Preparation of Cyclodextrin Precipitates with Volatile Hydrocarbons in Aqueous Media, and Determination of Hydrocarbon/Cyclodextrin Molar Ratios in the Precipitates

Isao SANEMASA,* Yue WU, Yuuki KOIDE, Masako SHIGENAGA, Kazunari ISHIBASHI, and Toshio DEGUCHI
Department of Chemistry, Faculty of Science, Kumamoto University, Kurokami 2-39-1, Kumamoto 860

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A technique is proposed to prepare cyclodextrin (CyD) precipitates with volatile hydrocarbons. On introducing the vapor of a liquid or solid hydrocarbon into α-, β-, or γ-CyD in an aqueous solution and circulating it in a closed system, CyD can be easily precipitated. The CyD precipitates isolated from aqueous solutions were analyzed for the hydrocarbon/CyD molar ratio. The concentration of CyD in the supernatant aqueous solution was also measured. The technique has been applied at 25 °C to such hydrocarbons as *n*-alkanes (C_5 to C_{10}), cyclohexane, halobenzenes(fluoro-, chloro-, bromo-, and iodo-benzene), *p*-difluorobenzene, benzene, naphthalene, biphenyl, and diphenylmethane. Precipitates were also prepared in β-CyD solutions with the vapor of C_5—C_9 mixtures at various mixing ratios.

Cyclodextrins (CyDs) themselves are rather hydrophilic and moderately soluble in water, with saturation concentrations 0.149 (α), 0.0163 (β), and 0.179 M (γ) (1 M≡1 mol dm^{-3}) at 25 °C.[1] In the presence of particular organic substances, however, the solubility decreases depending on the nature of substance used. This decrease in solubility is mainly due to the formation of a CyD inclusion complex with the guest substance of low solubility in water. In view of this, the solubilities of CyDs in water in the presence of guest substances have been studied.[1]

Several workers prepared precipitates from aqueous CyD solutions by adding guest substances, and studied the CyD : guest molar ratios in the precipitates.[2,3] The precipitates are easily prepared with water-soluble guests, but such preparations are difficult with guests sparingly soluble in water. A "shake-flask" technique is usually employed in such cases: An excess quantity of a guest substance, which is present in the liquid or solid phase, is added to water containing CyD, the mixture is mechanically shaken, and then the CyD precipitate is separated.

It seems difficult to isolate CyD precipitates in a pure form by this technique, because the excess guest phase is present in the solution. This problem is serious under such circumstances that the specific gravity of the guest phase is larger than that of water. Saenger prepared CyD precipitates with substances of low solubility in water by dissolving them in organic solvents such as ether, chloroform, benzene, and the like.[4] The precipitates thus prepared were washed with an organic solvent to remove any adhering substances. However, as was pointed out by Schlenk and Sand,[3] washing with organic solvents may partly extract the guest substance included in CyD and the solvent molecules themselves may be included.

We recently found that β-CyD is readily precipitated from an aqueous solution on introducing a cyclohexane vapor into the solution and circulating it in a closed system.[5] In that work, our object was to see whether hydrophobic solutes present in the aqueous solution can be coprecipitated by β-CyD. The present work is concerned with a further application of the vapor-introducing and circulating technique to α- and γ-CyD as well as β-CyD. Here we used a vapor of volatile liquid or solid hydrocarbons to make the technique applicable to prepare pure CyD precipitates with such guest hydrocarbons. The solubility of such hydrocarbons in water is quite low, so that it is necessary to supply the hydrocarbon molecules continuously into the aqueous solution to get CyD precipitates. The technique proposed here is effective for this purpose.

Experimental

Reagents. All of the reagents used were of analytical reagent grade. Deionized distilled water was used throughout the experiments. An appropriate amount of α-, β-, or γ-CyD of guaranteed grade (Nacalai Tesque Co.), dried over phosphorus pentoxide under vacuum, was dissolved in water.

Preparation of CyD precipitates. The apparatus used to prepare CyD precipitates was described in a previous paper.[5] The apparatus consists of three parts: a cylindrical separatory funnel (6.5 cm i.d., 600 cm^3 capacity), a test tube (2.8 cm i.d.×25 cm), and a Viton diaphragm airpump (Iwaki Co., AP-032Z). The three parts were connected with PTFE tubings to make a closed system. A CyD solution of less than 500 cm^3 was placed in the funnel and ca. 10 cm^3 of a liquid hydrocarbon was placed in the test tube. In the case of solid hydrocarbon, the crystal was roughly powdered in an agate mortar, and dispersed in ca. 10 cm^3 water in the test tube. By operating the airpump, air was introduced into the liquid phase in the test tube via a glass capillary, and the resulting hydrocarbon vapor was dispersed in the CyD solution via a glass capillary, and circulated in the closed system. The flow rate was controlled by changing the voltage applied to the airpump.

The temperature of the CyD solution was held at 25.0±0.1°C and that of liquid hydrocarbon at 24.5±0.1°C by circulating water from two separate thermostated water baths through each jacket surrounding the funnel and the test tube. It is necessary to hold the temperature of the liquid hydrocarbon phase lower than that of the CyD solution, otherwise condensation of hydrocarbon occasionally takes place in the CyD solution; condensation of water occurs in the hydrocarbon phase under such conditions, but this makes no problem. For a solid hydrocarbon, it is unnecessary to lower the temperature of the suspended water phase in the test tube than that of the CyD solution.

Treatment of CyD Precipitates and Measurements of CyD and Guest Hydrocarbons in the Precipitates. Vapor-circulation was continued for 6, 12, or 24 h, and within this period ca. 10 cm^3 portions of the turbid sample solution were withdrawn to centrifuge tubes (conical type with stopper) of 10 cm^3 capacity from the separatory funnel via a stopcock attached to its bottom. (In order to facilitate this sampling, the funnel position was slightly lowered so as to allow air to enter the funnel through its top opening.) After the sample solution was centrifuged for 10 min at 4000 rpm, the supernatant was removed, and a 5 cm^3 portion of cyclohexane was immediately added to the precipitate and then a small amount of water was added. The centrifuge tube was vigorously hand-shaken to disperse the contents; for this purpose, ultrasonic vibrations are effective. The dispersed contents was transferred into an extraction funnel with the aid of water, another 5 cm^3 portion of cyclohexane was added, and the mixture was shaken after diluting it to 100 cm^3 with water. The cyclohexane extract was analyzed, after dilution with cyclohexane, if necessary, with a Hitachi 100-50 spectrophotometer at an appropriate wavelength for each hydrocarbon.

After extraction with cyclohexane, ca. 10 cm^3 portion of the aqueous phase in the extraction funnel was transferred to a test tube and N_2 was bubbled through the solution to expel the dissolved cyclohexane. Then, after dilution with water, if necessary, the solution was applied to a Shodex Model SE-11 differential refractometer (Showa Denko Co.), and the amount of CyD in the precipitate was determined based on a calibration curve made up with a series of CyD aqueous solutions of known concentration. The concentration of CyD in the supernatant solution removed from the centrifuge tube was also determined; prior to refractometric measurements, aeration with N_2 was indispensable, because the supernatant solution was nearly saturated with the guest hydrocarbon.

For cyclohexane and *n*-alkanes used as guest hydrocarbons, chloroform was employed as an extractant and the extracts were analyzed with a Shimadzu GC-14A gas chromatograph with a 3.2 mmϕ×2.1 m column packed with DEGS-HG (Wako Pure Chemicals Co., 80—100 mesh). As internal standards, decane was used for C_5—C_9, and cyclohexane and octane for C_{10}. Aqueous solutions after extraction with chloroform cannot be used for CyD determinations, because it is difficult to expel ethanol completely. Ethanol, spiked into chloroform as a stabilizer by manufacturing, dissolves in the aqueous solution during extraction and interferes with refractometric measurements of CyD. Therefore, refractometric measurements of CyD of precipitates were made on a separate 10 cm^3 portion of the turbid CyD sample solution after dilution to 100 cm^3 with water and aeration with N_2.

Results and Discussion

Experimental Conditions to Prepare CyD Precipitates. The solubility of various volatile nonelectrolytes in water has been observed to obey the Henry's law. Namely, a liquid or solid solute itself is not necessarily in contact with water to prepare an aqueous solution saturated with the solute, and the vapor of solute can be used instead. Based on this idea, we proposed a method which involves a circulation of a solute vapor in a closed system through water. This technique has proved useful for saturating water with various liquid or solid hydrocarbons in a relatively short time without any need to remove the excess solute phase (solute droplets or microcrystals).[6—8] The technique has been used in this work to prepare CyD precipitates. By applying this technique, it is recommended to hold the temperature of the liquid hydrocarbon phase slightly lower than that of the CyD solution. Otherwise, as the circulation is continued, an increase in the molar ratio of the guest hydrocarbon to CyD in the precipitate is occasionally observed (Fig. 1). This is probably due to adsorption of hydrocarbon droplets to the precipitate. Minute droplets of the liquid hydrocarbon may occasionally appear in the CyD solution due to condensation of the hydrocarbon vapor.

The effect of the initial CyD concentration on the guest/CyD molar ratio and the CyD concentration in

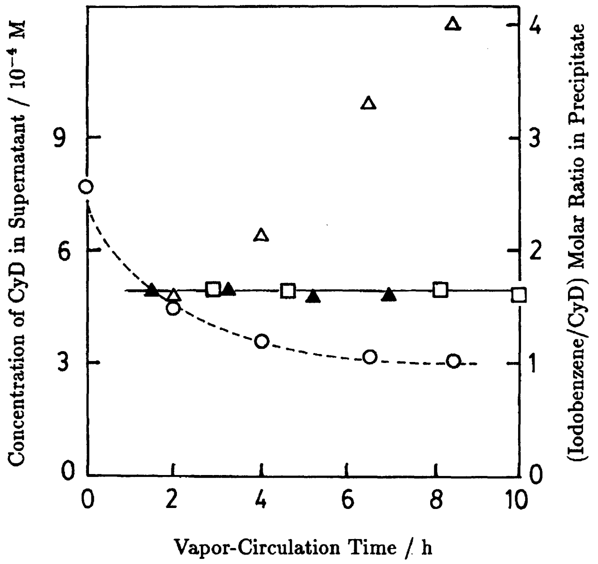

Fig. 1. Change in the CyD concentration in the supernatant solution (○) and the guest/CyD molar ratio in the precipitate as a function of vapor-circulation time for the iodobenzene (guest) –β-cyclodextrin (host) system. The temperature of the liquid iodobenzene phase was set at 24.5 (□) or at 25.0 °C (△, ▲) equal to that of the CyD solution. Note that different molar ratios are observed under equivalent conditions (△, ▲).

the supernatant solution were studied on *n*-alkane–β-CyD systems (Table 1). After a prescribed vapor-circulation period, the β-CyD concentration in the supernatant aqueous solution reaches a constant value which depends on the guest used but almost independent of the initial CyD concentration. The guest/CyD molar ratio is also independent of the initial CyD concentration. The amount of the CyD precipitate itself depends of course on the initial CyD concentration.

The aqueous solution becomes turbid owing to CyD precipitation just after the introduction of the vapor of liquid hydrocarbon into the CyD solution; in the case of a solid hydrocarbon, it takes more time. It can be judged from a change in the CyD concentration of the supernatant solution during the course of vapor-circulation whether the precipitation is almost complete. During the course, the guest/CyD molar ratio in the precipitate remains constant (Table 2).

Guest Hydrocarbon/CyD Molar Ratio in Precipitate. The vapor-circulation technique was applied to the guest hydrocarbons listed in Table 3. Normal alkanes (C_5 to C_{10}) and cyclohexane were studied only in β-CyD solutions. Biphenyl[9] and diphenylmethane[10] have been reported to associate with α-CyD, but there observed no precipitation for these host–guest combinations even after a 10-h circulation of the guest vapor at relatively high α-CyD concentrations.

The guest/CyD molar ratio in the precipitate rarely takes an integer. A fractional molar ratio has been observed for CyD precipitates with various guest molecules.[2,3]

It is important to discuss here the errors inherent in the determination of guest/CyD molar ratios. The errors mainly originate from the water content in the CyD precipitate; it is difficult to remove water completely from the precipitate by centrifugation. Let us take α-CyD–benzene as an example (see Table 2). In this case, the volume of the precipitate, after centrifugation, is ca. 0.2 cm^3, which can be read off by the scale on the conical type centrifuge tube. The α-CyD concentration in the supernatant aqueous solution is 1.2×10^{-2} M, and hence, if the precipitate contains water maximally 0.2 cm^3, the contribution of α-CyD in the water contained in the precipitate (2.4×10^{-3} mmol) to the precipitate itself (0.15 mmol) is 2%. The solubility of benzene in water, that is largest among the guest hydrocarbons used in this study, is 2.1×10^{-2} M at 25 °C, and hence, its contribution amounts to 2 to 4%.

Table 1. Effect of Initial Concentration of β-CyD on the Concentration of β-CyD in the Supernatant Aqueous Solution, and the Guest/CyD Molar Ratio in the Precipitate (*n*-Alkanes as Guest Hydrocarbons)[a]

Guest	Initial Concentration of β-CyD/10^{-3} M	Concentration of β-CyD in supernatant/ 10^{-3} M	Molar ratio of guest/CyD in precipitate
C_5	3.08	2.8	1.1
	3.80	2.9	1.0
	4.47	2.9	1.1
	4.99	2.7	1.1
C_7	2.85	2.3	0.87
	3.25	2.5	0.86
	3.86	2.2	0.90
	4.50	2.4	0.90
C_9	3.13	2.0	0.59
	3.77	2.0	0.69
	4.07	1.8	0.66

a) Vapor-circulation time; 1 h (C_5), 2 h (C_7), 4 h (C_9).

Another factor which may cause errors in determining guest/CyD molar ratios is that the CyD precipitate may partly dissolves in the supernatant aqueous solution during the course of centrifugation. The temperature of the room where centrifugal operations were carried out was held constant at 25±2 °C, but it was difficult to suppress a rise in temperature of the solution being centrifuged; the temperature was found to rise to ca. 31 °C at the end of centrifugation. We did not assess how and to what extent guest/CyD molar ratios of precipitates were influenced by this temperature increase. Vaporization of the guest hydrocarbon during centrifugation does not seem to occur, because the centrifuge tube was almost full of a sample solution and tightly stoppered.

As mentioned above, a fractional molar ratio of 1.1 determined in this work for α-CyD–benzene is not due to the errors originated from the water content in the precipitate. If the molar ratio of 1.0 is true for this host–guest system, the 10% excess may be attributed to the coprecipitation phenomenon, that is, benzene molecules dissolved in aqueous solution are brought to the precipitate by adsorption. Our previous work shows that CyD (we used β-CyD) can coprecipitate various organic substances.[5] However, this may not apply to all the circumstances. Organic substances present in CyD solutions in relatively small amounts are certainly coprecipitated with CyD, but it is not certain that relatively large amounts of guest hydrocarbons, say 10% of the benzene precipitated, are coprecipitated by adsorption to the CyD precipitates.

In contrast to the α-CyD–benzene system, there are several systems which guest/CyD molar ratios are just below an integral value: α-CyD–(chlorobenzene, bromobenzene, iodobenzene, and *p*-difluorobenzene), β-CyD–(benzene, fluorobenzene, biphenyl, diphenylmethane, *p*-difluorobenzene), and γ-CyD–naphthalene. In these cases, the following explanation may be possible. Take the γ-CyD–naphthalene as an example. If γ-CyD can accept maximally two naphthalene molecules inside its cavity, 90% of the CyD molecules precipitated may exist as 2 : 1 (guest : CyD) and 10% as 1 : 1, thus the overall stoichiometry becomes 1.9 : 1 (guest : CyD). We have, however, no experimental evidence to support this

Table 2. Effect of Vapor-Circulation Time on the Concentration of CyD in the Supernatant Aqueous Solution, and the Guest/CyD Molar Ratio in the Precipitate for Typical CyD–Guest Systems

Vapor-circulation time/h	Concentration of CyD in supernatant /M	Amount of CyD in precipitate /mmol	Amount of guest in precipitate /mmol	Molar ratio of guest/CyD in precipitate
α-CyD–Benzene (C_0=2.71×10^{-2} M)[a]				
0.5	1.2×10^{-2}	0.15	0.16	1.1
1	1.2×10^{-2}	0.16	0.17	1.1
2	1.2×10^{-2}	0.16	0.17	1.1
3	1.1×10^{-2}	0.16	0.17	1.1
5	1.1×10^{-2}	0.17	0.18	1.1
8	1.1×10^{-2}	0.17	0.18	1.1
13	1.1×10^{-2}	0.17	0.18	1.1
24	1.0×10^{-2}	0.18	0.20	1.1
β-CyD–Fluorobenzene (C_0=2.09×10^{-3} M)				
1.5	5.0×10^{-4}	0.015	0.036	2.4
2.5	4.7×10^{-4}	0.014	0.034	2.4
4	4.5×10^{-4}	0.014	0.033	2.4
5	4.7×10^{-4}	0.012	0.028	2.3
6	4.9×10^{-4}	0.012	0.029	2.4
12	4.4×10^{-4}	0.012	0.027	2.3
γ-CyD–Naphthalene (C_0=9.81×10^{-3} M)				
5	4.5×10^{-4}	0.098	0.19	1.9
15	2.6×10^{-4}	0.10	0.19	1.9
19	2.5×10^{-4}	0.10	0.18	1.8
24	2.6×10^{-4}	0.099	0.19	1.9

a) C_0 denotes the initial CyD concentration in water.

idea.

Guest hydrocarbon/CyD molar ratios determined in this work are not consistent with those by Cramer and Henglein[2)] who used the shake-flask technique. Their data related to the present guest-host systems are as follows: benzene–α (0.12), –β (0.2), and –γ (0.3); fluorobenzene–α (0.47), –β (0.32), and –γ (0.55); chlorobenzene–α (1), –β (0.42), and –γ (0.54); bromobenzene–α (1), –β (1), and –γ (0.44); iodobenzene–α (0.8), –β (1), and –γ (1); naphthalene–β (0.77) and –γ (0.27); cyclohexane–α (0.5), –β (1.33), and –γ (2); biphenyl–α (0.35), –β (0.52), and –γ (0.13). These guest/CyD molar ratios are considerably smaller than our estimations. Cramer and Henglein washed and dried the CyD precipitates, prior to their measurements, to remove the excess guest hydrocarbon. Because of high volatility, the loss of guest molecules associated with CyD will occur during such operations and this may lead to a lower guest/CyD molar ratio.

Schlenk and Sand, who prepared α- and β-CyD precipitates with fatty and benzoic acids, employed a distillation technique to remove free acids incorporated into the precipitates while keeping back acids associated with CyD.[3)] Their data for the acid/β-CyD molar ratio in the precipitate are as follows: The ratio for fatty acids, which depends on the C number of acid, decreases from 1.6 (C_6) to 0.64 (C_{10}), that for benzoic acid is 1.1, and that for *p*-iodobenzoic acid is 0.97. The data for fatty acids are in reasonable agreement with the present work on *n*-alkanes, but those for benzoic and *p*-iodobenzoic acids, if we take these acids as analogues of benzene and iodobenzene, respectively, are inconsistent with ours. It is well established by X-ray crystallographic studies that benzene derivatives are included in the cavity of α-CyD.[11,12)] Considering the thickness of the benzene ring of 3.4 Å,[13)] the cavity of β-CyD (ca. 7 Å in diameter) can afford to accept two benzene molecules piled on together.

CyD Concentration in Supernatant Aqueous Solutions. The solubilities of CyDs in water in the presence of hydrocarbons have been determined by French et al.[1)] Their data are in fair agreement with ours concerning the CyD concentrations in supernatant aqueous solutions. It is interesting to see whether there is any relationship between the CyD concentration in the supernatant solution and the solubility of guest hydrocarbon in water or the 1 : 1 association constant of the guest–CyD. These are plotted in Figs. 2 and 3. No clear correlations are seen, but very roughly, the concentration of CyD in the supernatant solution increases with a decrease in the guest solubility (for α- and β-CyDs) and with an increase in the association constant

Table 3. CyD Precipitates and Supernatant Aqueous Solution Obtained by Guest Hydrocarbon Vapor Circulation Technique

Guest hydrocarbon	Type of CyD	Initial concentration of CyD/M	Concentration of CyD in supernatant solution/M	Guest/CyD molar ratio of precipitate	Solubility of guest hydrocarbon in water$^{a)}$/M	Association constant$^{b)}$ K_1/M^{-1}	K_2/M^{-1}
Pentane	β	$(3.08—4.99)\times10^{-3}$	2.8×10^{-3}	1.1	5.8×10^{-4}	50	
Hexane	β	$(3.15—5.02)\times10^{-3}$	2.1×10^{-3}	1.1	1.3×10^{-4}	60	
Heptane	β	$(2.85—4.50)\times10^{-3}$	2.3×10^{-3}	0.88	2.4×10^{-5}	69	
Octane	β	$(2.45—2.91)\times10^{-3}$	1.4×10^{-3}	1.0	6.2×10^{-6}	76	
Nonane	β	$(3.13—4.07)\times10^{-3}$	1.9×10^{-3}	0.65	1.3×10^{-6}	90	
Decane	β	$(3.84—4.75)\times10^{-3}$	2.7×10^{-3}	0.78	1.1×10^{-7}		
Cyclohexane	β	1.90×10^{-3}	7.4×10^{-4}	1.4	1.90×10^{-3}	156	
Benzene	α	2.71×10^{-2}	1.0×10^{-2}	1.1		17	
	β	5.43×10^{-3}	9.1×10^{-4}	1.9	2.05×10^{-2}	120	
	γ	4.00×10^{-3}	9.8×10^{-4}	2.7		12	
Fluorobenzene	α	1.97×10^{-2}	1.3×10^{-2}	1.1		34	1.7
	β	2.05×10^{-3}	4.0×10^{-4}	1.9	1.58×10^{-2}	70	
	γ	1.13×10^{-2}	3.1×10^{-4}	3.0		14	
Chlorobenzene	α	2.40×10^{-2}	1.7×10^{-2}	0.94		100	8
	β	2.22×10^{-3}	3.5×10^{-4}	2.0	4.37×10^{-3}	160	
	γ	7.90×10^{-3}	1.1×10^{-4}	2.3		33	
Bromobenzene	α	3.00×10^{-2}	2.4×10^{-2}	0.91		510	3.1
	β	2.22×10^{-3}	3.5×10^{-4}	2.0	2.82×10^{-3}	310	
	γ	1.00×10^{-2}	1.1×10^{-4}	2.3		39	
Iodobenzene	α	7.00×10^{-2}	3.6×10^{-2}	0.90		1100	1
	β	1.02×10^{-3}	3.0×10^{-4}	1.6	1.66×10^{-3}	800	
	γ	1.00×10^{-2}	1.8×10^{-4}	2.3		30	
p-Difluorobenzene	α	2.00×10^{-2}	1.2×10^{-3}	0.98		20	4.9
	β	2.00×10^{-3}	6.1×10^{-4}	1.9	1.19×10^{-2}	40	110
	γ	1.12×10^{-2}	2.5×10^{-4}	2.3		28	
Naphthalene	α	5.64×10^{-2}	4.8×10^{-2}	0.30		83	23
	β	2.10×10^{-3}	2.9×10^{-4}	1.3	2.42×10^{-4}	630	
	γ	9.81×10^{-3}	2.5×10^{-4}	1.9		130	
Biphenyl	α	5.64×10^{-2}	No precipitation			24	120
	β	1.06×10^{-2}	9.4×10^{-4}	0.90	4.30×10^{-5}	2100	
	γ	1.02×10^{-2}	6.3×10^{-3}	1.5			
Diphenylmethane	α	2.72×10^{-2}	No precipitation			19	13
	β	2.09×10^{-3}	1.2×10^{-4}	0.98	8.38×10^{-6}	11000	
	γ	8.93×10^{-3}	4.1×10^{-4}	1.7			

a) Solubility data at 25 °C are taken from "Solubility Data Series," ed by A. S. Kertes, Pergamon Press, Oxford (1989), Vols. 37 and 38. The solubility of *n*-alkane in water at 25 °C measured in this work by the present vapor-circulation technique; 5.86×10^{-4} (C_5), 1.50×10^{-4} (C_6), 3.22×10^{-5} (C_7), 9.36×10^{-6} (C_8), and 2.24×10^{-6} M (C_9). b) Association constants, K_1 (1 : 1) and K_2 (1 : 2), of (guest : CyD) at 25 °C are taken from our previous works (*Bull. Chem. Soc. Jpn.*, **60**, 2059 (1987), **61**, 1163 (1988), **63**, 1246, 2814 (1990), and **64**, 480, 2705 (1991), those of biphenyl and diphenylmethane are unpublished).

(for α-CyD).

CyD precipitates with Mixed Guest Hydrocarbon. In this work, CyD precipitates of mixed hydrocarbons were also prepared: The vapor of the mixed hydrocarbon was introduced in an aqueous CyD solution and circulated in the closed system. Here we used mixtures of the homologs, C_5 and C_9, at various ratios as a mixed guest and β-CyD as a host. The 1 : 1 association constant of C_9–β-CyD is roughly twofold larger than that of C_5–β-CyD,[14] while the solubility in water of C_5 is much larger than that of C_9 (see Table 3). The C_5—C_9 mixture is, therefore, an interesting combination to study which, in the presence of two different guest hydrocarbons, is the predominant factor governing the guest/CyD molar ratio in the CyD precipitate, the association constant (affinity of guest for CyD) or the solubility (population of guest molecule). The concentrations of C_5 and C_9 in water were estimated from their mole fractions in the mixed hydrocarbon phase by assuming an ideal mixing.

The mole fraction of C_5 in the mixed hydrocarbon was set in the range of 0.21 to 0.70 (Table 4). The (C_5+C_9)/CyD molar ratio in the precipitate was found in a range of 0.71 to 1.1. The C_5 mole fraction in the guest precipitated was in a range of 0.39 to 0.65 and found to be almost parallel to that in the mixed hydrocarbon except at the smallest C_5 molar ratio in the mixture. This seems to indicate that the composition of the guest in the CyD precipitate is determined by the concentration of the guest in the CyD solution. It

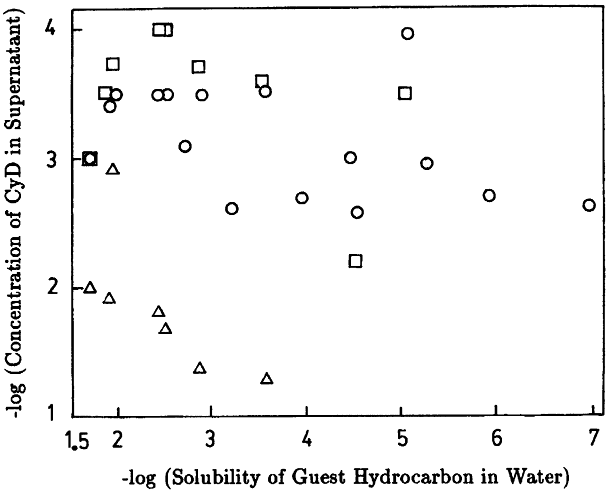

Fig. 2. Relationship between the CyD concentration in supernatant and the solubility of guest hydrocarbon in water for α-CyD (△), β-CyD (○), and γ-CyD (□).

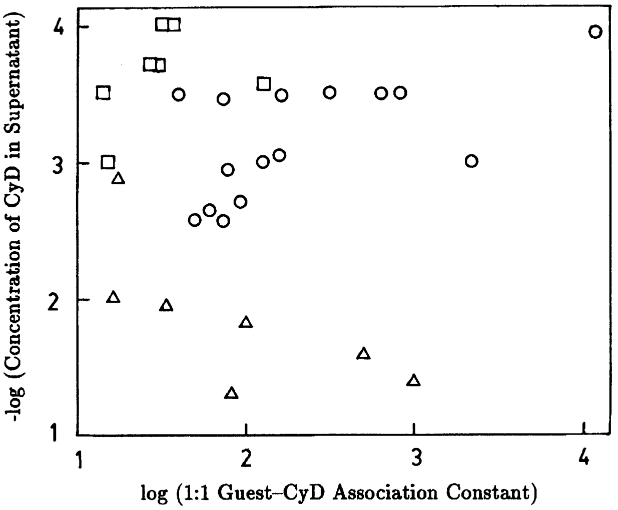

Fig. 3. Relationship between the CyD concentration in supernatant and the 1 : 1 (guest : CyD) association constant for α-CyD (△), β-CyD (○), and γ-CyD (□).

Table 4. Preparation of β-CyD Precipitates with C_5—C_9 Mixed Guest Hydrocarbon

Mole fraction of C_5 in mixed hydrocarbon[a]	Molar ratio in precipitate $(C_5+C_9)/CyD$	Mole fraction of C_5 in precipitate	Concentration of guest in water[b]	
			C_5 10^{-4} M	C_9 10^{-6} M
0.21	0.71	0.39	1.6	1.6
0.40	0.75	0.49	2.2	1.4
0.53	0.90	0.48	2.5	1.3
0.70	1.1	0.65	4.4	0.56

a) Vapor of the mixed hydrocarbon was circulated for 3 h, and then the composition of the mixture was determined.
b) We employed our data as the solubility of C_5 and C_9 in water (see footnote to Table 3) and assumed that the concentration of each constituent component in water is directly proportional to its mole fraction in the mixed hydrocarbon phase).

should be noted, however, that even at a C_5 mole fraction of 0.70 in the mixed hydrocarbon, C_9 molecules are incorporated into the CyD precipitate to a considerable extent, though the concentration of C_9 in the CyD solution is ca. three orders-of-magnitude smaller than that of C_5. This observation indicates that the affinity of guest molecules to CyD plays an important role in precipitating CyD. In order to generalize, however, to what extent these affinity and population of guest molecules contribute to the composition in CyD precipitates, further studies are needed using various mixed hydrocarbon combinations.

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