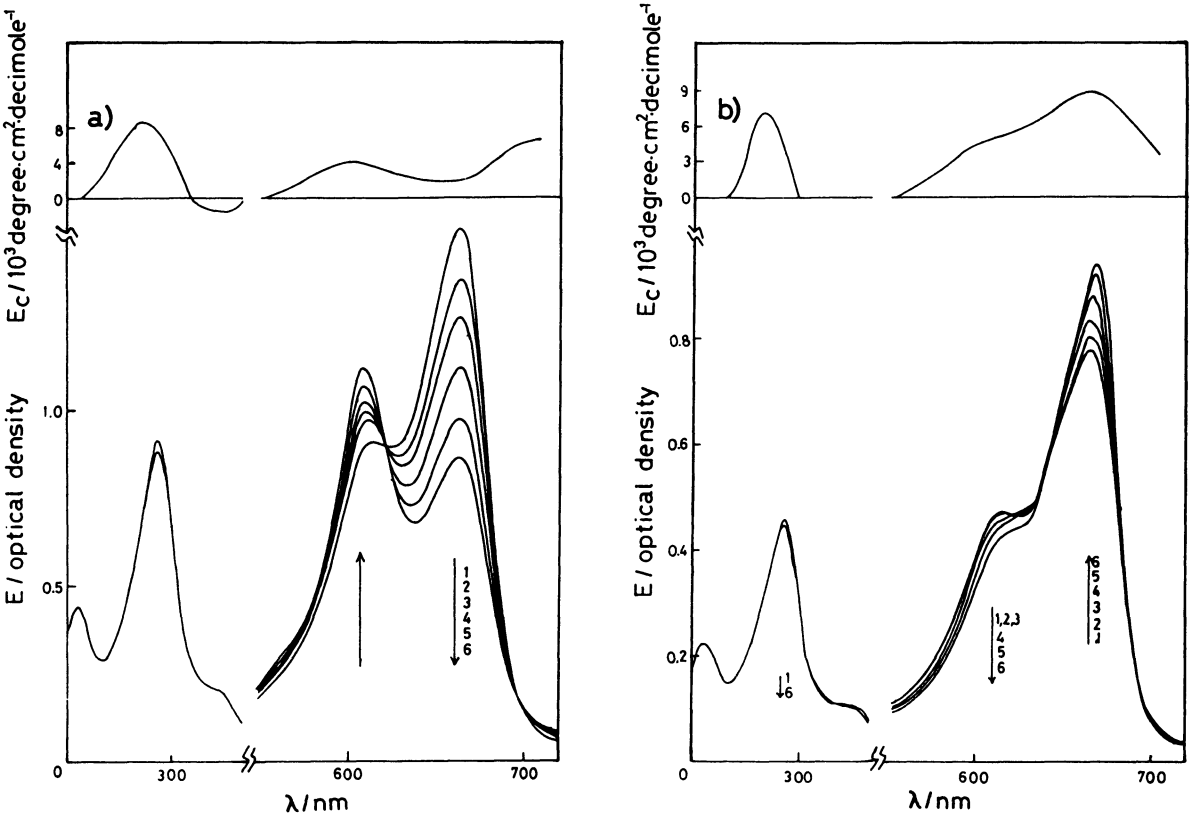


Fig. 2. Induced circular dichroism and absorption spectra of Methylene Blue (2.5×10^{-5} mol dm^{-3}) at various concentrations of γ-cyclodextrin (a: ICD, [γ-CyD]=2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.4; 3, 0.8; 4, 1.6; 5, 3.2; 6, 6.4×10^{-3} mol dm^{-3}) and β-cyclodextrin (b: ICD, [β-CyD]=2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.25; 3, 0.5; 4, 1.0; 5, 2.0; 6, 4.0×10^{-3} mol dm^{-3}).

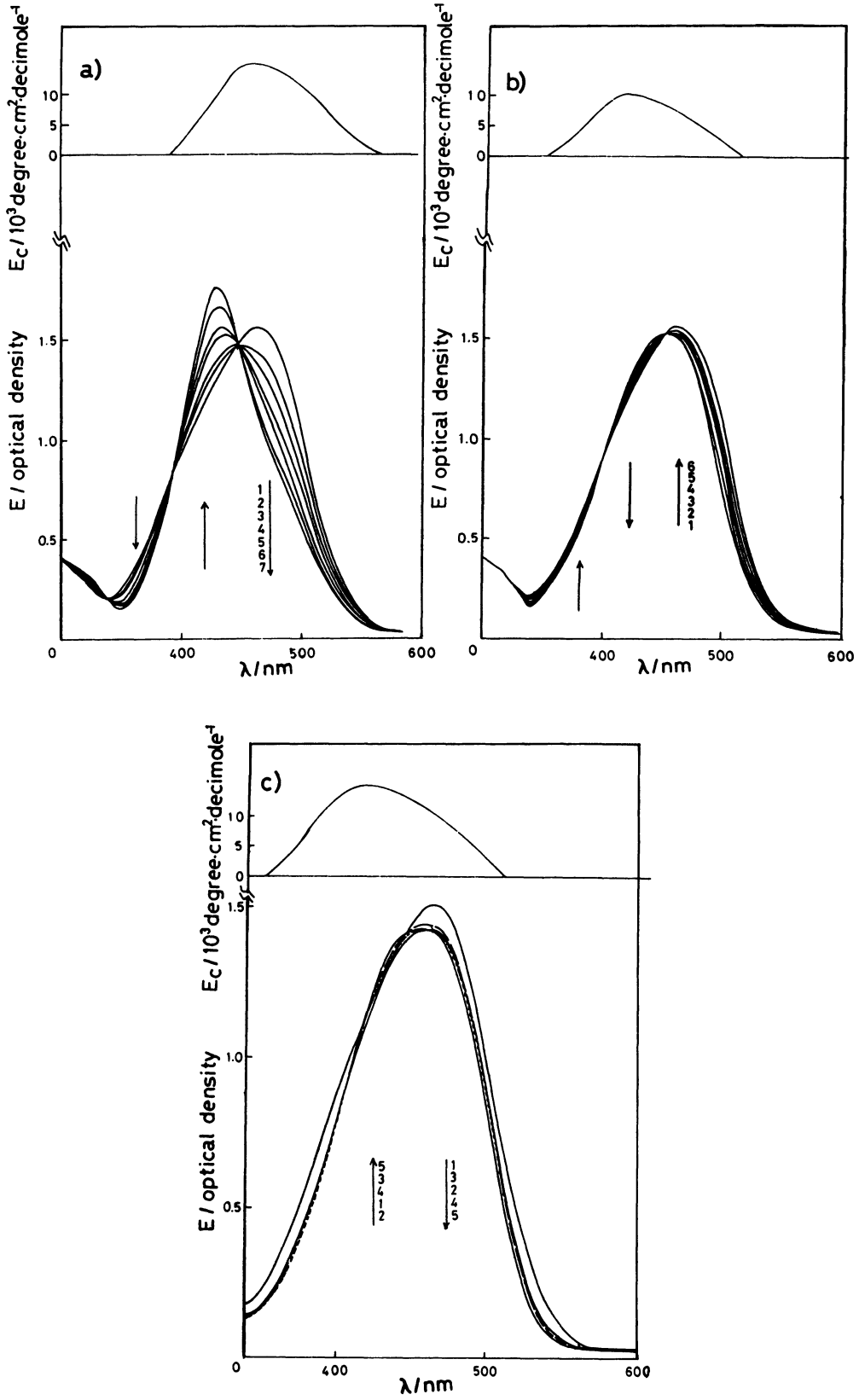


Fig. 3. Induced circular dichroism and absorption spectra of Methyl Orange (5×10^{-5} mol dm^{-3}) at various concentrations of γ-cyclodextrin (a: ICD, [γ-CyD]= 2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.25; 3, 0.5; 4, 1.0; 5, 2.0; 6, 4.0; 7, 8.0×10^{-3} mol dm^{-3}), β-cyclodextrin (b: ICD, [β-CyD]=2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.25; 3, 0.5; 4, 1.0; 5, 2.0; 6, 4.0×10^{-3} mol dm^{-3}) and α-cyclodextrin (c: ICD, [α-CyD]= 2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.25; 3, 0.5; 4, 2.0; 5, 4.0×10^{-3} mol dm^{-3}).

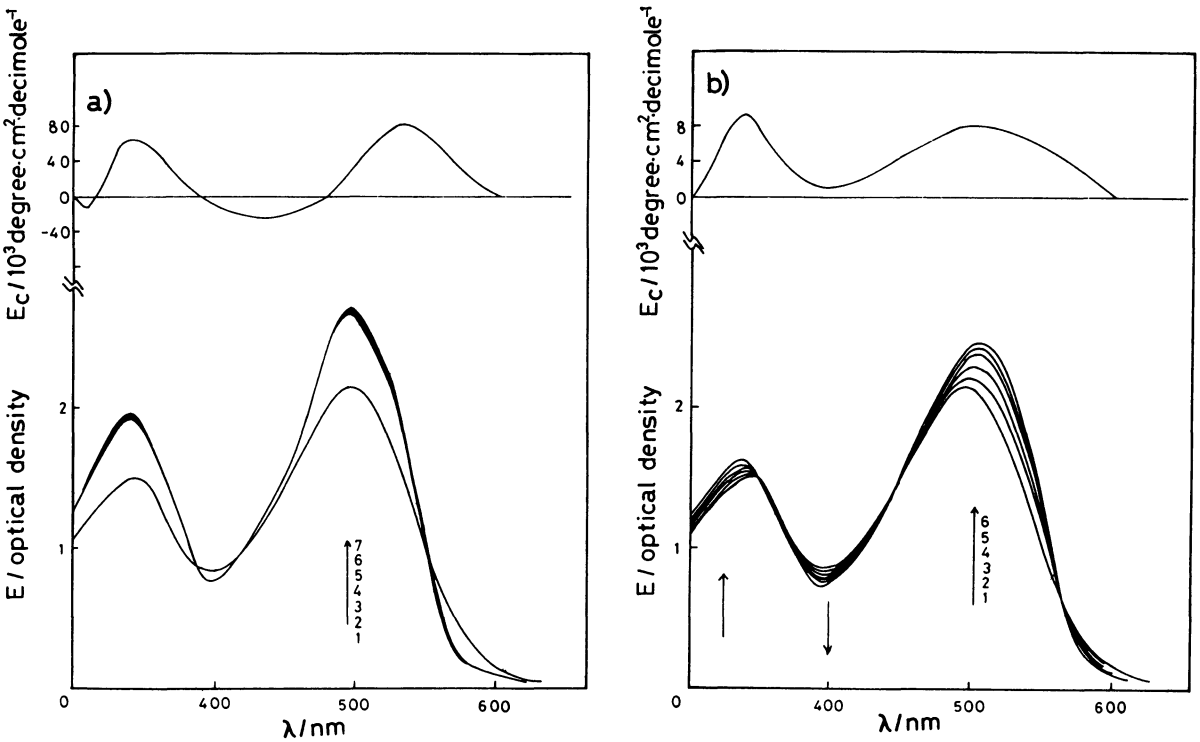


Fig. 4. Induced circular dichroism and absorption spectra of Congo Red (5×10^{-5} mol dm^{-3}) at various concentrations of γ-cyclodextrin (a: ICD, [γ-CyD]=2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.25; 3, 0.5; 4, 1.0; 5, 2.0; 6, 4.0; 7, 8.0×10^{-3} mol dm^{-3}) and β-cyclodextrin (b: ICD, [β-CyD]=2.5×10^{-3} mol dm^{-3}; AB, 1, 0.0; 2, 0.25; 3, 0.5; 4, 1.0; 5, 2.0; 6, 4.0×10^{-3} mol dm^{-3}).

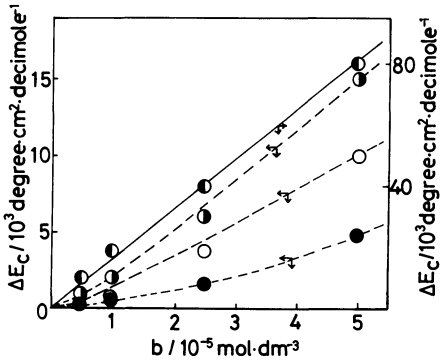


Fig. 5. The plot of the initial concentration of the dye (*b*) *vs.* the magnitude of circular dichroism (ΔE_c) induced by the complexes between the γ-cyclodextrin (2.5×10^{-3} mol dm^{-3}) and dyes (○, Crystal Violet, CD at 530 nm; ●, Methylene Blue, at 600 nm; ◑, Methyl Orange, at 456 nm; ◐, Congo Red, at 533 nm) in water. The broken curves follow the Eq. B-10, and the solid straight line follows the Eq. B-1.

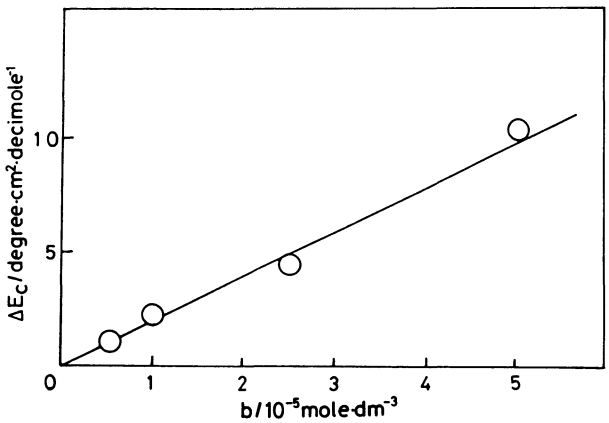


Fig. 6. The plot of the initial concentration of Methyl Orange (*b*) *vs.* the magnitude of circular dichroism (ΔE_c) induced by the Methyl Orange-β-cyclodextrin complex. [β-CyD]=2.5×10^{-3} mol dm^{-3} in water. ICD at 418 nm. The straight line follows Eq. B-1.

the linear relationship was shown in Fig. 5, the maximum ellipticity was obtained at the point of $f=1/2$ (Fig. 7), indicating the 1:1 complex formation of CR and γ-CyD. On the other hand, the peak was observed at $f=2/3$ in the case of the mixed solution of CV, MB, or MO (Fig. 7) with γ-CyD, suggesting the 1:2 complex formation of these three dyes with γ-CyD according to the Appendix C.

Dissociation Constant of the Inclusion Complex. The general Benesi-Hildebrand equation was applied to determine the dissociation constant of the 1:1 inclusion complex. For the complex of CV, MB, and MO with γ-CyD, the improved Benesi-Hildebrand equation shown in Appendix A was used for calculating the dissociation constant by assuming the 1:2 complex formation. These results are summerized in Table 1 as well as the electronic spectral data and the maximum apparent molar ellipticities of the circular dichroisms.

Discussion

Electronic Spectral Change of Dye by Addition of Cyclodextrin. Change in the electronic spectrum of

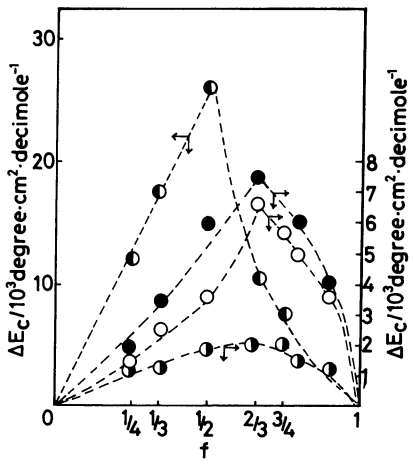


Fig. 7. Continuous variation method with induced circular dichroism (ΔE_c) for the complexes between γ-cyclodextrin and dyes (●, Crystal Violet, ◑, Methylene Blue, ○, Methyl Orange, ◐, Congo Red). [CyD]+[Dye]=6.0×10^{-4} mol dm^{-3}.

Table 1. Dissociation contants of the complexes between cyclodextrins and dyes[a)]

Dye	$K_d\times10^5$ [b)]	
	β-CyD	γ-CyD
Congo Red	60	5.7
Methyl Orange	26	0.0062[c)]
Crystal Violet	38	0.0046[c)]
Methylene Blue	110	0.019[c)]

a) [Cyclodextrin (CyD)]=2.5×10^{-3} mol dm^{-3}, [Dye]=5×10^{-5} mol dm^{-3} in H_2O. b) Dissociation constant of the 1:1 complex (mol dm^{-3}) except c). c) Dissociation constant of the 1:2 complex (mol^2 dm^{-6}).

a guest molecule by inclusion with CyD is generally explained by the effect of environment around the guest molecule, *i.e.*, so-called solvent effect. Among the present investigations, the small shifts, observed by addition of α- or β-CyD and by addition of γ-CyD to CR could take place due to the similar effect of the hydrophobic cavity of CyD. However, the spectral change observed by addition of γ-CyD to CV, MB, or MO is too large to be explained by the similar effect and must be caused by the completely different effect.

Dye can exist in a monomer form in a very dilute aqueous solution like 10^{-5} mol dm^{-3}. In a more concentrated solution, however, dye can aggregate each other. For example, the direct dye like CR forms the large ionic micelle,[17] whereas the basic dye like CV and MB stays as a stable dimer in a usual aqueous solution. The absorption peak of the dimer in an aqueous solution is well investigated. For example, the dimer peaks appear at 532 nm and 605 nm for CV and MB, respectively.[18,19] In the absence of CyD, CV, and MB stay mostly as a monomer form and some fractions as a dimer form in the present experimental conditions (5 or 2.5×10^{-5} mol dm^{-3}) as shown in curves 1 of Figs. 1 and 2. With addition of β-CyD, the absorbance at the wavelength of the dimer peak slightly decreases, as indicated in Figs. 1 and 2. On the other hand, the addition of γ-CyD increases the absorbance at the dimer peak and decreases the monomer peak dramatically to result in an appearance of a new peak. These spectral changes strongly suggest that γ-CyD includes the dimer forms of CV and MB while β-CyD does the monomer forms.

In case of MO, any sharp peak has not clearly been assigned as the dimer peak in literatures, but the peak at 462 nm has been reported to be the overlap peak of two bands of monomer and dimer forms.[20] The large blue shift observed with addition of γ-CyD is considered due to the inclusion of the species which have the absorption maximum at the shorter wavelength between the two species. Consequently, the unique change in the electronic spectra suggests that γ-CyD forms the 1:2 complex with MO as well as CV and MO.

Relationship between the Magnitude of the Induced Circular Dichroism and the Concentration of the Inclusion Complex. The dyes examined in the present paper do not indicate any circular dichroism by themselves. CyDs also have no circular dichroic absorption in the visible region. When the dye is included by CyD to form the inclusion complex, the circular dichroism is induced in the visible region where the complex has the electronic absorption. The magnitude of the induced circular dichroism is proportional to the amount of the complex produced. In case of the 1:1 complex formation, the amount of the complex is expected to increase with charged concentration of the dye. If the charged concentration of CyD is kept constant and is large excess compared with that of the dye, the linear relationship is expected as shown in Appendix B. Therefore, when the magnitude of the circular dichroism, ΔE_c, is plotted against the charged concentration of the dye, b, the linear relationship between ΔE_c and b is observed as shown in Eq. B-1 in Appendix B. Thus, the linear relationship is often used as the evidence of the 1:1 complex formation.[20] In the 1:n complex, however, ΔE_c is not proportional to b but the correlation is more complicated than that for the 1:1 complex as shown in Eq. B-10 of Appendix B.

The present results about the dependence of induced ellipticity on the initial concentration of the dye shown in Figs. 5 and 6 are well consistent with the above consideration. The linear relationship was observed indeed for the case of the 1:1 complex formation which had been suggested from the electronic spectral changes (solid lines in Figs. 5 and 6). Nonlinear relationship was observed for the case of the 1:2 complex formation (broken curves in Fig. 5). In the latter case, the induced ellipticity increased at the rate proportional to b^2 when the charged concentration of the dye, b, was small enough as estimated from Eq. B-10. In fact, the data plotted in Fig. 5 are considerably well fit to the broken curves calculated from Eq. B-10.

The 1:2 Complex Formation. Recently much attention has been paid to the inclusion of two guest molecules with the large cavity of γ-CyD. Enhance-

ment of excimer fluorescence by addition of γ-CyD has suggested the 1:2 complex formation.[4,6] However, no direct evidence for the 1:2 complexation had not been reported before the preliminary communication.[5] In the present investigation, the large blue shift in the electronic absorption spectrum and nonlinear relation between the ellipticity and the concentration of the dye suggest the complexation between two dye molecules (CV, MB, and MO) and one γ-CyD. The 1:2 complex formation is strongly supported by the continuous variation method by using the induced circular dichroism. As shown in Fig. 7, the maximum ellipticity was observed at the point that the molar fraction of the dye, f, equals to 2/3 for CV, MB, and MO. In the case of CR, the maximum appeares at f=0.5.

From the present results, CV, MB, and MO are concluded to form the 1:2 complex with γ-CyD, while CR results in the 1:1 complexation. These differences could be explicable from the viewpoint of aggregation character of the water-soluble dye, because the former three dyes are known to form the corresponding dimers by aggregation in water, while no report on dimer formation has been presented in case of CR. The fitness of the size of the guest molecules to the cavity of CyD is also an important factor for complexation, which will be discussed in the next section.

These observations could also suggest the complexation mechanism. The 1:2 complex could be produced not by including one more dye molecule with the 1:1 complex, but by directly including the dimer of the dye with γ-CyD (as Eq. A-3 in Appendix A).

Fitness of the Size for Inclusion Complex. The molecular size of the guest molecule has to fit to the size of the cavity of the host molecule for the inclusion complex formation.[1] Here the fitness of the molecular size is considered based on the CPK model.

Congo Red contains the naphthyl moiety having the large sulfonato group. The CPK model shows that CR is too large to pass through the small cavity of α-CyD. This result is consistent with the fact that CR cannot be included by α-CyD. In case of β-CyD, the CR molecule is still unable to be included completely within the cavity according to the CPK model. Thus, the induction of the circular dichroism in this case suggests the partial inclusion of CR by β-CyD.[21] On the other hand, γ-CyD can include a CR molecule completely. The sulfonato groups of CR could interact with the hydroxyl groups of γ-CyD and could lock the CyD molecule from both sides by a hydrogen bond. The model structure of the inclusion complex seems to suggest the very strong complex formation. This consideration is supported by the small dissociation constant observed for the complex of CR with γ-CyD.

Methyl Orange is an enough small molecule to be included with α-CyD.[22-24] The electronic spectra of MO with different concentrations of α-CyD have not an isosbestic point. This fact could be explicable by the simultaneous formation of both the 1:1 complex and the 2:1 complex (two molecules of α-CyD and one molecule of MO).[25-26] The CPK models of MO and CyDs suggest that β-CyD includes one molecule of MO, while γ-CyD does two molecules but never three molecules of MO. The apparent isosbestic point was observed in the electronic spectra of MO with different concentrations of γ-CyD as shown in Fig. 3a. This fact suggests the direct formation of a single complex, *i.e.*, the 1:2 complex of γ-CyD with MO.

With regard to MB and CV, the CPK molecular model indicates that (1) the cavity of α-CyD is too small to include these dyes, (2) β-CyD has an enough large cavity to include one molecule of MB or CV but not to include two molecules, and (3) the cavity size of γ-CyD does well fit to the two molecules of MB or CV. These demonstrations with a CPK model are completely consistent with the experimental results. The difference in the dissociation constants of CV and MB cannot arise from the molecular size, but from another character of the molecules.

Appendix A.

Improved Benesi-Hildebrand Equation for Determination of Dissociation Constant, K_d, of the 1:2 Inclusion Complex. The dissociation constant for the 1:1 complex has been determined by the Benesi-Hildebrand equation (A-1)[27] where a and b are the concentrations of CyD and the dye, respectively, l is the length of a cell, and ΔE and $\Delta\varepsilon$ present the differences of absorbance and molar absorption coefficient between the complex and the dye, respectively. It is assumed in the equation that all the dye molecules in the solution

$$\frac{a \cdot b \cdot l}{\Delta E} = \frac{K_d}{\Delta\varepsilon} + \frac{a}{\Delta\varepsilon} \qquad \text{(A-1)}$$

form the complexes with CyD if the concentration of CyD is large excess to that of the dye.

For the 1:2 complex, there are two way of formation. One case is the direct equilibrium between two molecules of the dye and a molecule of CyD (Eq. A-2) and the other is the coexistance of two equilibriums shown in Eq. A-3.

$$\mathrm{CyD} + 2\,\mathrm{Dye} \overset{K_d}{\rightleftharpoons} \mathrm{Complex} \qquad \text{(A-2)}$$

$$\left.\begin{aligned} 2\,\mathrm{Dye} &\overset{k_1}{\rightleftharpoons} (\mathrm{Dye})_2, \\ (\mathrm{Dye})_2 + \mathrm{CyD} &\overset{k_2}{\rightleftharpoons} \mathrm{Complex} \end{aligned}\right\} \qquad \text{(A-3)}$$

When a and b are used as the initial concentrations of CyD and the dye, respectively, and x is the concentration of the complex, the dissociation constant, K_d, for the former Eq. A-2, is presented by the following Eq. A-4.

$$K_d = \frac{(a-x)(b-2x)^2}{x} \qquad \text{(A-4)}$$

Even though the separate equations A-5 and A-6 exist instead of Eq. A-2 among these species, the overall dissociation constant from Eqs. A-5 and A-6 is the same with that of Eq. A-4.

$$\text{Dye} + \text{CyD} \rightleftharpoons (\text{Dye–CyD}) \quad \text{(A-5)}$$

$$\text{Dye} + (\text{Dye–CyD}) \rightleftharpoons (\text{Dye}_2\text{–CyD}) \quad \text{(A-6)}$$

In other words, the dissociation constant does not depend on whether the two molecules of the dye are included stepwise or at once.

On the other hand, the latter case (Eq. A-3) gives the little different results. The dissociation constants for dimer of the dye, k_1, and for the ternary complex, k_2, are given by the following by using y as the concentration of the dimer.

$$k_1 = \frac{(b-2y-2x)^2}{y}, \quad k_2 = \frac{y(a-x)}{x} \quad \text{(A-7)}$$

Thus, the over-all dissociation constant, K_d', is led as follows.

$$K_d' = \frac{(a-x)(b-2x-2y)^2}{x} \quad \text{(A-8)}$$

The difference between K_d and K_d' is very small since the concentration of the free dimer, y, is very small under the experimental conditions. Moreover, it is not so easy to determine the concentration of the free dimer of the dye or the dissociation constant, k_1. Therefore, K_d has been used in the present paper for the practical discussion. When a is much larger than b, $(a-x)$ and x can be replaced with a and $b/2$, respectively. So, the Eq. A-4 can be rewritten as follows:

$$K_d = \frac{ab^2}{x} - 4ab + 4ax = \frac{ab^2}{x} - 2ab \quad \text{(A-9)}$$

Since the concentration of the complex, x, is proportional to $\Delta E/(\Delta\varepsilon \cdot l)$, the Eq. A-9 can be rearranged as Eq. 10 like the Benesi-Hildebrand equation.

$$\frac{a \cdot b^2 \cdot l}{\Delta E} = \frac{K_d}{\Delta\varepsilon} + \frac{2b}{\Delta\varepsilon}a \quad \text{(A-10)}$$

Thus, the dissociation constant, K_d, for the 1:2 complex can be obtained from the intercept of the straight line by plotting $(a \cdot b^2 \cdot l)/\Delta E$ against a under the conditions that a is much larger than b and that b is constant.

Appendix B.

Nonlinear Relationship between the Concentration of Dye and the Induced Circular Dichroism for the 1:n *Complex.* The magnitude of induced circular dichroism changes with the concentration of the dye, when the concentration of CyD is much larger than that of the dye for the 1:1 complex, the linear relationship is given between ΔE_c and b according to Eq. B-1, where a and b are the initial concentration of CyD and the dye, respectively, and ΔE_c, θ_{tr}, and K_d are the measured magnitude of induced circular dichroism, the molar ellipticity of the complex, and the dissociation constant for the complex, respectively.

$$\Delta E_c = \frac{\theta_{tr}a}{100 \cdot (K_d + a)}b \quad \text{(B-1)}$$

For 1:n complex formation, the following equilibriums would be kept.

$$n\,\text{Dye} \overset{k_1'}{\rightleftharpoons} (\text{Dye})_n \quad \text{(B-2)}$$

$$(\text{Dye})_n + \text{CyD} \overset{k_2'}{\rightleftharpoons} \text{Complex} \quad \text{(B-3)}$$

Here,

$$k_1' = \frac{(b-ny-nx)^n}{y} \quad \text{(B-4)}$$

$$k_2' = \frac{y(a-x)}{x} \quad \text{(B-5)}$$

The term x and y present the concentrations of the CyD complex and of the n-mer of the dye, respectively.

When the concentration of CyD is much larger than that of the dye, ny is negligible small compared with b and nx. Therefore, the Eq. B-4 can be rewritten as Eq. B-6.

$$k_1' = \frac{1}{y}(b^n - {}_nC_1 \cdot nx \cdot b^{n-1} + {}_nC_2 \cdot (nx)^2 \cdot b^{n-2} - \cdots) \quad \text{(B-6)}$$

By using Eq. B-5, Eq. B-6 is rearranged as follows.

$$k_1' = \frac{(a-x)}{x \cdot k_2'}(b^n - {}_nC_1 \cdot nx \cdot b^{n-1} + {}_nC_2 \cdot (nx)^2 \cdot b^{n-2} - \cdots) \quad \text{(B-7)}$$

When the first and the second terms of the development equation are used, and $(a-x)$ is replaced with a since the concentration of CyD is very large, the overall dissociation constant for the CyD complex of the n-mer is presented as follows.

$$K_d'' = k_1' \cdot k_2' = \frac{a \cdot b^n}{x} - n^2 \cdot a \cdot b^{n-1} \quad \text{(B-8)}$$

Rearrangement of Eq. B-8 leads Eq. B-9.

$$x = \frac{a \cdot b^n}{K_d'' + n^2 \cdot a \cdot b^{n-1}} \quad \text{(B-9)}$$

Since the magnitude of the induced circular dichroism is proportional to the concentration of the complex, x can be replaced with $100 \cdot \Delta E_c/\theta_{tr}$. Then, Eq. B-9 can be rewritten as Eq. B-10.

$$\Delta E_c = \frac{\theta_{tr} \cdot a \cdot b^n}{100(K_d'' + n^2 \cdot a \cdot b^{n-1})} \quad \text{(B-10)}$$

One can compare Eq. B-10 for the 1:n complex with Eq. B-1 for the 1:1 complex to find the difference.

Appendix C.

Continuous Variation Method to Determine the Molar Ratio of the Inclusion Complex. When more than two equilibrium exist under experimental conditions, the continuous variation method is complicated. Therefore, one equilibrium (Eq. C-1) is considered in place of n equilibriums since the concentration of the intermediate $(\text{Dye})_n$ is thought negligible.

$$n\,\text{Dye} + \text{CyD} \rightleftharpoons \text{Complex} \quad \text{(C-1)}$$

The continuous variation method in the equilibrium C-1 is led as follows. When the concentrations of the dye, CyD, and the complex in the equilibrium are represented as c_1, c_2 and c_3, respectively, and the sum of the initial concentrations of the dye and CyD is constant equaling to m, then the following relations are obtained.

$$c_1 = m \cdot f - n \cdot c_3 \quad \text{(C-2)}$$

$$c_2 = m(1-f) - c_3 \quad \text{(C-3)}$$

$$K_d = \frac{c_1^n \cdot c_2}{c_3} \quad \text{(C-4)}$$

Here, f indicates the molar fraction of the dye.

The value f, that makes the concentration of the complex, c_3, maximum, can be obtained from the condition of $(dc_3/df)=0$. Differentiation of Eqs. C-2, C-3, and C-4 with f gives the following Eqs.

$$\frac{dc_1}{df} = m - n\frac{dc_3}{df} \quad \text{(C-5)}$$

$$\frac{dc_2}{df} = -m - \frac{dc_3}{df} \quad \text{(C-6)}$$

$$K_d \cdot \frac{dc_3}{df} = c_1^n \cdot \frac{dc_2}{df} + c_2 \cdot \frac{dc_1}{df} n \cdot c_1^{(n-1)} \quad \text{(C-7)}$$

Substitution of the condition $(dc_3/df)=0$ for the Eqs. C-5, C-6, and C-7, and rearrangement give the Eq. C-8.

$$n = \frac{f}{1-f} \text{ or } f = \frac{n}{1+n} \quad \text{(C-8)}$$

Therefore, if the concentration of the intermediate $(\text{dye})_n$ is assumed negligible, the concentration of the complex becomes maximum at the condition satisfying the Eq. C-8.

The fraction at the maximum induction of the circular dichroism observed gives the composition of the complex.

References

1) M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry," Springer-Verlag, New York, 1978; Japanese translation by H. Hirai and M. Komiyama, Japan Scientific Societies Press, 1979.

2) W. Saenger, *Angew. Chem. Int. Ed.*, **19**, 344 (1980).

3) K. Uekama, *Kagaku to Yakugaku no Kyoshitsu*, **59**, 43 (1978).

4) A. Ueno, K. Takahashi, and T. Osa, *J. Chem. Soc., Chem. Commun.*, **1980**, 921.

5) H. Hirai, N. Toshima, and S. Uenoyama, *Polymer J.*, **13**, 607 (1981).

6) K. Kano, I. Takenoshita, and T. Ogawa, *Chem. Lett.*, **1980**, 1035.

7) R. Breslow, M. F. Czaruiecki, J. Emert, and H. Hamaguchi, *J. Am. Chem. Soc.*, **102**, 762 (1980).

8) A. Ueno, K. Takahashi, Y. Hino, and T. Osa, *J. Chem. Soc., Chem. Commun.*, **1981**, 194.

9) N. Kobayashi, A. Ueno, and T. Osa, *J. Chem. Soc., Chem. Commun.*, **1981**, 340.

10) N. Kobayashi, R. Sato, Y. Hino, A. Ueno, and T. Osa, *J. Chem. Soc., Chem. Commun.*, **1982**, 706.

11) K. Kano, I. Takenoshita, and T. Ogawa, *Chem. Lett.*, **1982**, 321.

12) K. Kano, I. Takenoshita, and T. Ogawa, *J. Phys. Chem.*, **86**, 1833 (1982).

13) J. Emert, D. Kodali, and R. Catena, *J. Chem. Soc., Chem. Commun.*, **1981**, 758.

14) K. Takemura, S. Inoue, and F. Kusu, *Chem. Lett.*, **1983**, 233.

15) I. Tabushi, *Yuki Gosei Kagaku Kyokai Shi*, **40**, 892 (1982).

16) M. Komiyama and H. Hirai, *J. Am. Chem. Soc.*, **105**, 2018 (1983); **106**, 174 (1984).

17) S. Ando, "Development of Modern Colloidlogy," Vol. 1, p. 138 (1948) Gakujutsu Tosho.

18) W. H. J. Stock, G. J. M. Lippits, and M. Mandel, *J. Phys. Chem.*, **76**, 1372 (1972).

19) K. Bergmann and C. T. O'Konski, *J. Phys. Chem.*, **67**, 2169 (1963).

20) R. T. Reeves and R. S. Kaiser, *Can. J. Chem.*, **51**, 628 (1973).

21) K. Takeo and T. Kuge, *Stärke*, **24**, 281 (1972).

22) K. Harata, *Bull. Chem. Soc. Jpn.*, **49**, 1493 (1976).

23) K. Yamada and J. Morimoto, *Research Report of Chiba Univ.*, **24-47**, 71 (1973).

24) W. Brocer and W. Lantsch, *Z. Naturforsch.*, **86**, 711 (1953).

25) F. Cramer, W. Saenger, and H.-Ch. Spatz *J. Am. Chem. Soc.*, **89**, 14 (1967).

26) R. L. VanEtten, J. F. Sebastian, G. A. Cloorves, and M. L. Bender, *J. Am. Chem. Soc.*, **89**, 3242 (1967).

27) T. Fujinaga, *Kagaku (Kyoto)*, **26**, 91 (1970).