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Spectrofluorimetric Study of Cyclodextrin Complexation in the Presence of Third and Fourth Components

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Abstract. The steady-state fluorescence profile of 4,5-benzopiaselenol (Se-DAN) has been investigated in mixed aqueous media comprising cyclodextrins (CDs) and sodium dodecyl sulfate (SDS). The emission intensity of Se-DAN, whereas virtually unaffected in the αCD/SDS medium, was enhanced synergistically by βCD and SDS and was accompanied by a marked blue shift of the maximum emission wavelength. The interactions involving γCD were rather interesting. While a mixed solution of γCD and SDS brought about a slight fluorescence increase of Se-DAN, addition of toluene, a fourth component, resulted in a pronounced emission enhancement. To ascertain the role of toluene in the system, a model study was carried out by use of pyrene (Py) as a fluorescence probe in place of Se-DAN/SDS. A $1:1:1$ γCD/Py/toluene ternary complex was detected and its association constant was determined to be 869 M^{-1}, significantly greater than the previously reported values of the γCD/Py binary complex. On the basis of the mechanistic study, the formation of a γCD/Se-DAN/SDS/toluene quaternary complex is proposed.

Key words: Quaternary, tertiary, surfactant, cyclodextrin complex, pyrene, fluorescence.

1. Introduction

In the last decade practical applications of cyclodextrins (CDs) have expanded dramatically [1, 2]. Because inclusion by CDs alters the photophysical properties of many fluorophores, spectrofluorimetry has been used extensively in investigating CD systems [3–7]. In many cases, the presence of CDs dramatically enhances the fluorescence of complexed solutes in aqueous solutions. The factors thought to be responsible include a favorable apolar microenvironment provided by the CD cavity [8, 9], shielding of the excited state from various external quenchers [6, 8–10], a decrease in the rotational freedom of the guest fluorophore in molecular motion [2, 8–10], an increase in the radiative rate constant [6], and prevention of collisional deactivation [11]. Enhanced luminescence may lead to practical applications, especially in diagnostic and analytical fields. For instance, Hinze and coworkers have evaluated the effectiveness of various water-soluble βCD systems as fluorescence enhancement media for 14 dansylamino acids and 33 organic/pharmaceutical compounds [12]. Ueno *et al.* have reported the use of naphthyl derivatives of βCD to detect organic compounds [13].

Most previous studies of CD–guest complexation were focused on binary systems, although recently more attention has been turned to ternary complexes in which third components such as alcohols, alkylamines and surfactants also participate in the inclusion processes [14–22]. Published results have revealed interesting intramolecular and intermolecular interactions in the ternary CD complexes. However, complexation involving more than three different components has not been well explored thus far owing to their higher levels of complexity. In this manuscript we report our findings from a recent investigation of the 4,5-benzopiaselenol (Se-DAN)/γCD/SDS/toluene system [23]. The current study provides evidence of the formation of a quaternary CD complex.

2. Experimental

β-Cyclodextrin (βCD) was purchased from Sigma Chemical Company while αCD and γCD were obtained from American Maize-Products Company. Deionized water was used for preparation of all aqueous solutions. Selenium stock solution (1 mg/mL) was prepared by dissolving 0.4089 g selenious acid (Beijing Chem. Works, A. R.) in 250 mL of 0.1 M HCl. This solution was then diluted 1000-fold with 0.1 N HCl to obtain the Se(IV) standard (1 μg/mL). The stock solution (1 mg/mL) of 2,3-diaminonaphthalene (DAN) was made by dissolving 0.1 g DAN (Koch-Light) in 100 mL of 0.1 N HCl. The solution was then extracted three times with cyclohexane and the organic layer was discarded. This stock solution, covered with a layer of cyclohexane to prevent oxidation, was stored at 4 °C and diluted 1000 times with 0.1 N HCl prior to use. Stock solutions (20 mM) of αCD, βCD and γCD were prepared in 0.4% $NH_2OH{\cdot}HCl$. The stock solution (5 μM) of pyrene (Py) (99+%, Aldrich) and the stock solutions (4% v/v) of toluene, cyclohexane, cyclohexanol, cyclohexaketone, benzene, p-nitrophenol and ethylnaphthol were made with anhydrous ethanol.

2.1. EFFECTS OF MEDIA ON THE FLUORESCENCE OF Se-DAN

Typically, to a 25.0 mL volumetric flask were added 12.5 mL of Se(IV) standard (1 μg/mL), 2.5 mL of DAN standard (1 μg/mL) and 5.0 mL of KCl-HCl buffer (pH 2.0). The mixture was heated in a warm water bath for 10 minutes and mixed well for a complete dissolution. Appropriate volumes of stock solutions of CD (20 mM) and sodium dodecyl sulfate (SDS, 0.1 M) were then added to the solution. For the solvent effect experiments, one of the ethanolic solvent solutions was also added. Each solution was then diluted to the final volume with deionized water. The fluorescence intensity of Se-DAN was measured on a Shimadzu Model RF-540 spectrofluorometer in 1-cm cell (slits: Ex 5 nm, Em 5 nm; excitation wavelength: 379 nm).

2.2. EFFECTS OF TOLUENE ON PYRENE/γCD COMPLEXATION

An aliquot (0.5 mL) of the pyrene stock solution was transferred to a 25.0 mL volumetric flask and the solvent was evaporated. To the flask 4.0 mL of γCD stock (20 mM) were added the necessary amount of toluene stock solution (4% v/v), as well as 5 mL of HCl-KCl buffer (pH 2.0), the components then being mixed thoroughly to obtain a homogeneous solution. The volume was then made up to the mark with deionized water. This procedure gave samples of 0.1 μM in pyrene, 3.2 mM in γCD, and 0–240 ppm in toluene. Each prepared sample was excited at 338.5 nm for the fluorescence emission measurement. The pyrene I/III band ratio was obtained from each spectrum by measuring emission intensities at 373 nm and 383 nm (slits: Ex 2 nm; Em 2 nm).

2.3. DETERMINATION OF THE APPARENT BINDING CONSTANT FOR THE PYRENE/γCD/TOLUENE TERNARY SYSTEM

Solutions were made in the same way as described above, except a fixed volume (80 μL) of 4% toluene was present while the content of γCD varied from 0 to 3.6 mM. Changes in the pyrene I/III ratio as the function of γCD concentration were analyzed on the basis of a modified Benesi–Hildebrand equation [24]. The apparent constant was determined from the slope and the intercept of the 1 : 1 linear plot.

2.4. DETERMINATION OF γCD : TOLUENE STOICHIOMETRIC RATIO FOR THE PYRENE/γCD/TOLUENE COMPLEX

The continuous variation method [25] was adopted. In our experiments, the concentration of pyrene was fixed at 0.1 μM whereas concentrations of γCD and toluene were changed systematically. Under the condition of ([γCD] + [toluene] = 1.5 mM), the observed fluorescence intensity was plotted as a function of the γCD molar fraction. As the relative fluorescence intensity reached its maximum value at the γCD molar function of 0.5, the 1 : 1 stoichiometry between γCD and toluene was concluded.

3. Results and Discussion

3.1. SYNERGISTIC ENHANCEMENT OF THE FLUORESCENCE INTENSITY OF Se-DAN BY CDS AND SDS

Selenium is an important microbioinorganic element. Trace selenium is generally determined fluorometrically based on the reaction [26, 27] depicted in Figure 1.

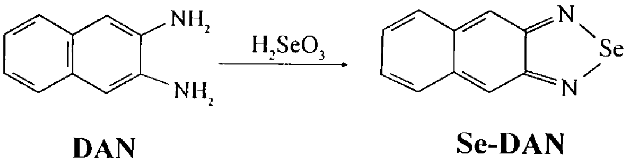

Figure 1. Formation of 4,5-benzopiaselenol (Se-DAN) in the fluorometric determination of trace selenium.

The resultant 4,5-benzopiaselenol (Se-DAN) is extracted into an organic solvent and its fluorescence intensity measured for the quantification of selenium.

Previous work by Zheng and Lu [28, 29] showed that addition of βCD and SDS to an aqueous solution of Se-DAN resulted in 5–6 times stronger fluorescence emission than that achieved by either βCD or SDS alone. Similar observations were made in the current study. However, when βCD was replaced either with αCD or γCD results were quite different (Figure 2). In the presence of SDS, γCD enhances the fluorescence intensity of Se-DAN to a lesser extent than βCD does, whereas αCD is practically ineffective. The fluorescence profile of Se-DAN has been found [29] to be very sensitive to the polarity of the medium, where the emission intensity increases rapidly with a decrease in polarity. Thus, the fluorescence enhancement brought about by βCD can be largely attributed to the transfer of the Se-DAN fluorophore from the bulk aqueous solution to the hydrophobic cavity of βCD upon inclusion. The differentiating results in Figure 2 reflect, at least in part, the geometric compatibility between Se-DAN and the cavity of each CD. The fluorophore of Se-DAN obviously fits well inside the cavity of βCD, as predicated on the basis of molecular modeling and evidenced by the significant fluorescence enhancement. In contrast, the cavity of αCD seems to be unable to include the Se-DAN ring structure since the presence of αCD does not affect the fluorescent behavior of Se-DAN. The moderate enhancing effect of γCD indicates a loose binding of its cavity with Se-DAN so that the fluorophore can move in and out with relative ease.

In addition to the observed fluorescence intensity enhancement, blue shifts of the maximum emission wavelength (λ_{ex}) of Se-DAN have also been induced by βCD and γCD. For example, λ_{ex}, of Se-DAN in water is about 650 nm, but it is shifted to around 535 nm in βCD (3.2 mM) and 545 nm in γCD (3.2 mM). Concomitant blue shifts of λ_{ex} with enhancements of fluorescence [15, 19] and phosphorescence [30] in mixed surfactant–CD media have been documented. It is known that a high electron density prevails inside the cavity of a CD molecule. The observed media effects therefore suggest a perturbation toward the electron clouds of an included guest by the hydrophobic CD cavity. The stronger the CD–guest binding, the greater the interaction of their electron clouds, and the more significant the change of the guest luminescence profile.

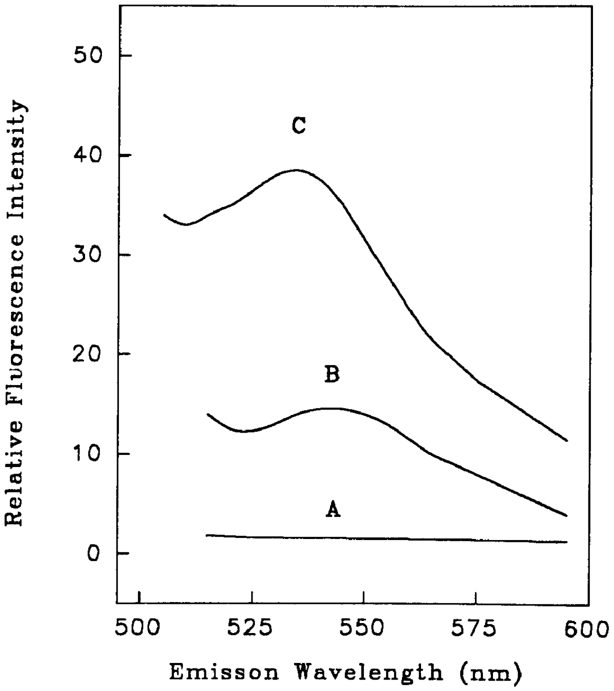

Figure 2. Influence of αCD (A), γCD (B), and βCD (C) on the fluorescence intensity of Se-DAN in the presence of SDS.

Figure 3 illustrates the influence of SDS concentration on the emission intensity of Se-DAN. At a fixed content of βCD, the synergistic enhancing effect of SDS increases rapidly as a function of its concentration when [SDS] is below 1 mM. The maximum emission is reached at around 1.4 mM. As the concentration of SDS rises further the fluorescence intensity of Se-DAN drops. Meanwhile, the solution starts to turn turbid. There are two species of SDS in aqueous solution, i.e. monomers and micelles, observed below and above the critical micelle concentration (cmc), respectively. It has been discovered that the cmc of SDS increases upon complexation with CDs [31], and that it is the SDS monomers, rather than the micelle, that function synergistically with CDs in enhancing the intensities of both fluorescence [29] and phosphorescence [30]. In the ternary system containing Se-DAN, βCD, and SDS at low concentration, Se-DAN is most likely to be included by the CD cavity. The synergistic effect of SDS may be explained by the formation of a more hydrophobic microenvironment around the fluorophore with the dodecyl tail of a SDS monomer either aggregating at the open ends of the βCD torus or partially protruding into the cavity. However, as the concentration of SDS increases beyond its cmc, premicellar aggregates will start to form, which can extract Se-DAN out of the hydrophobic cavity of βCD, leading to a decrease in fluorescence intensity.

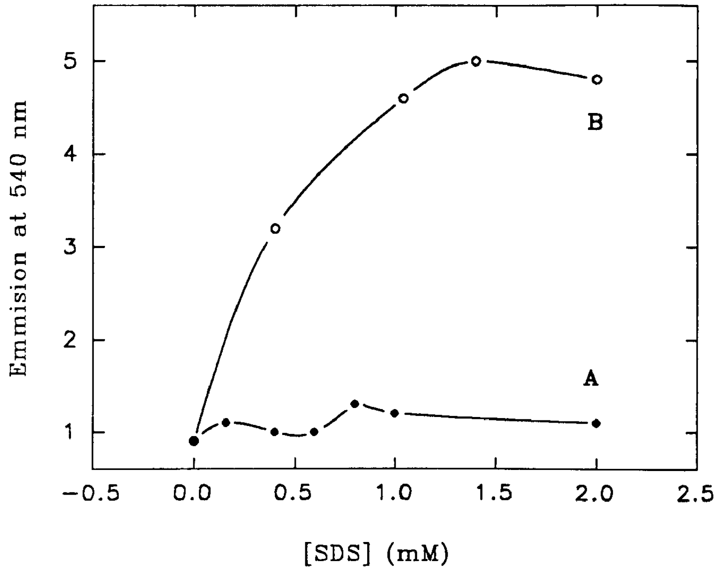

Figure 3. Effect of SDS concentration on the fluorescence intensity of Se-DAN in the presence of γCD (A) and βCD (B).

Data in Figure 3 suggest that in the γCD medium the effect of SDS concentration on the Se-DAN fluorescence is essentially negligible. It may be inferred from the observation that unoccupied space remains inside the γCD cavity even when Se-DAN and SDS are both complexed. If this speculation is true, the extra space in the γCD cavity may be filled with a third guest of appropriate polarity and dimension. In this regard, Ueno *et al.* previously reported a marked fluorescence enhancement of a γCD/α-naphthyloxyacetic acid complex upon addition of cyclohexanol [15]. The function of cyclohexanol was interpreted as a space regulator that narrows the γCD cavity. More recently, Jin *et al.* [32] observed a space-matching effect of cyclohexane, which induced a significantly stronger room temperature phosphorescence of phenanthrene in the presence of βCD than n-hexane did.

3.2. INFLUENCE OF TOLUENE ON THE FLUORESCENCE OF THE Se-DAN/γCD/SDS TERNARY COMPLEX

To resolve our postulate on the binding mode of the Se-DAN/γCD/SDS ternary system, a series of organic solvents were investigated as fourth components. Among the compounds tested, including cyclohexane, cyclohexanol, cyclohexaketone, benzene, toluene, p-nitrophenol and ethylnaphthol, only toluene showed an impact on the fluorescent profile of Se-DAN. As illustrated in Figure 4, the fluorescence emission of Se-DAN is significantly intensified upon the addition of toluene in increasing amounts. This result is consistent with the formation of a γCD/Se-DAN/SDS/toluene complex, in which toluene contributes to a stronger binding

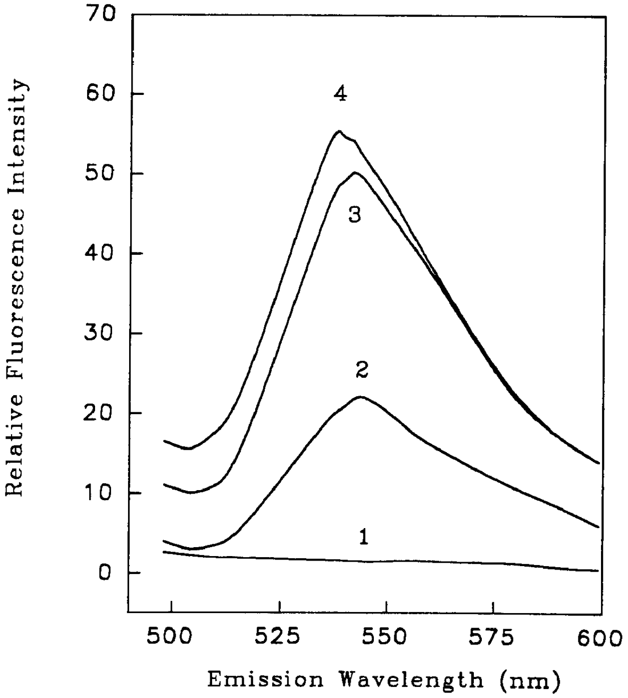

Figure 4. Effect of toluene concentration on the fluorescence intensity of Se-DAN in the presence of SDS and γCD. Toluene content: 0.0 (1); 96 ppm (2); 160 ppm (3), and 240 ppm (4).

of Se-DAN inside the γCD cavity. In view of the organic compounds tested in the current study, it has become clear that the formation of a quaternary complex depends critically on the geometric compatibility of the fourth component. Since benzene shows no similar effect to that exhibited by toluene, the methyl group of toluene most likely has contributed to the binding interaction in forming the quaternary complex. Although the participation of all four components appeared evident, it was difficult to ascertain the mode of binding owing to the complexity of the quaternary system. A simplification was thus made by using pyrene (Py) in place of Se-DAN and SDS.

3.3. MECHANISTIC STUDY OF THE γCD/PY/TOLUENE SYSTEM

Py is a widely used fluorescent probe because its vibronic fine structure is very sensitive to changes in its surrounding microenvironment. Py has been taken as the model compound in the current study on the basis of its characteristics as well as its molecular dimension. The fluorescence emission of Py changes dramatically upon addition of γCD and toluene. The changes are readily monitored by examining the I/III vibronic band ratio of Py. This ratio has been recognized as a sensitive

Table I. Effect of toluene on the I/III band ratio of pyrene in the presence of γCD (3.2 mM).

Toluene (ppm)	0	64	96	128	160	240
I/III band ratio	0.653	0.612	0.565	0.450	0.392	0.630

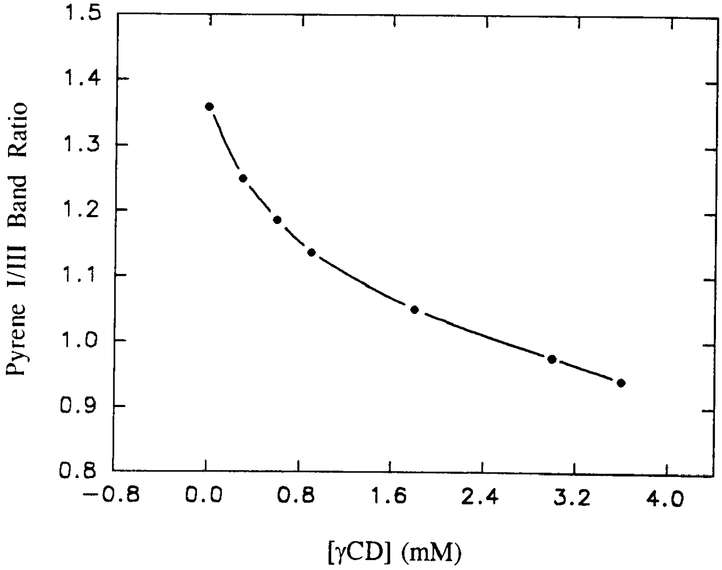

Figure 5. Effect of γCD concentration on the I/III band ratio of pyrene in the presence of toluene (128 ppm).

indicator of the molecular environment around Py, where a reduction in polarity is reflected by a decrease in the I/III ratio. The effect of toluene concentration on the fluorescence emission of Py has been examined in the presence of 3.2 mM of γCD (Table I). The I/III band ratio initially drops as the toluene concentration increases until it reaches a minimum (0.392) at around 160 ppm toluene. As the toluene content increases further, however, so the I/III ratio rises. Moreover the solution turns slightly turbid after the toluene concentration exceeds 160 ppm. This trend indicates that the microenvironment surrounding Py is more hydrophobic in the presence of toluene at appropriate concentrations. The increase of the I/III ratio at a higher toluene level is presumably due to a slight displacement of Py from the cavity of γCD, and its subsequent exposure to the bulk aqueous solution [33].

The effect of γCD concentration on the fluorescence spectrum of Py has been examined in the presence of 128 ppm toluene (Figure 5). The I/III band ratio of Py diminishes as the γCD content increases. The variation of the Py I/III ratio not only provides qualitative evidence of inclusion complexation, it also allows one to graphically determine the stoichiometry and the binding constants of Py/CD binary [34, 35] or ternary [21, 22, 36, 37] complexes. Figure 6 shows the same

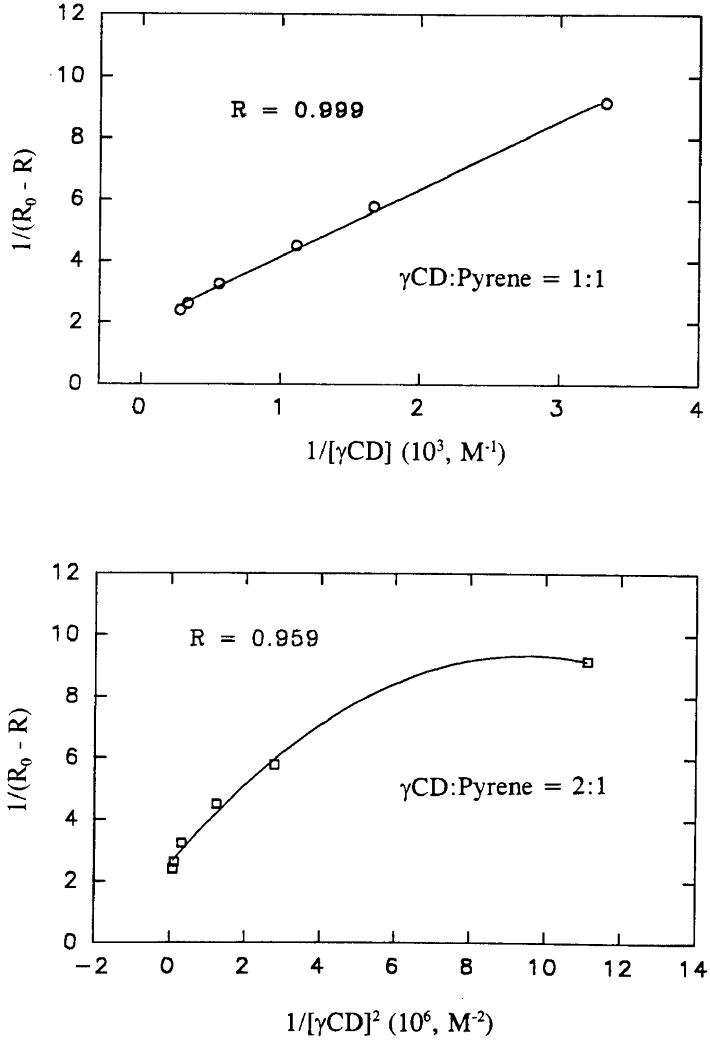

Figure 6. Double reciprocal plots for the γCD/pyrene/toluene complex at a constant toluene concentration (128 ppm), comparing 1 : 1 (top) and 2 : 1 (bottom) γCD/pyrene stoichiometries.

set of fluorescence data processed as double-reciprocal plots for determining the stoichiometric ratio between γCD and Py. The fact that the 1 : 1 plot is linear (R = 0.999) and that the 2 : 1 plot is curved (R = 0.959) indicates that the molar ratio of Py and γCD in the presence of toluene remains 1 : 1. The stoichiometric ratio between γCD and toluene in the ternary system has been determined separately to be 1 : 1 by use of the continuous variation method [25]. Therefore, the stoichiometric composition of the Py/γCD/toluene system is 1 : 1 : 1. The measured apparent association constant of this ternary complex is 869 M^{-1}. The binding strength in the ternary system is thus significantly enhanced in comparison with that of the Py/γCD binary complex (reported K values: 270 M^{-1} [19], 250 M^{-1} [35], 300 M^{-1} [38]). Similar results have been reported previously [21, 22]. The stability-enhancing function of the third components has been interpreted as space-filling, or space-regulating [15]. In this regard, proper size matching among the interacting species seems to be of crucial importance. Depending on its property and concentration, the role of the additional component can be either a space regulator or a competitor for the CD–guest binding. Toluene enhances the binding of Py and γCD by filling the remaining space inside the γCD cavity, thus acting as a space regulator. It should be noted that toluene exhibits the space-regulating effect at a remarkably low concentration range.

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