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The Solubility and Cyclodextrin Complexations of Fluorene, Fluoranthene, and Pyrene in Aqueous Medium

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The vapor saturation method was applied to prepare aqueous solutions saturated with a volatile solid solute such as fluorene, fluoranthene, or pyrene. Henry's law was found to hold for their aqueous solubility, and sublimation enthalpy was evaluated for each solid solute. Complex formation constants with cyclodextrins at 25 °C have been determined by the vapor saturation method. The applicability and limitation of the method are discussed.

A large number of data concerning aqueous solubility of various hydrocarbons have been accumulated. They have been obtained mainly by the "shake-flask method": an excess quantity of a solute is added to water, the mixture is mechanically shaken, and then the excess solute phase is separated. An alternative technique named the "generator column method," which, recently proposed, involves continuous pumping of water through a column filled with a support material coated with the solute of interest.[1] This technique has an advantage over the shake-flask method: saturated solutions can be prepared quickly without any excess solute phase, and moreover, if necessary, the solute in the aqueous solution can be easily concentrated by connecting a second column packed with a hydrophobic support.

We recently proposed a new technique which we call the "vapor saturation method," and can be applied to volatile solid solutes such as biphenyl, naphthalene, anthracene, and phenanthrene.[2] An excess quantity of a solid solute is dispersed in water (which we call the "solute reservoir"), and then air is bubbled through the aqueous phase to produce the solute vapor. The vapor is introduced into a separate container (which we call the "solubility flask"), where water to be saturated is placed and circulated in a closed system. The water in the solubility flask can thus be saturated with the solute in a relatively short time without any need to remove solute microcrystals. The main advantage of this vapor saturation method over the preceding two methods is that we can determine directly whether the solubility obeys Henry's law. The temperature of the solute reservoir can be changed up to that of the solubility flask while the latter temperature is kept constant: the solubility can be measured as a function of the solute vapor pressure.

Another important feature of the vapor saturation method is its potential applicability to studying associations which may occur between solutes of different kinds in aqueous medium, such as hydrophobic interaction. A simple and probably more direct way to estimate such associations is to measure the aqueous solubility of one solute (1), excess of which may be present in water as a solid or a liquid phase, in the absence and presence of the other solute (2); the association constant can be evaluated from the difference in solubility. It is desirable that such solubility runs be made in the absence of excess solute (1), otherwise coexisting solute (2) molecules will be adsorbed on the solid or liquid solute (1) or, in an extreme case, can penetrate into the excess solute phase to cause modification of its physicochemical properties.

This work is concerned with a further application of the vapor saturation method to preparing aqueous solutions saturated with a volatile solid solute such as fluorene, fluoranthene, or pyrene and to studying associations of these solutes with cyclodextrins in aqueous medium.

Cyclodextrins (CyDs, cyclic oligosaccharides consisting of six, seven, or eight glucopyranose units, called α, β, or γ, respectively, with a central cavity of the corresponding size) have attracted interests of many workers due to their unique properties. It is well-established that CyDs (hosts) form inclusion complexes with a variety of molecular species (guests) in aqueous medium. In this host-guest association, the size (and shape) and hydrophobicity of a guest molecule play an important role. We have recently proposed a method which makes use of the volatility of a guest molecule and can be used to determine association constants of CyDs with various volatile guests.[3,4] The method, however, requires such experimental conditions that host molecules should always be in excess over guest molecules, and hence allows estimation of only 1 : 1 and 2 : 1 (host : guest) complexes. The solubility measurements, on the other hand, can be used to estimate not only 1 : 1 and 2 : 1 but also 1 : 2 complexations, because the guest substance is always supplied to the association system.

Experimental

Materials. Distilled deionized water was used throughout the experiments. Fluorene, fluoranthene, and pyrene of analytical reagent grade (Wako) and CyDs of guaranteed grade (Nakarai) were used as received. A suitable amount of CyD, which had been dried over phosphorus pentaoxide under vacuum, was weighed and dissolved in water to prepare CyD solutions of desired concentrations.

Apparatus and Procedures. The apparatus and procedure to prepare aqueous solutions saturated with solute vapor are the same as those previously described.[2] Solute concentrations were measured after extraction with cyclohexane. A JASCO FP-770 spectrofluorometer was used for fluorene (274.8/310.2) and fluoranthene (360.4/463.0) by setting such (excitation/emission) wavelengths, nm as indicated in the parentheses. In the case of pyrene, fluorescence spectra were also taken, but in determining its concentrations in water, some unknown impurities were observed and thus the cyclohexane extracts were analyzed on a Shimadzu LC-6A HPLC: packed column, Shim-pack FLC-ODS 4.6 mm×5 cm (particle size 3 μm); autoinjector, SIL-6B; UV detector, SPD-6AV (operated at 334 nm); mobile phase, ethanol (80%)/water (20%).

For CyD solutions saturated with solute vapor, prior to the extraction with cyclohexane, the saturated solutions were in advance diluted with water so that any turbidity due to deposit of CyD cannot be observed in the presence of the organic extract.

Results and Discussion

The Time Required for Saturating Water with Solute Vapor. At a constant circulation rate of solute vapor, ca. 0.5 $dm^3 min^{-1}$, the aqueous phase reaches solubility equilibrium, depending on the solutes as shown in Fig. 1, at 3 (fluorene), 7 (fluoranthene), and 16 h (pyrene).

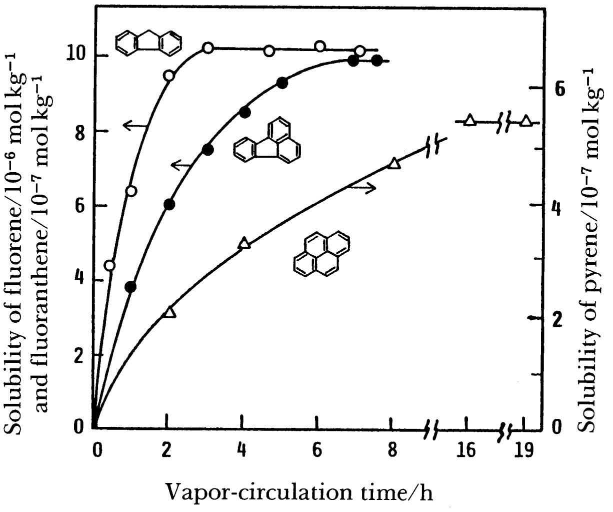

Fig. 1. Solute concentrations in water at 25 °C vs. solute-vapor-circulation time. Fluorene (○), fluoranthene (●), and pyrene (△).

Equilibrium Solubility and Solute Vapor Pressure. The term "equilibrium solubility" is used to describe the solubility obtained under the conditions that the temperature of the solute reservoir differs from that of the solubility flask. In Fig. 2 is shown the equilibrium solubility of each solute in the solubility flask kept constant at 25 °C; the vapor pressure data have been taken from the literature.[5] Henry's law holds for the solutes investigated. Fluorescence spectra taken for pyrene in aqueous solutions of any equili-

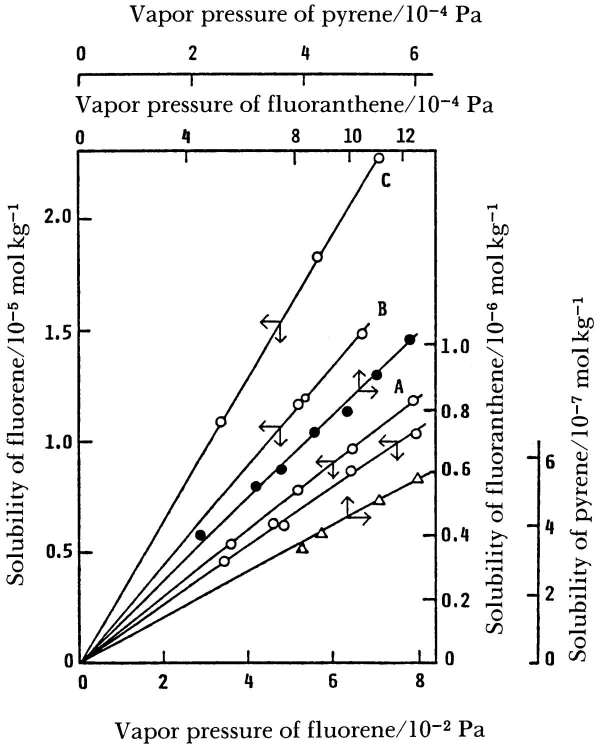

Fig. 2. Equilibrium solubility vs. solute vapor pressure. The temperature of solute was changed, while that of solvent water was held constant at 25 °C. Signs are the same as those shown in Fig. 1. Equilibrium solubility of fluorene measured in the presence of β-CyD at 1.07×10^{-4} (A), 5.02×10^{-4} (B), 1.00×10^{-3} mol dm^{-3} (C) are also shown.

Table 1. Henry's Law Constant, Aqueous Solubility, and Enthalpy of Sublimation (Data at 25 °C)

Solute	K_H[a]/10^4 Pa	S[b]/10^{-6} mol kg^{-1} This work (Literature data)[d]	ΔH_{sub}[c]/kJ mol^{-1} This work (Literature data)[e]
Fluorene	43.0	10.2 (10.15, 11.9, 11.4)	86.8 (83.0, 88.8)
Fluoranthene	6.75	1.02 (1.02, 1.3, 1.32)	78.5 (103, 84.5)
Pyrene	5.89	0.569 (0.64, 0.653, 0.668, 0.733, 0.77)	97.5 (91.3, 93.8, 100)

a) Henry's law constant determined in this work. b) The solubility in water estimated from K_H. c) The enthalpy of sublimation estimated from equilibrium solubility. d) Taken from Ref. 13. e) Taken from Ref. 14.

brium solubility did not show a peak at 472 nm, an emission wavelength at which the pyrene excimer appears (see Fig. 6).

At 25 °C, the Henry's law constant K_H $(=P/X_2)$, where P is the partial pressure of the solute and X_2 is the mole fraction of the dissolved solute, and the aqueous solubility determined from K_H are shown for each solute in Table 1. Our value for pyrene seems somewhat smaller than the literature data, while those for the other two solutes are in reasonable agreement.

Estimation of Sublimation Enthalpy. The enthalpy of sublimation of each solid solute at 25 °C has been estimated in the same way as described in our previous paper:2) as shown in Fig. 3, the logarithm of the equilibrium solubility (mole fraction), as determined in the solubility flask kept at 25 °C, is plotted against the reciprocal of the absolute temperature of the solute reservoir. The sublimation enthalpies thus estimated for each solute are listed in Table 1. Taking it into account that our values have been estimated based on rather narrow temperature ranges, fluorene (18.1—25 °C), fluoranthene (16.5—24.9 °C), and pyrene (21.6—25 °C), their agreements with the literature are reasonable. It is noteworthy that the sublimation enthalpy, which usually requires vapor pressure measurements as a function of temperature, can be simply estimated by the vapor saturation method.

Complex Formations with Cyclodextrins in Aqueous Medium. For determining formation constants of molecular species with CyDs in aqueous medium, the solubility method has been demonstrated to be usuful, particularly for sparingly soluble guests.6,7)

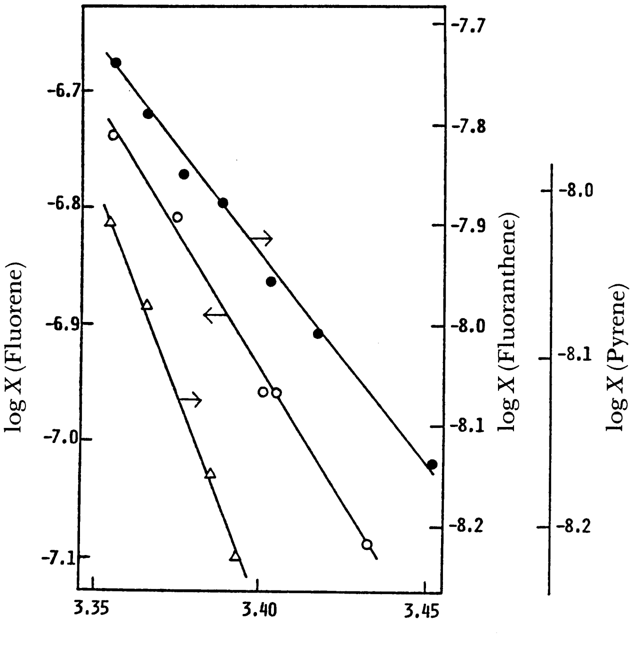

Fig. 3. log X vs. T^{-1}. X: equilibrium solubility at 25 °C in mole fraction; T: absolute temperature of solute. Signs are the same as those shown in Fig. 1.

Let S be a substrate (guest) and L be a ligand (CyD, host), and let us consider the 1 : 1, 1 : 2, and 2 : 1 complex formations

$$\mathrm{S}+\mathrm{L} \rightleftarrows \mathrm{SL}\ K_1, \tag{1}$$

$$\mathrm{SL}+\mathrm{L} \rightleftarrows \mathrm{SL_2}\ K_2, \tag{2}$$

$$\mathrm{SL}+\mathrm{S} \rightleftarrows \mathrm{S_2L}\ K_3. \tag{3}$$

Taking the law of mass action into account, we can get an equation using the substrate solubility in water in the absence (S_0) and presence (S_t) of CyD:

$$\mathrm{S_t}=S_0+K_1S_0[\mathrm{L}]+K_1K_2S_0\,[\mathrm{L}]^2+2K_1K_3S_0{}^2\,[\mathrm{L}], \tag{4}$$

where [L] is the equilibrium concentration of free ligand. As a first approximation, the total concentration of L, L_t, can be used as [L], because L_t is sufficiently large compared with the sum of the concentration of each complexed species. Rewriting of Eq. 4 yields

$$(S_t-S_0)/S_0=K_1L_t+K_1K_2L_t{}^2+2K_1K_3S_0L_t. \tag{5}$$

In Fig. 4 is plotted $(S_t-S_0)/S_0$ against S_0 at constant

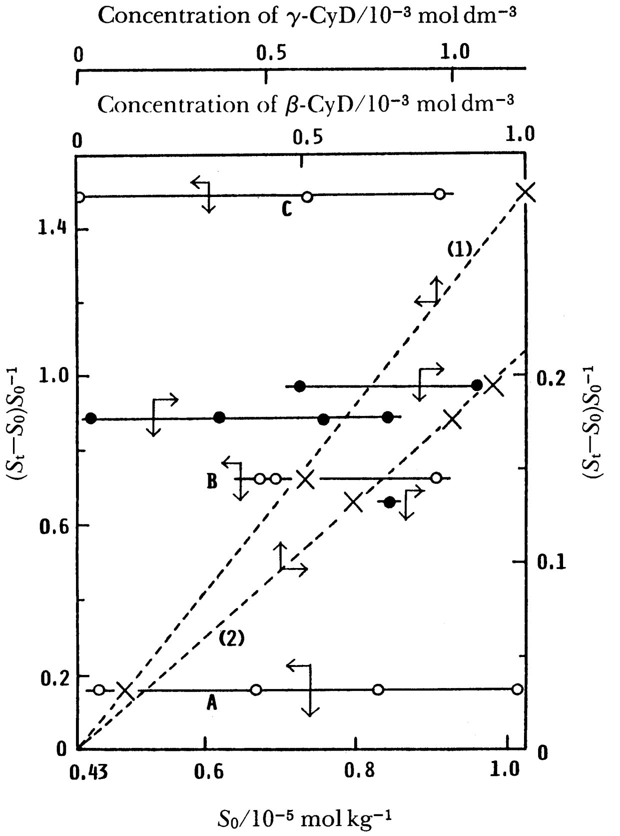

Fig. 4. $(S_t-S_0)/S_0$ plots against S_0 or L_t for fluorene. Equilibrium solubility in the absence (S_0) and presence (S_t) of β-CyD (○) or γ-CyD (●). Plots indicated by symbols, A, B, and C are based on the results given by the corresponding symbols shown in Fig. 2. $(S_t-S_0)/S_0$ vs. L_t plots are shown by dotted lines: β-CyD(1), γ-CyD(2).

L_t or against L_t for fluorene-β- and γ-CyD systems. Let us take one case, fluorene-β-CyD. As can be seen from Fig. 2, the equilibrium solubility of fluorene obeys Henry's law in the presence of β-CyD. At a β-CyD concentration of 1.00×10^{-3} mol dm^{-3}, the equilibrium solubility was measured at 25 °C by setting the solute reservoir at three different temperatures, 18.0 (3.39×10^{-2}), 22.2 (5.70×10^{-2}), and 24.0 °C (7.09×10^{-2} Pa) (the solute vapor pressure is given in parentheses) to be 1.09, 1.82, and 2. 27×10^{-5} mol kg^{-1}, respectively; each value refers to S_t at the constant L_t. The equilibrium solubility in the absence of CyD can be estimated to be 0.436, 0.732, and 0.910×10^{-5} mol kg^{-1} at the corresponding solute vapor pressure; each value refers to S_0. Then, the term $(S_t-S_0)/S_0$ is calculated to be 1.50, 1.49, and 1.49 at the three different solute temperatures. The term being independent of S_0 at a constant β-CyD concentration (as indicated in Fig. 4 by a horizontal line connecting open circles), we assume $K_3=0$. In the same way, the term $(S_t-S_0)/S_0$ is calculated to be 0.725 and 0.161 at β-CyD concentrations of 5.02 and 1.07×10^{-4} mol dm^{-3}, respectively. The term being linearly proportional to L_t, (as indicated in Fig. 4 by the dotted line (1) connecting the points on each horizontal line at the corresponding L_t), we assume $K_2=0$.

The vapor saturation method allows us to determine whether the 2:1 (guest:host) complexation is involved in the association system of interest by plotting $(S_t-S_0)/S_0$ against S_0 at a constant L_t. The conventional shake-flask method or its improved generator-column method cannot be used for such measurements, because the aqueous phase is always in contact with excess solute and, then, the concentration of free substrate, S_0, is unequivocally determined by the temperature of the solute-solvent mixture.

Particular attention should, however, be called to the use of the vapor saturation method. When it needs a long time to saturate water with the solute vapor, as is the case with fluoranthene and pyrene, the temperature difference between the solute reservoir and the solubility flask should be small. Otherwise, the concentration of CyD in the solubility flask increases gradually during the vapor circulation period due to vaporization of water in the solubility flask; the vapor is to be condensed in the solute reservoir. This is a limitation to the vapor saturation method.

The complex formation constants of fluoranthene and pyrene have been determined by keeping the temperature of the solute reservoir equal to that of the solubility flask. On the assumption that the 2:1 (guest:host) complexations are not involved in the fluoranthene-β- or -γ-CyD or the pyrene-β-CyD system, $(S_t-S_0)/S_0$ was plotted against L_t, and linear relationships were found for these systems. In the case of pyrene, the solubility has been measured also by the shake-flask method in the presence and absence of CyD, based on which the formation constants were estimated to check the reliability of the vapor saturation method.

Table 2. Complex Formation Constants (25 °C)

Formation constant/dm^3 mol^{-1}		
β-CyD		γ-CyD
This work	Literature data	This work
Guest: Naphthalene		
900±200	$(6.3\pm0.4)\times10^{2}$ [15] 685,[16] 730,[17] 850[18]	
Guest: Fluorene		
$(1.49\pm0.03)\times10^{3}$	$(1.1\pm0.1)\times10^{3}$ [19] $(1.2\pm0.1)\times10^{3}$ [20]	178±4
Guest: Fluoranthene		
160±10		170±10
Guest: Pyrene		
160 (175±25)[a]	7.6,[19] 44,[21] 128,[22] 190,[23] 277,[24] 528.3±48.9 (459.0±14.7)[7]	

a) The value given in the parentheses was determined by the shake-flask method.

The complex formation constants determined in this work are listed in Table 2. Using the vapor saturation method, we have also studied the naphthalene-β-CyD system, which has so far been studied by many workers with different techniques. The present result for this system is somewhat large compared to the literature data, but, in view of the large variation with our value, the difference is not so large. Complexations of fluorene and fluoranthene with α-CyD were studied. In the presence of α-CyD, however, the solubility increases of these solutes were found to be too small to be given significant values. There have been published no data concerning CyD complexes with fluoranthene or γ-CyD complexes with fluorene, while as for pyrene considerably abundant data are available. They are, however, scattered over so wide a range that the present result cannot be compared with them.

Because of its characteristic property, pyrene has attracted interests of many workers. This substrate associates with each other to form a dimer in some circumstances and its excimer fluorescence reflects the microenvironment where the dimer exists.[8,9] It is now well-known that pyrene forms a 1:1 complex with β-CyD, whereas two pyrene molecules can together enter the cavity of γ-CyD (excimer emission from pyrene is markedly enhanced by γ-CyD). There are, however, some arguments with respect to whether the excimer is formed in a three-component (two-pyrene-one-γ-CyD) complex or a four-component (two-pyrene-two-γ-CyD) complex. Some pieces of experimental evidence presented by recent investigators have supported the latter case.[10–12] We cannot decide it experimentally by the present vapor saturation method which is correct, the three- or four-component complex. This is another limitation to the vapor saturation method.

According to an opinion of recent investigators, we assume that pyrene-γ-CyD exists only in the four-component complex and employ, instead of Eq. 3, the equilibrium

$$2SL \rightleftarrows S_2L_2 \quad K_4. \tag{6}$$

Then, Eq. 5 is to be modified to

$$(S_t-S_0)/S_0=K_1L_t+K_1K_2L_t^2+2K_1{}^2K_4S_0L_t^2. \tag{7}$$

With the solubility flask kept at 25 °C, the solubility at 25 °C and the equilibrium solubility at 24.0 °C have been measured as a function of γ-CyD concentration by the vapor saturation method; this small temperature difference between the solubility flask and the solute reservoir was found not to cause any serious changes in CyD concentrations in the solubility flask. In Fig. 5 is plotted $(S_t-S_0)/S_0$ vs. L_t. By the curve-fitting method, we have estimated K_1, K_2, and K_4. The results are listed in Table 3 together with literature data.

We have also estimated K_1 by the shake-flask method. The solubility of pyrene at 25 °C, S_t, was measured over the range of γ-CyD concentration, L_t, (0.15—2.50)×10^{-3} M (M=mol dm^{-3}). The plot of $(S_t-S_0)/S_0$ against L_t was found to be parabolic similarly to the curve shown in Fig. 5. A curve-fitting treatment according to Eq. 7 has evaluated the two terms K_1 and $K_1K_2+2K_1{}^2K_4S_0$. We cannot evaluate K_2 or K_4 in the second term because S_0 cannot be changed.

The K_1 values thus determined by the two methods are found to be in resonable agreement with each other.

As can be seen from the K_4 being very large, the 2:2 complex of pyrene with γ-CyD is easily formed in the

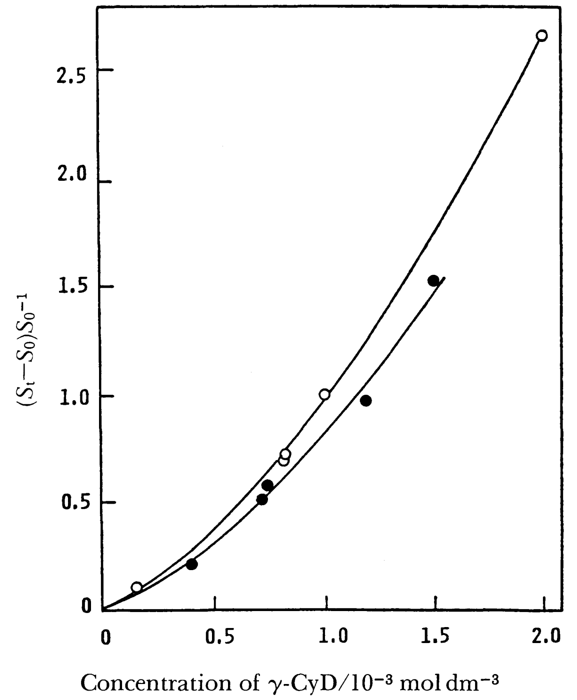

Fig. 5. $(S_t-S_0)/S_0$ vs. L_t plots for pyrene -γ-CyD system. The temperature of solute was set at 25.0 °C (○) or at 24.0 °C (●), while that of solvent water was kept constant at 25.0 °C.

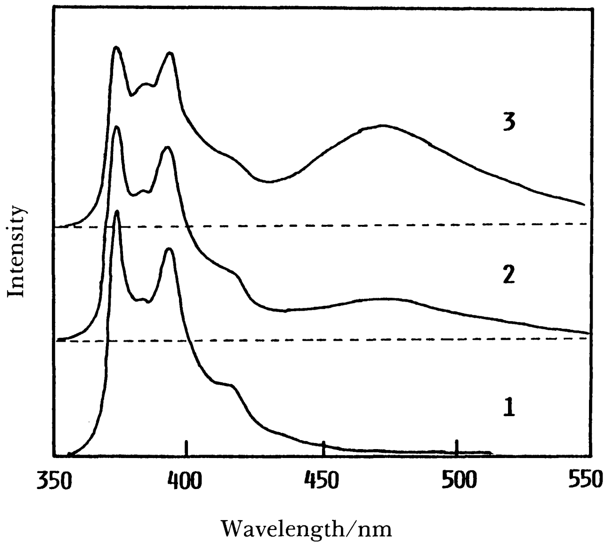

Fig. 6. Fluorescence spectra of aqueous pyrene solutions prepared by vapor saturation method in the absence (1) and presence of 7.04×10^{-4} (2) and 3.68×10^{-3} mol dm^{-3} (3) γ-CyD.

Table 3. Complex Formation Constants of Pyrene with γ-CyD (25 °C)

	Formation constant[a]/dm^3 mol^{-1}					
	This work	Literature data				
		Ref. 23	Ref. 24	Ref. 11	Ref. 22	Ref. 7
K_1	560±40 (552)[b]	20	35	300	399	1104±66 (1146±9)
K_2	365	200	310	170		
K_3		5×10^6	1.9×10^7			
K_4	4.45×10^5		1.1×10^8	1.3×10^6		

a) K_1, K_2, K_3, and K_4 refer to the formation constants of 1:1, 1:2, 2:1, and 2:2 (pyrene: γ-CyD) complexes, respectively. b) The value given in the parentheses was determined by the shake-flask method.

aqueous medium. Ueno et al.[12] have presented a model of the complex and pointed out that a hydrophobic interaction between two pyrene molecules is responsible for its dimer formation. In the present work, too, the excimer was confirmed. The fluorescence spectra observed in the aqueous solutions of pyrene prepared by the vapor saturation method in the presence of γ-CyD of relatively high concentrations, are shown in Fig. 6.

Fluorene and pyrene are suitably fitted in the cavities of β- and γ-CyD, respectively, which may be responsible for the large K_1 values observed for fluorene-β-CyD and pyrene-γ-CyD complexations.

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