Spectroscopic Studies on Molecular Recognition of Modified Cyclodextrins

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Abstract: This review describes the spectroscopic studies on molecular recognition of a wide variety of guest molecules with native and modified cyclodextrins, including mono-modified cyclodextrins, and functional group-bridged bis(cyclodextrin)s. Steady-state and time-resolved fluorescence spectroscopy, circular dichroism spectroscopy, UV-vis spectroscopy, and two-dimensional NMR spectroscopy can be taken to determine the absolute conformation and inclusion complexation behavior of native and modified cyclodextrins. To investigate quantitatively molecular recognition of modified cyclodextrins, different spectrophotometric titration and/or competitive inclusion approach are used to determine the binding constant of inclusion complexes of various guest molecules with native and modified cyclodextrins.

1. INTRODUCTION

Supramolecular chemistry is a promising newly founded field [1], in which molecular recognition [2,3,4,5] is of current interest, and is fundamental basis of molecular assembly and its function. Molecular recognition is the selective binding of the host (receptor) compound to the guest (substrate) molecules through the simultaneous operation of several weak intermolecular non-covalent forces, involving dipole-dipole (ion), hydrophobic, electrostatic, van der Waals, and hydrogen-bonding

Therefore, at present, there are lots of techniques to measure the molecular recognition in supramolecular systems, such as, nuclear magnetic resonance (NMR), (micro)calorimetry, ultraviolet-visible spectroscopy, fluorescence (steady-state and time-resolved), circular dichroism, infrared, and solubility measurement [6].

Cyclodextrins are cyclic oligosaccharides comprising of six, seven, or eight (1 4)-linked glucopyranose units (-, -, or -cyclodextrin, respectively). The oligosaccharide ring forms a torus, whose openings are not identical; the primary

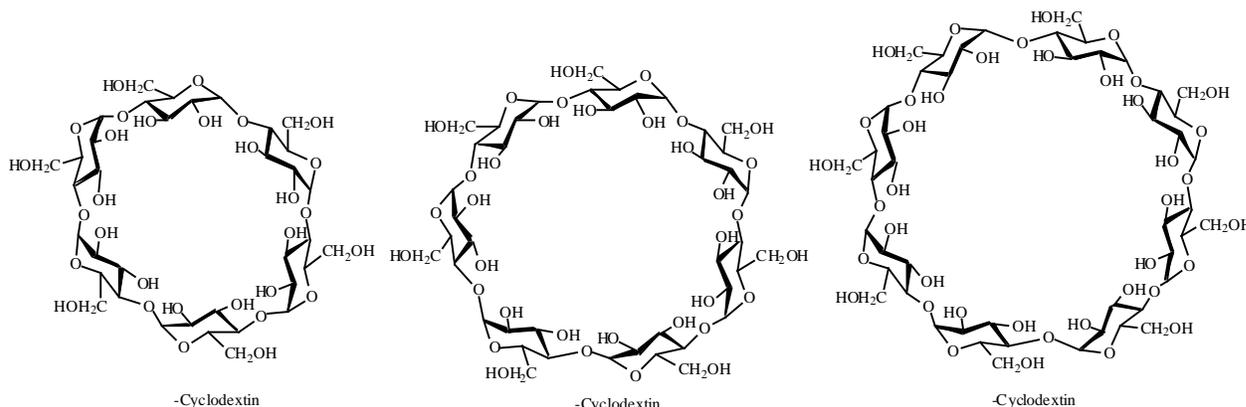**Fig. (1).** Schematics of -, -, and -cyclodextrins.

interactions on the basis of size/shape-fit concept. During the molecular recognition process, besides the several interactions in the host-guest systems mentioned above, the solvation, environment, and conformation for both the host compounds and guest molecules will definitely change. This certainly leads to some accompanying changes in thermodynamics and/or spectroscopy upon the host-guest complexation, on which people can base to determine or estimate the complex stability constant and corresponding thermodynamic parameters.

hydroxy groups on C-6 of the glucose residues lie on the narrow side, while the wider opening contains the secondary hydroxyl groups on C-2 and C-3. The inner surface of the toroidal cavity is hydrophobic; whereas, the outer surface is hydrophilic. Figure 1 schematically shows the structures of -, -, and -cyclodextrins. The strong binding affinity of cyclodextrins for hydrophobic molecules in aqueous media makes them naturally occurring receptors for organic molecules, inorganic molecules, and biological molecular substrates [7,8,9,10,11]. During the past decades, lots of modified cyclodextrins have been prepared to attempt to enhance their binding ability and/or the molecular selectivity or enantioselectivity [12,13].

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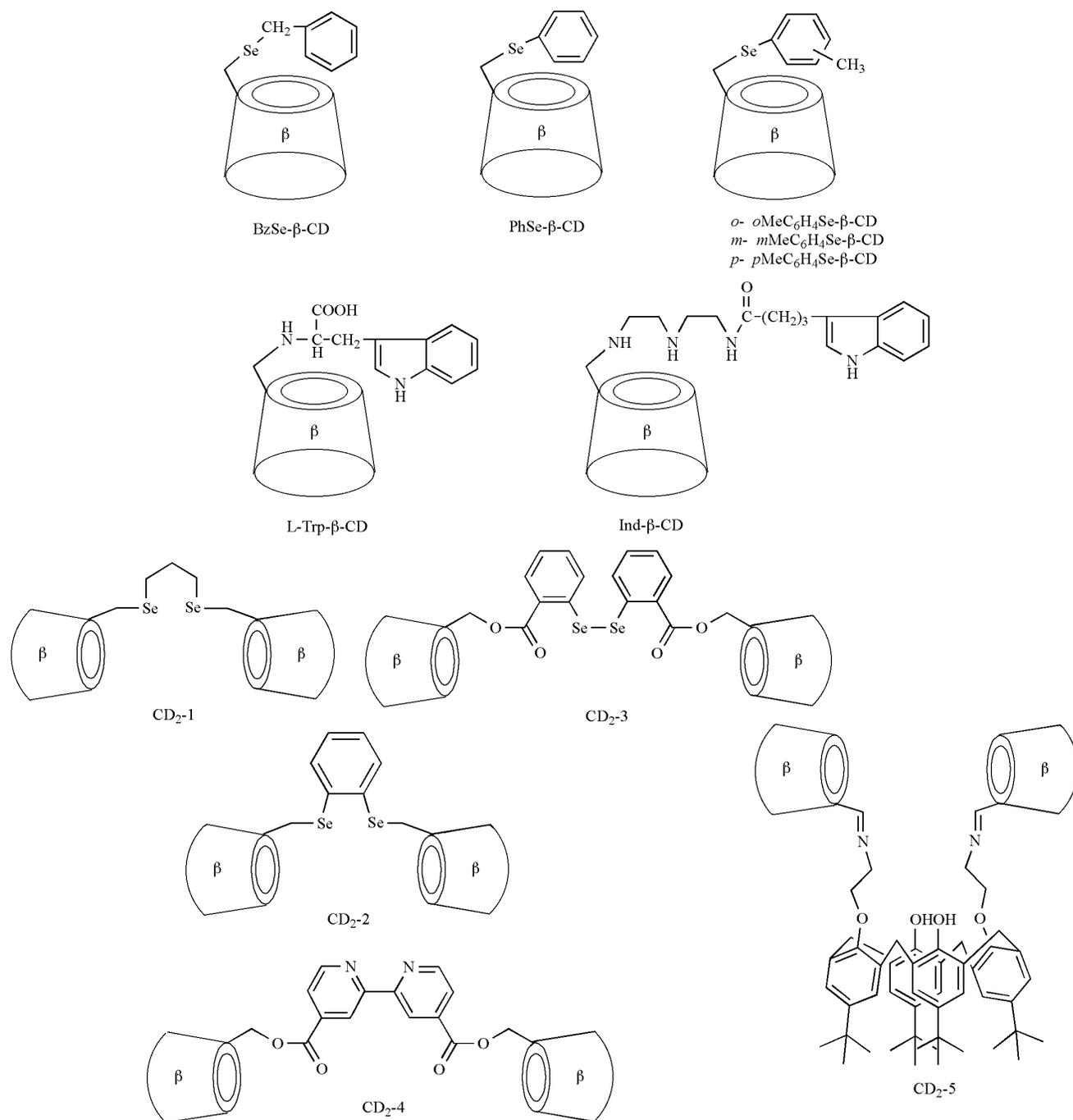
BzSe-β-CD   PhSe-β-CD

*o- o*MeC$_6$H$_4$Se-β-CD
*m- m*MeC$_6$H$_4$Se-β-CD
*p- p*MeC$_6$H$_4$Se-β-CD

L-Trp-β-CD   Ind-β-CD

CD$_2$-1   CD$_2$-3

CD$_2$-2

CD$_2$-4   CD$_2$-5

Fig. (2). Selected Cyclodextrin Derivates.

Calorimetric titration [14,15,16,17,18,19,20] and NMR techniques [21] are general methods which could apply to almost any systems of cyclodextrin hosts and various guests. However, due to the expensive instruments and special expertise are needed, theses techniques, sometimes, are not the first choice to investigate the molecular recognition in supramolecular systems involving cyclodextrins. Thus, spectrophotometric titration is a good alternative approach. However, there certainly are some limitations in these spectrometric methods, i.e. there should be some responsive group to a specific technique in either the cyclodextrin derivatives or the guest molecules, although the competitive inclusion method could overcome the limitations [22,23,24,25,26]. The data process of the spectrophotometric titration mainly includes approximate linear approach [27,28,29] and nonlinear curve-fitting method [30,31,32,33,34]. In this review, we will concentrate on the conformation analysis on self-inclusion and guest-inclusion involving various cyclodextrin derivatives (some selected ones are shown in Figure 2) and a wide range of guest molecules (Figure 3), and spectroscopic studies on the molecular recognition.
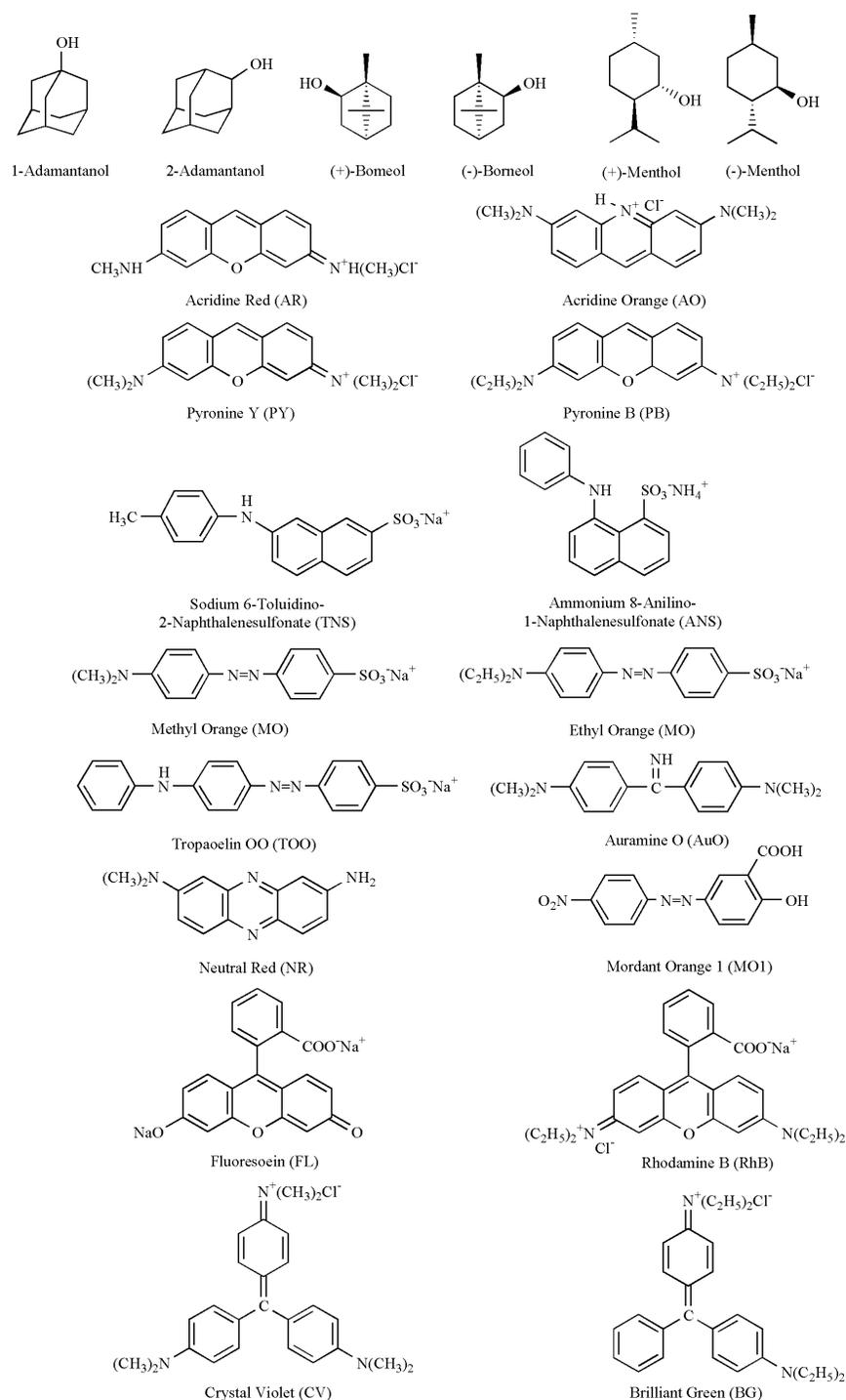
Fig. (3). Representative Guest Molecules.

2. CONFORMATION ANALYSIS ON GUEST-INCLUSION OR SELF-INCLUSION OF CYCLO-DEXTRIN DERIVATIVES

The molecular structures of cyclodextrins are shows in Figure **4**. In the NMR technique, [21] when guest molecules include into the hydrophobic cavity of cyclodextrin, the changes in the chemical shift for C3-H and C5-H, as well as the proton in the guest molecule occur upon the interaction of the guest molecule and the cyclodextrin. From comparing the changes in chemical shifts for C3-H and C5-H, it is easy

to determine the inclusion orientation, i.e. the guest molecules were included from the primary side or the secondary side of the cyclodextrins. If the substitutent group self-included into the hydrophobic cavity of cyclodextrins, it is also easy to observe from the chemical shift changes of related proton in the host and/or the guest molecules. In general, if the spacer linking group between the substitutent moiety and cyclodextrin ring is not long enough, the intramolecular self-inclusion is from the primary side. However, the substitutent group certainly can include into
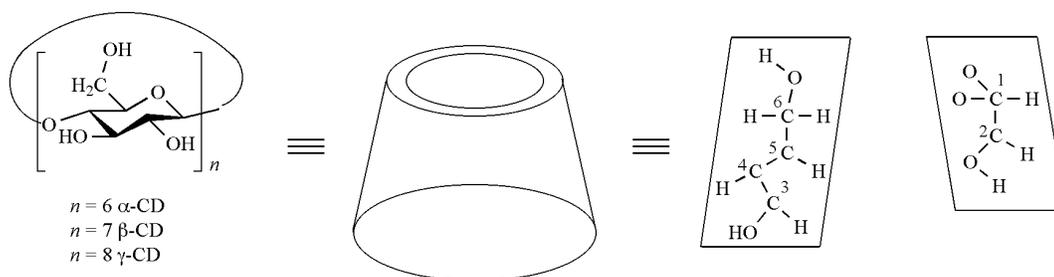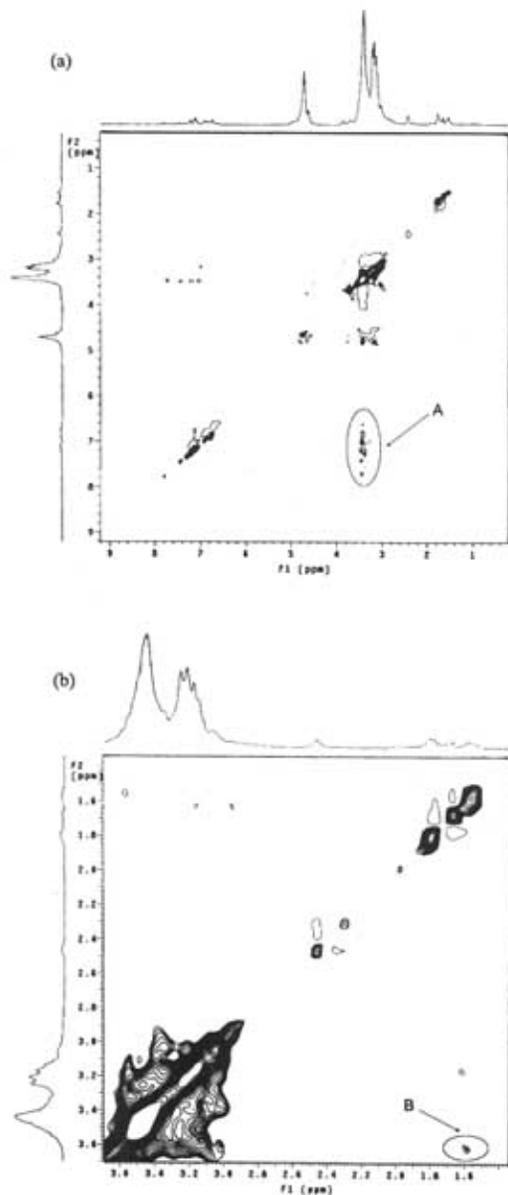
n = 6 α-CD
n = 7 β-CD
n = 8 γ-CD

Fig.

Fig. (5). [1]H NOESY spectra (300 MHz) of Ind- -CD in buffer solutions in D_2O at pH = 7.20 (a) and pH = 2.00 (b) with a mixing time of 800 ms.

the hydrophobic cavity of another cyclodextrin ring [35]. Furthermore, two-dimensional NMR techniques could give more clear evidences [36,37,38,39]. For instance, Figure **5** shows a couple of representative ^{1}H NOESY spectra of Ind-

-CD in buffer solutions at pH 7.20 and 2.00, respectively [38]. As illustrated in Figure 5a, the NOESY spectrum of Ind- -CD displays clear NOE cross-peaks between the C5-H of cyclodextrin and aromatic protons of the indolyl group in compound Ind- -CD (peaks A), which distinctly indicates that the indolyl group in Ind- -CD is shallowly self-included in the cavity from the primary side of cyclodextrin. In contrast, no cross-peaks were observed between aromatic protons of the indolyl group and the C5-H and C3-H protons of cyclodextrin in the NOESY spectrum of Ind- -CD in a buffer solution at pH 2.00, shown in Figure 5b, however, a NOE cross-peak between the C5-H of cyclodextrin and the -proton in the alkyl chain of the indol-3-ylbutyric substituent signifies that the alkyl chain in Ind- -CD is partially included in the cavity while the indolyl group is exposed outside. Therefore, the indolyl substituent switches between the inside form and the outside form upon the change in the pH value of the system, in which the protonation/deprotonation of the indolyl moiety plays a key role.

If the guest molecules are UV-visible responsive, when it includes into the hydrophobic cavity of cyclodextrin, the absorbance generally decreases. If the substitutent chromophoric group intramolecularly self-includes or intermolecularly includes into the hydrophobic cavity of cyclodextrin, the absorbance of the system will gradually increase upon the addition of guest molecule, due to the formation of complexes of the guest molecules and cyclodextrins, the guest molecules undoubtedly expel the included substitutent chromophoric group gradually.

When a fluorophoric guest molecule included into the hydrophobic cavity of cyclodextrin derivatives, the fluorescence intensity shows remarkable enhancement. However, for the cyclodextrin derivatives possessing a fluorophoric subsitutent, which could self-include into its cavity, the fluorescence intensity will decrease upon the addition of guest molecule.

Time-resolved fluorescent measurements prove the self-inclusion and the fluorescence weakening effect upon the complex formation of guest molecules with the hydrophobic cavity. Figures **6**, **7**, and **8** show time-resolved fluorescence decay measurements for L-Trp- -CD involving systems. The decay curve for L-Trp- -CD in the presence and absence of guest adamantanols could not be fitted to a single exponential function, but could be fitted well to a linear combination of two exponential functions. In contrast, the decay curve of L-tryptophan in aqueous solution gave a good fit to a single exponential function. The two-component decay observed for L-Trp- -CD in the presence and absence of guest molecules indicates that the indolyl moieties of the
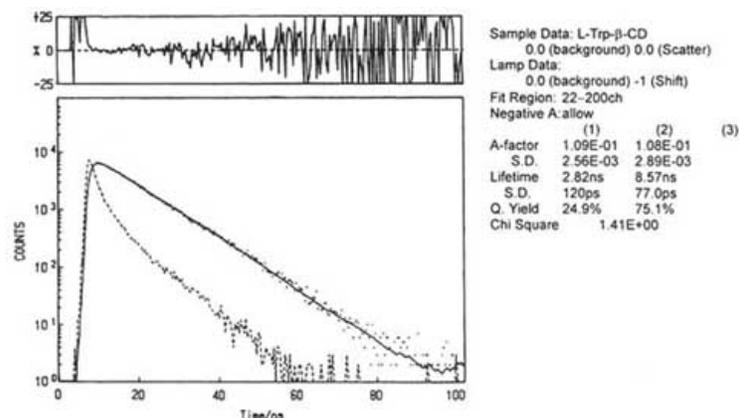
Fig. (6). The lifetime measurement of L-Trp- -CD in phosphate buffer solution at pH 7.20.
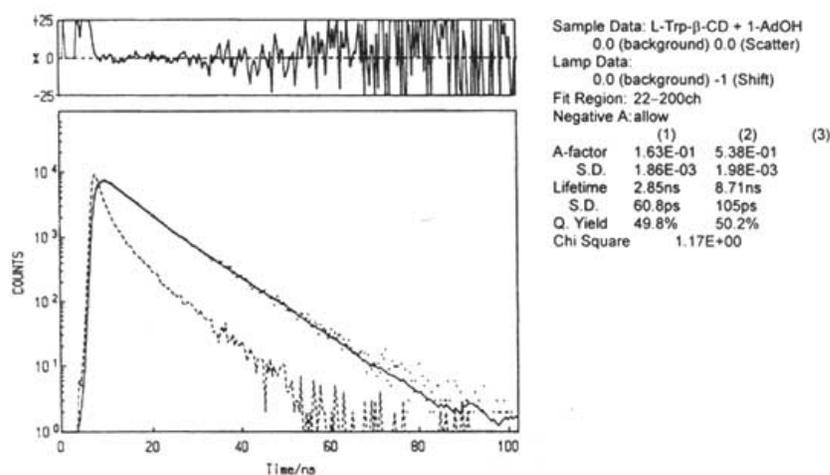

Fig. (7). The lifetime measurement of L-Trp- -CD in the presence of 1-adamantanol in phosphate buffer solution at pH 7.20.
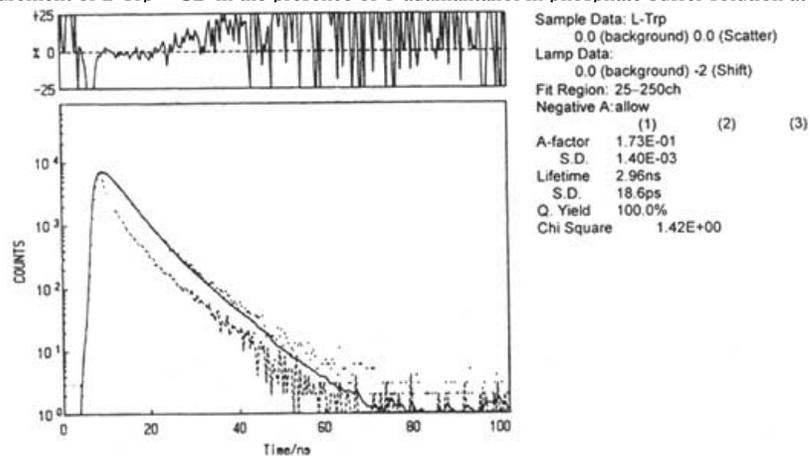

Fig. (8). The lifetime measurement of L-Trp in phosphate buffer solution at pH 7.20.

L-tryptophan residue are located in two different environments, one of which is polar and the other non-polar, and also that the interconversion of the two species is much slower than the fluorescence decay, which occurs on the ns time scale. The shorter lifetimes indicate that those indolyl moieties are exposed to the bulk aqueous solution; whereas, the longer fluorescence lifetimes for L-Trp- -CD in the presence and absence of guest adamantanols show that the indolyl moieties are located in an identical hydrophobic environment, i.e. within the cyclodextrin cavity. With compared to the relative quantum yields listed in Figures 6, 7, and 8, it is clear that the guest adamantanol competes with the self-included indolyl moiety for the cyclodextrin cavity and expels the indolyl moiety from the cavity, resulting in the increase of the $_s$ component. Figure **9** schematically shows the complexation mechanism, i.e. the free and self-included indolyl moieties are in a dynamic equilibrium and the addition of a guest leads to further equilibrium with the inclusion complex [34,36,40 ,41].
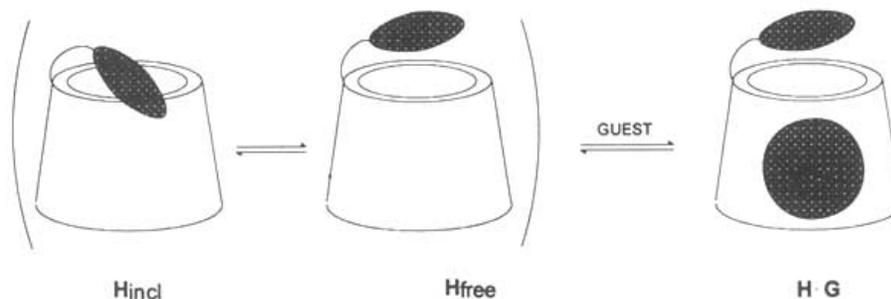
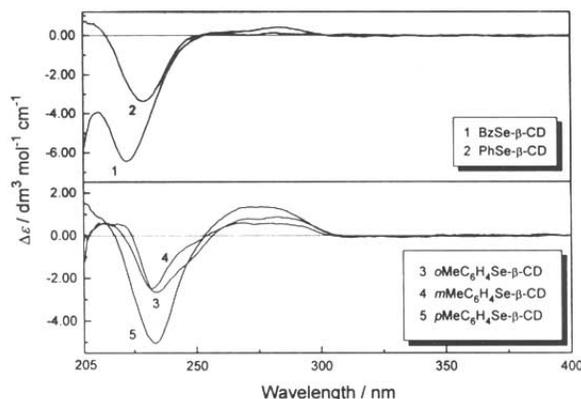**Fig. (9).** Schmatics for self-inclusion and guest-inclusion complexation.

Fig. (10). The circular dichroism of organoselenium modified -cyclodextrins in phosphate buffer solution (pH 7.20) at 25 ˚C.
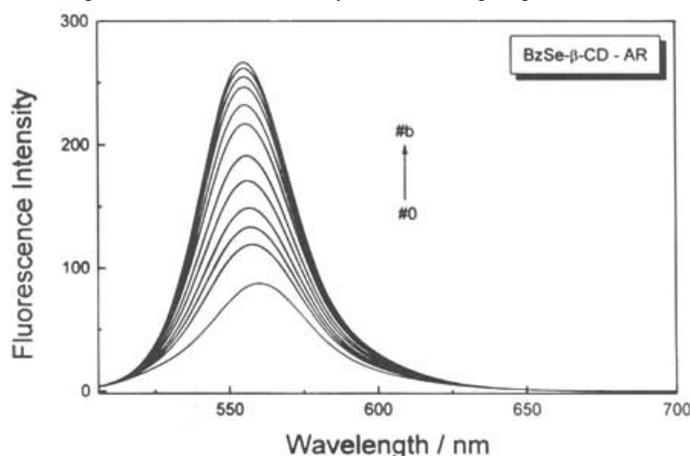

Fig. (11). Fluorescence spectra of acridine red dye (AR) in the presence and absence of -cyclodextrin derivative (BzSe- -CD) in aqueous citrate buffer solution (pH 6.00) at 25.0 ˚C: the fluorescence intensity increases upon the addition of the host cyclodextrin derivative.

Achiral organic compounds can show an induced circular dichroism signal in the corresponding transition band in cases where there is a chiral microenvironment. Cyclodextrins, which possess inherent chiral cavities, may provide such a microenvironment for the included achiral chromophore. Therefore, guest molecules or substitutent groups are included or self-included into the cyclodextrin cavity, induced circular dichroism could be observed [28,29,30,34,42]. According to the sector rule [42], the inclusion mode and orientation of the guest molecules or substitutent groups could be determined via the sign and intensity of Cotton effect. Figure **10** shows the circular dichroism of several organoselenium modified -cyclodextrins in phosphate buffer solution at pH 7.20. A strong negative Cotton effect peak, corresponding to the ^1L_a band around 230 nm and a weak positive Cotton effect for the ^1L_b band were observed, which indicates that the aromatic moiety penetrates shallowly into the hydrophobic cavity of cyclodextrin. Furthermore, the induced circular dichroism intensity regularly changes upon the gradual addition of guest molecules.

3. DETERMINING THE BINDING CONSTANT BY SPECTROPHOTOMETRIC TITRATION

3.1 Spectrofluorometric Titration

In a spectrofluorometric titration of a fluorophoric guest molecule with a host compound, e.g. cyclodextrin derivative, the concentration of fluorophore guest is kept constant, while the host compound is added gradually, resulting in regular changes in the fluorescent intensity of the host-guest system. Typically, Figures **11** and **12** show the fluorescence spectra
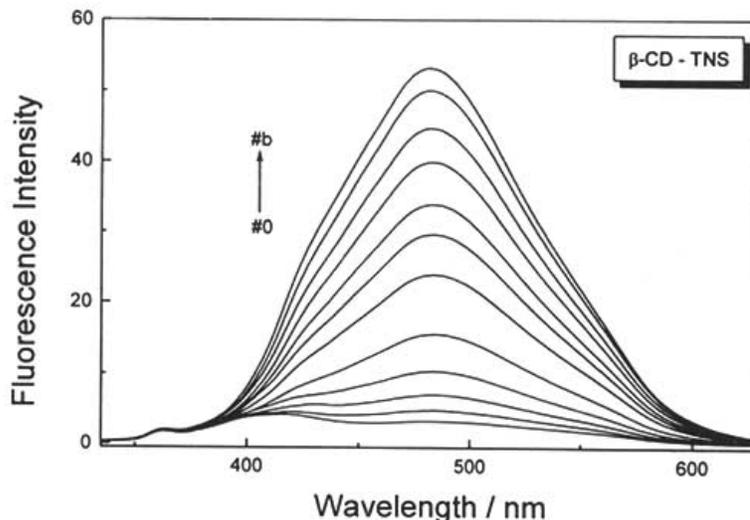
Fig. (12). Fluorescence spectra of sodium 6-toluidino-2-naphthalene sulfonate (TNS) in the presence and absence of -cyclodextrin (-CD) in aqueous citrate buffer solution (pH 6.00) at 25.0 °C: the fluorescence intensity increases upon the addition of -cyclodextrin.

of acridine red dye in the presence and absence of -cyclodextrin derivative (BzSe- -CD), as well as sodium 6-toluidino-2-naphthalene sulfonate (TNS) in the presence and absence of -cyclodextrin (-CD) in aqueous citrate buffer solution at pH 6.00.

For the spectrofluorometric titration, assuming a 1:1 stoichiometry, the inclusion complexation of a fluorescent guest molecule (G) with a host compound (H) is expressed by eq 1, and the complex stability constant (K_s) is given by eq 2.

$$H + G \underset{}{\overset{K_s}{\rightleftharpoons}} H \cdot G \tag{1}$$

$$K_s = \frac{[H \cdot G]}{[H] \cdot [G]} \tag{2}$$

where [H], [G], and [H·G] represent the equilibrium concentration of the host, the guest, and the formed complex, respectively.

The fluorescence intensity (F) is proportional to the concentration of the fluorophore (c) in dilute solution (eq 3), [43]

$$F = \varepsilon_F c \tag{3}$$

From eq 3, we can obtain the following equations. (In this case, the guest is the fluorophore).

$$F_0 = \varepsilon_F [G]_0 \tag{4}$$

$$F = \varepsilon_F [G] + \varepsilon_F'[H \cdot G] = \varepsilon_F [G]_0 + (\varepsilon_F' - \varepsilon_F)[H \cdot G] \tag{5}$$

where $[G]_0$ signifies the initial concentration of the guest fluorophore, ε_F and ε_F represent the molar fluorescence intensity of the free guest and the complexed guest, i.e. the formed complex (H·G).

Substracting eq 4 from eq 5, we obtain,

$$\Delta F = F - F_0 = (\varepsilon_F' - \varepsilon_F)[H \cdot G] = \Delta \varepsilon_F [H \cdot G] \tag{6}$$

where F and $_F$ denote the changes in the fluorescence intensity and molar fluorescence intensity of guest molecule upon complexation with host compounds.

We also have the following equations,

$$[H] = [H]_0 - [H \cdot G] \tag{7}$$

$$[G] = [G]_0 - [H \cdot G] \tag{8}$$

where the initial concentrations were designated $[H]_0$ and $[G]_0$ for the host and the guest, respectively.

Combining eqs 2, 6, 7 and 8, the following equation can be derived,

$$\Delta F'^2 - \Delta \varepsilon_F ([H]_0 + [G]_0 + \frac{1}{K_s}) \Delta F + \Delta \varepsilon_F^2 [H]_0 [G]_0 = 0 \tag{9}$$

and solving eq 9 for F, we can obtain,

$$\Delta F = \frac{\Delta \varepsilon_F ([H]_0 + [G]_0 + \frac{1}{K_s}) \pm \sqrt{\Delta \varepsilon_F^2 ([H]_0 + [G]_0 + \frac{1}{K_s})^2 - 4 \Delta \varepsilon_F^2 [H]_0 [G]_0}}{2} \tag{10}$$

Using the nonlinear curve-fitting approach, the complex stability constant (K_s) and the change in molar fluorescence intensity ($_F$) were determined according to eq 10 using the data of F observed at each initial host concentration $[H]_0$. Good curve-fitting plots further verify the 1:1 complex stoichiometry for each host–guest system. Typical curve-fitting plots are shown in Figure **13** for several systems.

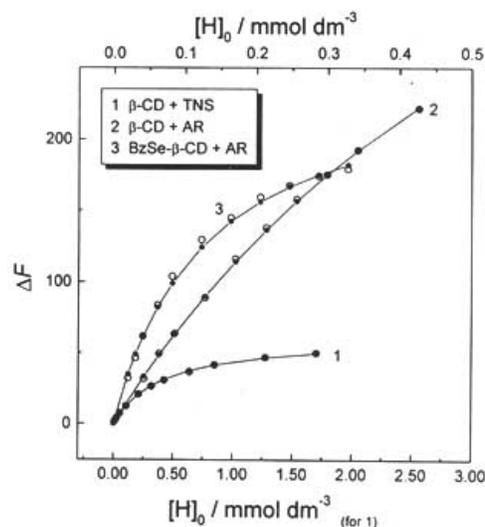**Fig. (13).** Curve-fitting plots for the spectrofluorometric titration: (1), -CD + TNS; (2) -CD + AR; (3) BzSe- -CD + AR.

If the initial host concentrations is much larger than that of fluorophoric guest molecules, i.e. $[H]_0 \gg [G]_0$, and $[H]_0 \gg [H \cdot G]$, then eq 7 reduces into,

$$[H] \approx [H]_0 \qquad (11)$$

from eqs 2, 6, 8 and 11, we can obtain

$$\frac{[G]_0[H]_0}{\Delta F} = \frac{1}{K_S \Delta \varepsilon_F} + \frac{[H]_0}{\Delta \varepsilon_F} \qquad (12)$$

This is the improved Benesi-Hildebrand equation [27]. Using the approximate linear plot $\frac{[G]_0[H]_0}{F} \sim [H]_0$, the complex stability constant (K_s) and the change in molar fluorescence intensity (ε_F) were determined using the data of F observed at each initial host concentration $[H]_0$.

If a host compound possesses a substituted fluorophoric moiety, same equations (eqs 10 and 12) could be deduced [44,45]. However, with regard to the self-inclusion of the host compound, ε_{incl} and ε_{free} denote molar fluorescence intensities for the two conformations of the host compound with the self-included fluorophore (H_{incl}) and with free fluorophore (H_{free}), respectively. Thus, we can obtain the following equations in the presence and absence of a guest molecule.

$$F_0 = \varepsilon_{free}[H_{free}]_0 + \varepsilon_{incl}[H_{incl}]_0 \qquad (13)$$

$$F = \varepsilon_{incl}[H_{incl}] + \varepsilon_{free}[H_{free}] + \varepsilon_{HG}[H \cdot G] \qquad (14)$$

With regard to the total amount of host compound, we have the following equation,

$$[H_{free}] + [H_{incl}] + [H \cdot G] = [H_{free}]_0 + [H_{incl}]_0 \qquad (15)$$

setting $\quad [H_{incl}] = [H_{incl}]_0 - x \qquad (16)$

then $\quad [H_{free}] = [H_{free}]_0 + x - [H \cdot G] \qquad (17)$

Combining eqs 14,16 and 17,

$$\begin{aligned} F &= \varepsilon_{incl}([H_{incl}]_0 - x) + \varepsilon_{free}([H_{free}]_0 + x - [H \cdot G]) + \varepsilon_{HG}[H \cdot G] \\ &= \varepsilon_{incl}[H_{incl}]_0 + \varepsilon_{free}[H_{free}]_0 + (\varepsilon_{free} - \varepsilon_{incl})x + (\varepsilon_{HG} - \varepsilon_{free})[H \cdot G] \\ &= F_0 + (\varepsilon_{free} - \varepsilon_{incl})x + (\varepsilon_{HG} - \varepsilon_{free})[H \cdot G] \end{aligned} \qquad (18)$$

where ε_{HG} signifies the molar fluorescence intensity of the formed complex ($H \cdot G$).

Supposing k be designated as the equilibrium constant between the two conformations of the host compound (H_{incl} and H_{free}),

$$k = \frac{[H_{free}]}{[H_{incl}]} = \frac{[H_{free}]_0}{[H_{incl}]_0} \qquad (19)$$

From eqs16, 17 and 19, we obtain:

$$x = \frac{[H \cdot G]}{1 + k} \qquad (20)$$

Combining eqs 18 and 20,

$$\begin{aligned} \Delta F &= F - F_0 = (\varepsilon_{free} - \varepsilon_{incl}) \cdot \frac{[H \cdot G]}{1 + k} + (\varepsilon_{HG} - \varepsilon_{free})[H \cdot G] \\ &= (\varepsilon_{HG} - \frac{k\varepsilon_{free} + \varepsilon_{incl}}{1 + k})[H \cdot G] = \Delta \varepsilon_F[H \cdot G] \end{aligned} \qquad (21)$$

Eq 21 is the same as eq 6, thus eqs 10 and 12 for curve-fitting approach and approximate linear plot approach, respectively, can be also obtained for this system.

Employing the spectrofluorometric titration, we have determined large amount of stability constants of inclusion complexes of various guest molecules (parts are shown in Figure **3**) with a wide variety of mono-substituted cyclodextrin derivatives. Furthermore, this approach has also successfully applied to the host-guest systems of bifunctional guest molecules and bridged bis(cyclodextrin)s [36,37,38,39,40,41,46,47,48,49,50,51,52,53,54,55,56,57,58, 59,60,61,62,63]. Similar method was also used to determine the complex stability constant by other groups [45,64,65,66,67].

3.2 Spectropolarometric Titration

Figures **14** and **15** shown the induced circular dichroism spectra of -cyclodextrin derivative (BzSe- -CD) in the presence and absence of cyclooctanol and (+)-borneol at various concentrations in aqueous phosphate buffer solution at pH 7.20.
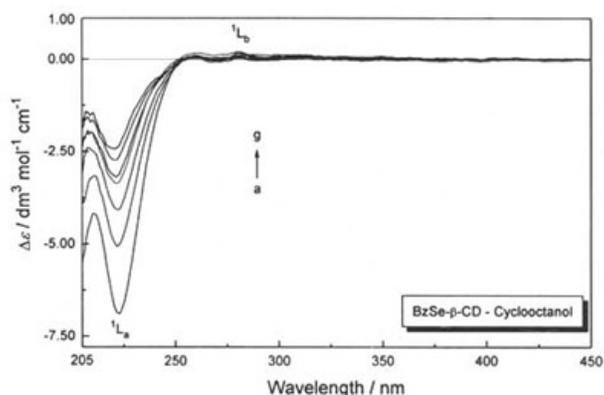

Fig. (14). The circular dichroism spectra of BzSe- -CD in the presence of cyclooctanol at different concentrations in phosphate buffer solution (pH 7.20) at 25 °C.
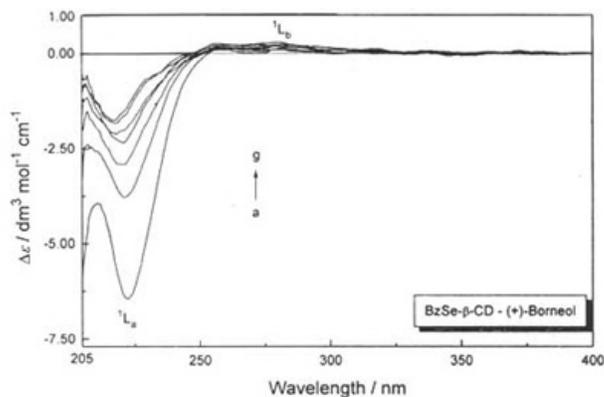

Fig. (15). The circular dichroism spectra of BzSe- -CD in the presence of (+)-borneol at different concentrations in phosphate buffer solution (pH 7.20) at 25 °C.

For the equilibrium equation 1, the intensity changes ( ) in the circular dichroism of L-Trp- -CD, for instance, upon the addition of guest molecule are assumed to be proportional to the concentration of inclusion complex (H·G) produced in the solution:

$$\Delta \Delta \varepsilon = \alpha[H \cdot G] \qquad (22)$$

where   denotes a sensitivity factor for the circular dichroism change, or a quantitative measure of the conformational change upon complexation. From eqs 2, 7, 8 and 22, the following equation can be derived.

$$\Delta\Delta\varepsilon^2 - \alpha([H]_0 + [G]_0 + \frac{1}{K_S})\Delta\Delta\varepsilon + \alpha^2[H]_0[G]_0 = 0 \qquad (23)$$

Solving eq 23 for   , we obtain:

$$\Delta\Delta\varepsilon = \frac{\alpha([H]_0 + [G]_0 + \frac{1}{K_S}) \pm \sqrt{\alpha^2([H]_0 + [G]_0 + \frac{1}{K_S})^2 - 4\alpha^2[H]_0[G]_0}}{2} \qquad (24)$$

The complex stability constant K_S and the sensitivity factor   were calculated by nonlinear curve-fitting using the value of   observed at each initial guest concentration $[G]_0$. Typical curve-fitting plots are shown in Figure **16** for several cyclic alcohols and organoselenium modified cyclodextrins.
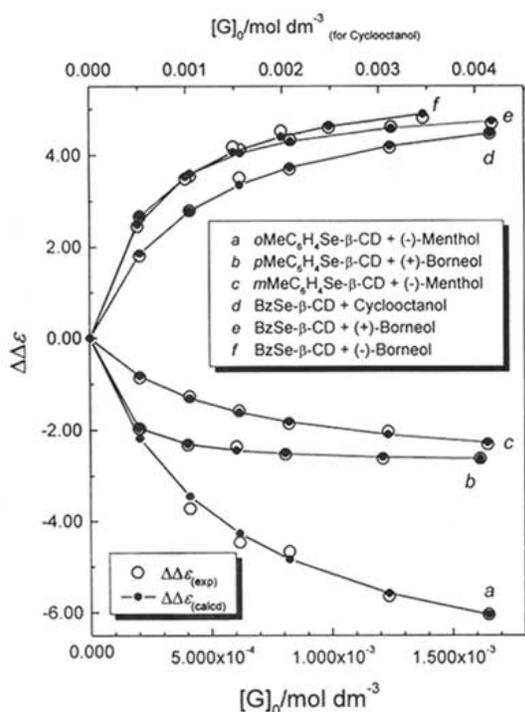

Fig. (16). Curve-fitting for spectropolarimetric titration.

Similarly, if the initial guest concentrations is much larger than that of host compound, i.e. $[G]_0 \gg [H]_0$, and $[G]_0 \gg [H \cdot G]$, then eq 8 reduces into,

$$[G] \approx [G]_0 \qquad (25)$$

from eqs 2, 7, 22 and 25, we can obtain extended Benesi-Hildebrand equation, [27].

$$\Delta\Delta\varepsilon = -\frac{1}{K_S} \cdot \frac{\Delta\Delta\varepsilon}{[G]_0} + \alpha[H]_0 \qquad (26)$$

Using the approximate linear   ~ $\frac{}{[G]_0}$ plot, the complex stability constant (K_s) and the sensitivity factor   can be determined using the data of   observed at each initial host concentration $[G]_0$.

The two data-processing method, i.e. curve-fitting approach and approximate linear Benesi-Hildebrand approach, were compared [33]. It is found that the larger stability constant, the more difference between these two methods. Therefore, it would be better to employ the curve-fitting approach when the stability constant is larger than 5000.

Employing the spectropolarometric titration, we have also determined many stability constants of inclusion complexes of various guest molecules (parts are shown in Figure 3) with a wide variety of cyclodextrin derivatives [36,37,38,40,41,46,55,68,69,70,71,72].

3.3 Other Spectrophotometric Titrations

For spectrophotometric titration employing ultraviolet-visible spectrophotometer, we have,

$$\Delta A = \Delta\varepsilon[H \cdot G] \qquad (27)$$

where A denotes the absorbance changes upon the inclusion complexation,   indicate the difference between the free chromophore and complexed chromophore.

We also can obtain the following improved Benesi-Hildebrand equation [27].

$$\frac{[G]_0[H]_0}{\Delta A} = \frac{1}{K_S\Delta\varepsilon} + \frac{[G]_0}{\Delta\varepsilon} \qquad (28)$$

We have determined some stability constants of inclusion complexes of cyclodextrin derivatives and some alcohols [36,55,73,74,75].

It should be noted that the errors in the stability constants determined via the spectrophotometric titration are larger than those from (micro)calorimetry. However, in general, the results with less than 5% errors could be obtained through correct design of the concentration range of the host−guest system and careful measurements. Furthermore, the spectrophotometric titration could apply to many suitable systems.

Tran and co-workers have successfully determined the complex stability constant between phenols and -, -, and -cyclodextrins employing spectrophotometric titration through near-infrared spectrometry and Benesi-Hildebrand approach [76,77].

NMR technique is employed in spectrophotometric titration to determine not only the host-guest complex stability constants, [6,21] but also the self-aggregation constant K and the self-aggregation number n of modified cyclodextrins [78,79].

Monomer-aggregate equilibrium equation in aqueous solution:

$$n \text{ Monomer} \xrightleftharpoons{K} \text{Aggregate}$$
$$\quad C_m \qquad\qquad\quad C_a \qquad\quad C_t$$
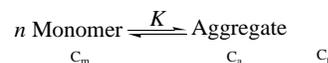
where C_t, C_m and C_a represent total, monomer and aggregate concentration; K and n are aggregate constant and aggregate number, respectively. Therefore,

$$C_t = C_m + nC_a \qquad (29)$$

$$\frac{C_m}{C_t} + \frac{nC_a}{C_t} = 1 \qquad (30)$$

The observed chemical shift δ_{obs} can be expressed as a weighted average of the corresponding monomer and aggregate shifts:

$$\delta_{obs} = N_m \delta_{mon} + N_a \delta_{agg}$$

$$= \frac{C_m}{C_t} \delta_{mon} + \frac{nC_a}{C_t} \delta_{agg} \qquad (31)$$

where δ_{mon} and δ_{agg} correspond to monomer and aggregate chemical shift, respectively.

Substitute eq 30 into eq 31 and give:

$$\delta_{obs} = (1 - \frac{nC_a}{C_t})\delta_{mon} + \frac{nC_a}{C_t}\delta_{agg} \qquad (32)$$

$$\delta_{obs} - \delta_{mon} = \frac{nC_a}{C_t}(\delta_{agg} - \delta_{mon}) \qquad (33)$$

$$C_a = \frac{\delta_{obs} - \delta_{mon}}{\delta_{agg} - \delta_{mon}} \cdot \frac{C_t}{n} \qquad (34)$$

Furthermore, the aggregate constant K used to characterize this equilibrium can be expressed as:

$$K = \frac{C_a}{(C_t - nC_a)^n} \qquad (35)$$

$$\ln K = \ln C_a - n\ln(C_t - nC_a) \qquad (36)$$

From eq 34 and eq 36, we can obtain:

$$\ln K = \ln \frac{\delta_{obs} - \delta_{mon}}{\delta_{agg} - \delta_{mon}} \cdot \frac{C_t}{n} - n\ln(C_t - n \cdot \frac{\delta_{obs} - \delta_{mon}}{\delta_{agg} - \delta_{mon}} \cdot \frac{C_t}{n})$$

$$= \ln \frac{\delta_{obs} - \delta_{mon}}{\delta_{agg} - \delta_{mon}} \cdot \frac{C_t}{n} - n\ln \frac{\delta_{agg} - \delta_{obs}}{\delta_{agg} - \delta_{mon}} \cdot C_t$$

$$= \ln \frac{\delta_{obs} - \delta_{mon}}{\delta_{agg} - \delta_{mon}} \cdot C_t - \ln n - n\ln \frac{\delta_{agg} - \delta_{obs}}{\delta_{agg} - \delta_{mon}} \cdot C_t \qquad (37)$$

$$\ln K + \ln N = \ln(\delta_{mon} - \delta_{obs})C_t - \ln(\delta_{mon} - \delta_{agg}) - n\ln(\delta_{obs} - \delta_{agg})C_t + n\ln(\delta_{mon} - \delta_{agg}) \qquad (38)$$

We can now obtain monomer-aggregate equilibrium equation expressed by δ, C_t, n and K give concluding equation:

$$\ln(\delta_{mon} - \delta_{obs})C_{tot} = n\ln(\delta_{obs} - \delta_{agg})C_{tot} + \ln k + \ln n - (n-1)\ln(\delta_{mon} - \delta_{agg}) \qquad (39)$$

Simultaneously, substitute $n = 2$ into monomer-aggregate equation can give monomer-dimer equilibrium equation as follow: [35,80].

$$\delta_{obs} = \delta_{dimer} + \{(\delta_{mon} - \delta_{dimer})[(-1 + (1 + 8K_{dimer}C_{tot})^{1/2}]/(4K_{dimer}C_{tot})\} \qquad (40)$$

Furthermore, the stability constants of the inclusion complexation of guest molecules with cyclodextrins could be determined via spectrophotometric titration at various temperatures. From the slope and ordinate-intercept of van't Hoff analysis $\ln K_s \sim \frac{1}{T}$ plots applying the following equation, the inclusion complexation thermodynamic parameters (ΔH and ΔS) would be obtained [31].

$$\ln K_S = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (41)$$

4. COMPETITIVE INCLUSION METHODS

The competitive inclusion method [22,23,24,25,26] is applicable to the systems in which both the host

cyclodextrins and the guest molecules are not spectrally sensitive. A chromophoric or fluorophoric molecule, which is taken as spectral probe, can form stable inclusion complex with cyclodextrin. Methyl orange is a common chromophoric probe used in ultraviolet-visible spectrometry. The stability constant is determined by spectrophotometric titration. To this cyclodextrin-spectral probe host-guest system, the gradual addition of another guest molecule will result in the regular changes in spectral intensity due to the exclusion of the spectral probe, which can be used to determine the stability constant of inclusion complex of the guest molecule with cyclodextrin.

5. CONFORMATION ANALYSIS OF INCLUSION MODE OF BRIDGED BIS(CYCLODEXTRIN)S

2D NMR spectroscopy has recently become an indispensable technique to investigate the interaction between guest molecules and host cyclodextrins, especially cyclodextrin dimers, since two protons C3-H and C5-H, which are closely located in space, can produce an NOE cross-peak between the relevant protons in NOESY or ROESY spectrum.

Fig. (17). ^{1}H NOESY spectrum (300 MHz) of a mixture of CD$_2$-4-Ni(II) with RhB in D$_2$O at 25 °C with a mixing time of 800 ms.

As illustrated in Figure **17**, the NOESY spectrum of an equimolar mixture of CD$_2$-4-Ni(II) (Figure **2**) with RhB (0.5 mM each) shows clear NOE cross-peaks between the C3-H and C5-H of cyclodextrin and the methyl protons of diethylamino fragments in RhB (peaks A), the cross-peaks between the C3-H and C5-H of cyclodextrin and the aromatic protons of diethylaminophenyl groups in RhB (peaks B), and the cross-peaks between the aromatic protons of bipyridine unit in CD$_2$-4-Ni(II) and the aromatic protons of the benzoate moiety in RhB (peaks C) [37]. This indicates that the diethylaminophenyl groups of RhB are accommodated in the cavities from the primary side of

cyclodextrin, while the benzoate branch of RhB penetrates into the pseudo cavity of CD_2-4-Ni(II) complex. Hence, the results of NOESY experiment strongly support the operation of the cooperative binding mode in the complexation of guest molecule by metal-ligated oligo(-cyclodextrin), as shown in Figure **18**.
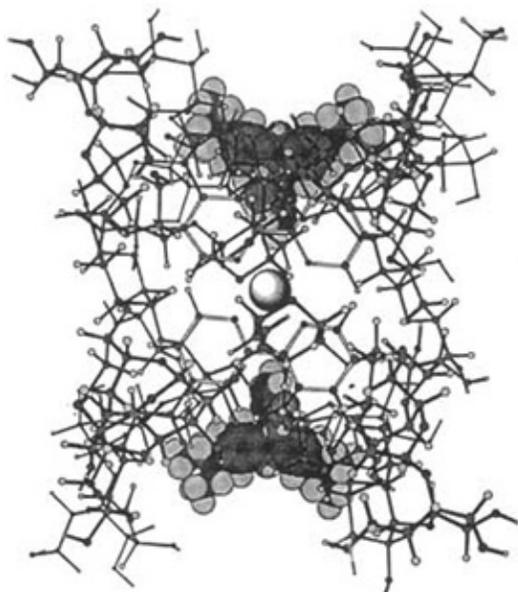

Fig. (18). Inclusion complexation modes of guest molecule RhB with metal-ligated bis(-cyclodextrin)s CD_2-4.
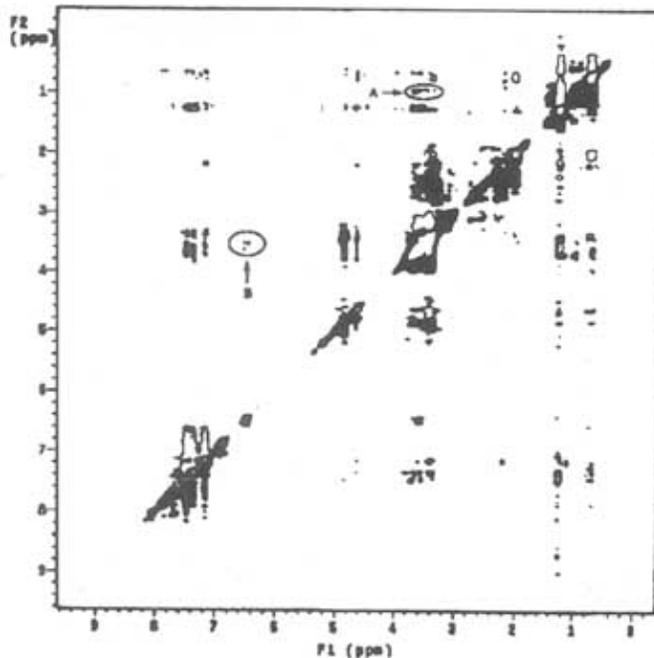

Fig. (19). [1]H NOESY spectrum (300 MHz) of a mixture of CD_2-5 with RhB in D_2O at 25 °C with a mixing time of 800 ms.

Figure **19** shows the NOESY spectrum of an equimolar mixture of triad host CD_2-5 (Figure **2**) with T-shaped guest RhB (0.5 mM each), which displays clear NOE cross-peaks between the methyl protons of the diethylamino moiety in RhB and the C3-H and C5-H of cyclodextrin (peaks A), as

well as the cross-peaks between the C3-H and C5-H and the aromatic protons of diethylaminophenyl in RhB (peaks B) [36]. This information indicates that the diethylaminophenyl groups of RhB are penetrating into the cavities from the primary side of cyclodextrin to form a sandwich complex.

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