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RETENTION CHARACTERISTICS OF SEVERAL COMPOUND CLASSES IN REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH β-CYCLODEXTRIN AS A MOBILE PHASE MODIFIER

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SUMMARY

β-Cyclodextrin was investigated as a mobile phase modifier in the reversed-phase liquid chromatography of polycyclic aromatic hydrocarbons, nitrogen heterocycles and hydroxyl aromatics. A wide range of β-cyclodextrin concentrations was employed, and the retention properties of the compound classes were compared in methanol–water and ethanol–water mobile phases at 25°C. β-Cyclodextrin–solute dissociation constants were obtained for the compounds in several methanol–water and ethanol–water mobile phase compositions, using the chromatographic data. The trends in the retention data and dissociation constants for the compound classes were discussed. Several conclusions were drawn concerning the mechanism of retention when β-cyclodextrin is present in the mobile phases. Also, comparisons were made between the structural features of the compounds and their capacity factors and dissociation constants.

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides, constructed from α-(1,4)-linked glucose units arranged in a torus[1]. The most common cyclodextrins are α-, β- and γ-cyclodextrin, containing six, seven and eight glucose units, respectively. Because the cavity of a CD molecule contains C–H groups and glucosidic oxygens, the interior part of the molecule is relatively hydrophobic[2]. Generally, the external part of the CD molecule is hydrophilic compared to its cavity. The hydrophilic nature of the external portion of the molecule is due to primary and secondary hydroxyl groups being located on the smaller and larger sides of the CD molecule, respectively. Cyclodextrins have the ability to form inclusion complexes with a variety of molecules. The formation of an inclusion complex depends upon shape, size and spatial geometry of the solute, the diameter of the CD cavity and other factors[1,2].

There are essentially two approaches for applying CDs in liquid chromatography (LC). The first involves the use of CDs bonded to silica gel, and the second involves the use of CDs as a mobile phase additive. Armstrong and DeMond[3] have reviewed cyclodextrin-bonded phases for LC separations. Tarr *et al.*[4] studied the influence of mobile phase alcohol modifiers on the separation of polynuclear aromatics

with a β-CD bonded phase column. However, relatively little work has been reported on the chromatographic aspects of CDs added to mobile phases when a C_{18} column is used. Armstrong[5] and Hinze and Armstrong[6] used CD mobile phase modifiers in thin-layer chromatography (TLC) for separating structural isomers. Debowski *et al.*[7,8] resolved enantiomers of mandelic acid and mandelic acid derivatives by using reversed-phase liquid chromatography (RPLC) for α- and β-CD inclusion complexes. Gazdag *et al.*[9] employed α-, β- and γ-CDs as mobile phase additives in RPLC for enantiomer separation of D,L-norgestrels. Gazdag *et al.*[10] added a mixture of CDs to the mobile phase to develop a simple optimization technique for the separation of isomeric compounds. They studied the effects of CD concentration, pH and ionic strength on the capacity factors of model compounds. Armstrong *et al.*[11] resolved a variety of racemic compounds, including drugs, nicotinoids, amino derivatives, sulfinates, metallocenes and crown ethers with β-CD in the mobile phase by RP-TLC.

The separation of structural isomers[12–14] and aromatic amino acids[15] has been achieved by the addition of CDs to the mobile phase. Bazant *et al.*[16] used either β-CD as the mobile phase additive or β-CD, chemically bonded to the stationary phase, for the separation of structural isomers of some biologically important hydroxy-, methoxy- and amino-substituted aromatic carboxylic acids by LC. They emphasized that CDs can be applied to the separation of compounds that are difficult to separate in other systems.

The formation of inclusion complexes between host/guest molecules is mainly responsible for altering solute retention in RPLC. There have been some reports relating the concentration of CD and stability constants of inclusion complexes to retention characteristics[17–21]. For example, Fujimura *et al.*[20] derived an equation relating capacity factor to CD concentration in the mobile phase and the stability constant of the CD complex. Zukowski *et al.*[19] derived a similar equation that relates the observed capacity factors to the total CD concentration.

Because very little retention data and mechanistic information have been published on the effects of β-CD added to the mobile phase in RPLC, the retention characteristics of several polycyclic aromatic hydrocarbons (PAHs), nitrogen heterocycles and aromatic hydroxyl compounds as a function of both β-CD concentration and organic modifier concentration were investigated. Furthermore, the dissociation constants of the inclusion complexes for the model compounds were obtained by using equilibrium concentrations of β-CD, and various mechanistic aspects of the chromatographic interactions with β-CD were investigated.

EXPERIMENTAL

Materials

The liquid chromatograph used was a Waters unit with a Model 6000A pump (Waters Assoc., Milford, MA, U.S.A.), a U6K injector, a dual-channel ultraviolet detector, set at 254 nm, and a dual-channel 10-mV strip-chart recorder. A Model FE Haake (Saddle Brook, NJ, U.S.A.) constant temperature bath was used to keep the temperature of the column at 25°C. The column employed was a μBondapack C_{18} (300 mm × 4 mm I.D.) purchased from Phenomenex (Torrance, CA, U.S.A.). PAHs and nitrogen heterocycles were obtained from commercial sources. The model compounds and β-CD were purchased from Aldrich (Milwaukee, WI, U.S.A.). Table I

lists the structures of the model compounds studied in this work. Methanol was of HPLC-grade and purchased from Baker (Phillipsburg, NJ, U.S.A.).

Methods

Methanol and water were prefiltered through a Millipore type FH 0.5-μm filter. β-CD was vacuum dried at 0.78 atm pressure at 75°C for 8 h prior to use. Then, β-CD was dissolved in purified water, and methanol was added to the β-CD solution. The maximum amount of β-CD that dissolved in all the mobile phases investigated was 6.81 g in 1 l of methanol–water (30:70). The largest analytical concentrations of β-CD in methanol–water mixtures of 30:70, 40:60, 50:50 and 60:40 were 6.0, 5.0, 4.0, 3.0 mM, respectively. The largest analytical concentration of β-CD in ethanol–water solvents was 5.0 mM. Eqn. 11 was used to calculate the equilibrium concentrations of β-CD. The formation constants for methanol and ethanol used in eqn. 11 were 0.32 and 0.93 M^{-1}, respectively[22]. The methanol–water mixtures used for the PAHs were 55:45, 60:40, 65:35, 70:30 and 75:25, and those for the nitrogen heterocycles were 50:50, 55:45, 60:40 and 65:35. The methanol–water compositions that were used for the hydroxyl aromatics were 30:70, 40:60, 50:50 and 60:40. The ethanol–water compositions used for the hydroxyl compounds were 35:65, 40:60, 45:55 and 50:50. The concentrations of all the samples injected were 1 mg/ml in methanol, except for the concentration of anthracene, which was 0.5 mg/ml. The temperature was kept constant at 25°C, and the column void volume was obtained by injecting a methanol solution of potassium nitrite.

RESULTS AND DISCUSSION

Theoretical considerations

The following equilibria occur when a solute (S) is involved in a reversed-phase chromatographic system with β-CD in the mobile phase:
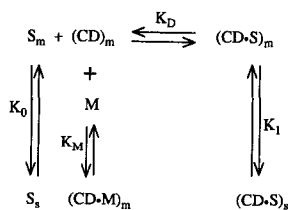
where subscripts m and s refer to the mobile and stationary phase, respectively, and M is the organic modifier. The distribution constants of the solute and the inclusion complex are given in eqns. 1 and 2, respectively.

$$K_0 = [S_s]/[S_m] \tag{1}$$

$$K_1 = [(CD \cdot S)_s]/[(CD \cdot S)_m] \tag{2}$$

The dissociation constant of the inclusion complex is given in eqn. 3, and

$$K_D = [S_m][(CD)_m]/[(CD \cdot S)_m] \tag{3}$$

TABLE I

NAMES AND STRUCTURES OF MODEL COMPOUNDS

Compound	Structure	Compound	Structure
(1) Naphthalene		(12) 1-Indanol	
(2) Biphenyl		(13) 5-Indanol	
(3) Acenaphthene		(14) 1,7-Dihydroxynaphthalene	
(4) Phenanthrene		(15) 2,3-Dihydroxynaphthalene	
(5) Anthracene		(16) o,o'-Biphenol	
(6) Quinoline		(17) p,p'-Biphenol	
(7) Isoquinoline		(18) 1-Naphthol	
(8) Benzo(f)quinoline		(19) 2-Naphthol	
(9) Benzo(h)quinoline		(20) 2-Phenylphenol	
(10) 1,3-Dihydroxybenzene		(21) 3-Phenylphenol	
(11) 1,4-Dihydroxybenzene		(22) 4-Phenylphenol	

the formation constant for the formation of $(CD \cdot M)_m$ is given in eqn. 4:

$$K_M = \frac{[(CD \cdot M)_m]}{[(CD)_m][M]} \tag{4}$$

The capacity factor (k') of the solute is defined as

$$k' = \varphi([S_s] + [(CD \cdot S)_s])/([S_m] + [(CD \cdot S)_m]) \tag{5}$$

where φ is the phase ratio of the column. Since the external part of the CD is hydrophilic, the interaction between CD and the stationary phase is considered negligible[20]. Thus, eqn. 5 can be written as

$$k' = \varphi[S_s]/([S_m] + [(CD \cdot S)_m]) \tag{6}$$

Substituting into eqn. 6 for $[S_s]$ and $[(CD \cdot S)_m]$ by using eqns. 1 and 3, respectively, results in

$$k' = \varphi K_0 K_D/(K_D + [(CD)_m]) \tag{7}$$

Since $k'_0 = \varphi K_0$, where k'_0 is the capacity factor with no β-CD present, eqn. 7 is written as

$$1/k' = 1/k'_0 + [(CD)_m]/k'_0 K_D \tag{8}$$

Eqn. 8 is similar to the equation derived by Fujimura et al.[20]. However, the equilibrium concentration of β-CD appears in the equation instead of total β-CD concentration. The main reason for using the equilibrium concentration of β-CD is that methanol weakly competes with the solute for the formation of an inclusion complex with β-CD[22].

To obtain the equilibrium concentration of β-CD, the mass balance for CDs is used:

$$[(CD)_T] = [(CD)_m] + [(CD \cdot M)_m] + [(CD \cdot S)_m] + [(CD \cdot S)_s] \tag{9}$$

If it is assumed that the last two terms on the right side of eqn. 9 are negligible compared to the first two terms, because the solute concentration is very small, then the following equation results:

$$[(CD)_T] = [(CD)_m] + [(CD \cdot M)_m] \tag{10}$$

By substituting $[(CD \cdot M)_m]$ from eqn. 4 into eqn. 10 the following equation is obtained:

$$[(CD)_m] = [(CD)_T]/(1 + K_M[M]) \tag{11}$$

Zukowski et al.[19] used eqn. 11 to obtain the equilibrium concentrations of β-CD in reversed-phase chromatographic systems.

Dependence of capacity factors on organic modifier at a given β-CD concentration

Methanol (used as the main organic modifier in this work) competed somewhat with the solute in occupying the β-CD cavity. This aspect has not been considered in detail in chromatographic systems. Matsui and Mochida[22] determined the stability constants of α- and β-CD complexes for several alcohols, using a spectrophotometric method. According to their data, the association constant of methanol with β-CD was $0.32 \ M^{-1}$. The small value for the association constant shows that methanol interacts weakly with β-CD, and the interaction with β-CD can normally be considered a constant effect at a given methanol composition. However, because of the relatively large concentration of methanol used in the mobile phase, a substantial amount of methanol can interact with β-CD. This aspect will be discussed in more detail later. Fig. 1 illustrates the effect of methanol concentration on log k' for the PAHs in the
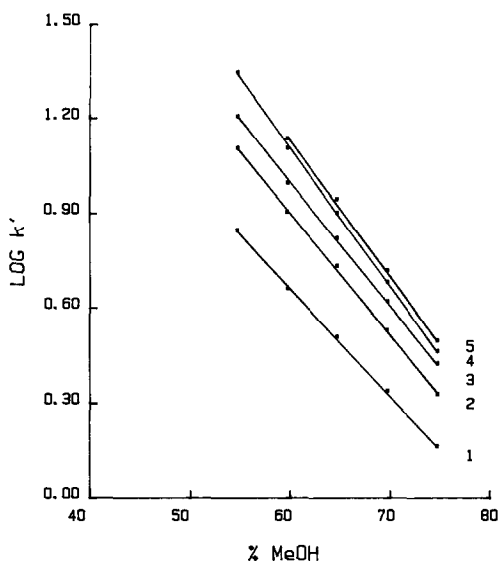
Fig. 1. Plot of log k' vs. percentage of methanol (MeOH) for PAHs on a C_{18} column with 1.5 mM β-CD.

presence of 1.5 mM β-CD. This figure shows that with increasing amounts of methanol the capacity factors decrease for a given compound in the presence of the same amount of β-CD, as would be expected. The other mobile phases containing different amounts of β-CD gave similar graphs. The dependence of log k' on the organic modifier concentration and β-CD concentration for quinoline, isoquinoline, benzo(f)quinoline [B(f)Q], and benzo(h)quinoline [B(h)Q] was also investigated. The same general retention characteristics were found for these compounds as were found for PAHs as the methanol concentration increased at a given β-CD concentration. The other set of compounds investigated was hydroxyl aromatics. Linear relationships were also obtained between log k' and methanol concentration in the presence and absence of β-CD for the hydroxyl aromatics.

Dependence of capacity factors on β-CD concentration
        The capacity factors of the PAHs and nitrogen heterocycles for different analytical concentrations of β-CD (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mM) in methanol–water were obtained. The methanol–water ratios employed were 75:25, 70:30, 65:35, 60:40 and 55:45. The k' values showed relatively small decreases for the PAHs on the C_{18} column with increasing concentration of β-CD. For example, the k' value of naphthalene changed from 3.51 with no β-CD in the mobile phase to 3.15 with 2.5 mM β-CD in the methanol–water (65:35) mobile phase. These results indicated that weak inclusion complexes were formed and that the uncomplexed PAHs interacted readily with the hydrocarbonaceous ligand of the stationary phase. The small change in the k' values was essentially the same for all the PAHs investigated. Pyrene had a long retention time with a methanol–water (65:35) mobile phase, and its chromatographic band was much broader compared to the other PAHs investigated. Pyrene only partially fits into the cavity of β-CD[23], and thus β-CD would have little influence on its retention under the experimental conditions used in this work.

Quinoline and isoquinoline showed little change in k' values with the addition of β-CD to the mobile phase. Also, addition of β-CD caused the bands to broaden to some extent. The broadening of the bands with the addition of β-CD could possibly be explained by an increased interaction between the nitrogen atom of the solute with residual silanol groups of the stationary phase[24]. However, additional work is needed to substantiate this. The broad bands and small k' values for quinoline and isoquinoline indicated that they did not form strong complexes with β-CD. The k' value for quinoline in the absence of β-CD was 1.36, whereas with 2.5 mM β-CD present it was 1.24. B(f)Q and B(h)Q gave well-defined chromatographic bands, although β-CD had little effect on the k' values for B(f)Q and B(h)Q. This indicates that these nitrogen heterocycles interacted with C_{18} more readily than with β-CD. For example, B(f)Q had a k' value of 3.13 with no β-CD, but a k' value of 3.01 with 2.5 mM β-CD.

The retention characteristics of thirteen hydroxyl aromatics over a wide range of methanol–water compositions with different concentrations of β-CD were also studied (Table I). This class of compounds showed much greater changes in k' values than the PAHs and nitrogen heterocycles. In fact, several of the hydroxyl aromatics showed rather dramatic changes in k' values as a function of β-CD concentration. Table II lists the capacity factors of the solutes in methanol–water (40:60) on a C_{18} column at 25°C with different amounts of β-CD. Fig. 2 shows the effect of β-CD on the retention of 2-, 3- and 4-phenylphenol and indicates that addition of β-CD to the mobile phase causes a substantial decrease in the retention of all three solutes. The relatively large changes in k' values occur because of the formation of strong inclusion complexes between the β-CD and the hydroxyl aromatics, and thus the solutes interact less with the hydrocarbonaceous ligand of the stationary phase.

TABLE II

k' VALUES OF HYDROXYL AROMATIC COMPOUNDS FOR METHANOL–WATER (40:60) WITH DIFFERENT CONCENTRATIONS (mM) OF β-CD

The names and structures of the compounds are given in Table I.

| Solute	β-CD (mM)					
	0.0	1.0	2.0	3.0	4.0	5.0
10	0.732	0.682	0.651	0.624	0.544	0.530
11	0.496	0.450	0.422	0.395	0.337	0.330
12	4.33	4.06	3.96	3.73	3.46	3.40
13	9.98	8.64	7.70	6.74	5.94	5.47
14	4.37	4.06	3.91	3.63	3.32	3.22
15	5.35	4.86	4.56	4.11	3.70	3.50
16	9.62	9.06	8.86	8.42	7.92	7.69
17	5.90	3.61	2.74	2.14	1.71	1.45
18	11.64	10.04	8.99	7.98	7.06	6.50
19	10.18	9.05	8.34	7.55	6.82	6.35
20	22.95	20.14	18.28	16.39	14.72	13.65
21	25.89	19.23	15.30	12.47	10.36	9.02
22	25.40	17.49	13.34	10.57	8.56	7.40
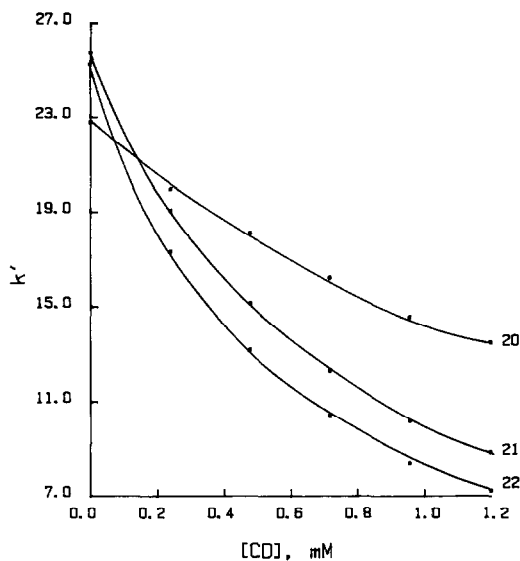
Fig. 2. Plot of k' values *vs.* equilibrium of β-CD concentration for 2-phenylphenol (20), 3-phenylphenol (21) and 4-phenylphenol (22) with methanol–water (40:60).

Dependence of K_D values on the methanol content

Eqn. 8 relates the capacity factor to the dissociation constant of the inclusion complex and the equilibrium concentration of β-CD and shows that a graph of $1/k'$ *vs.* $[(CD)_m]$ would give a straight line with an intercept of $1/k'_0$ and a slope of $1/k'_0 K_D$. Therefore, K_D values can be obtained by using eqn. 8. The linear relationship between $1/k'$ and β-CD concentration also indicates a 1:1 ratio between the solute and β-CD[20].
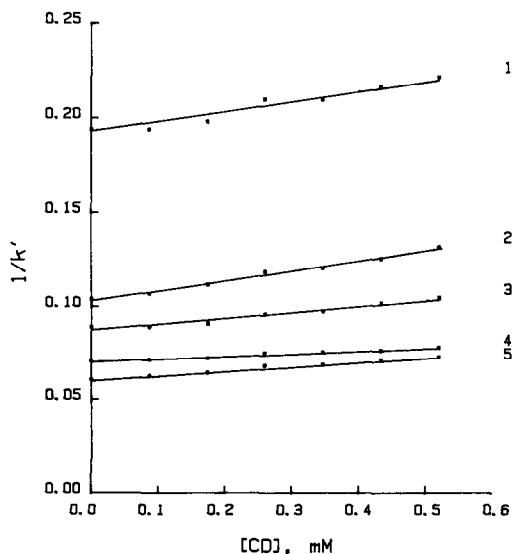

Fig. 3. Plot of $1/k'$ *vs.* equilibrium of β-CD concentration for PAHs with methanol–water (60:40).

TABLE III

K_D (M) VALUES OF PAHs FOR METHANOL–WATER MOBILE PHASES IN THE PRESENCE OF β-CD

Solute	Methanol–water				
	55:45	60:40	65:35	70:30	75:25
Naphthalene	$3.14 \cdot 10^{-3}$	$3.30 \cdot 10^{-3}$	$3.25 \cdot 10^{-3}$	$3.87 \cdot 10^{-3}$	$2.53 \cdot 10^{-3}$
Biphenyl	$1.62 \cdot 10^{-3}$	$1.95 \cdot 10^{-3}$	$2.27 \cdot 10^{-3}$	$2.91 \cdot 10^{-3}$	$2.12 \cdot 10^{-3}$
Acenaphthene	$2.02 \cdot 10^{-3}$	$2.65 \cdot 10^{-3}$	$3.57 \cdot 10^{-3}$	$4.00 \cdot 10^{-3}$	$2.99 \cdot 10^{-3}$
Phenanthrene	—	$4.88 \cdot 10^{-3}$	$5.11 \cdot 10^{-3}$	$5.52 \cdot 10^{-3}$	$3.33 \cdot 10^{-3}$
Anthracene	—	$2.58 \cdot 10^{-3}$	$3.38 \cdot 10^{-3}$	$4.20 \cdot 10^{-3}$	$2.50 \cdot 10^{-3}$

In addition, it is important to mention that the equilibrium concentration of β-CD should be used in obtaining K_D values rather than the analytical concentration of β-CD. This is because the effect of methanol interaction with β-CD can be substantial due to the large amount of methanol present in the mobile phase. For example, a methanol–water (75:25) mobile phase is 18.4 M in methanol.

Fig. 3 shows the linear relationships between $1/k'$ values and the equilibrium concentration of β-CD for the PAHs, investigated with methanol–water (60:40). The equilibrium concentrations of β-CD were calculated from eqn. 11. The slopes of these lines were small, indicating that β-CD concentration had little effect on the retention of the solutes. The nitrogen heterocycles showed fairly good linear relationships between $1/k'$ and β-CD concentration. However, as stated above, the change in k' values was even smaller than for PAHs. Table III lists dissociation constants of the inclusion complexes of the PAH model compounds obtained from eqn. 8 for several different methanol–water compositions. Table III shows that by increasing the methanol
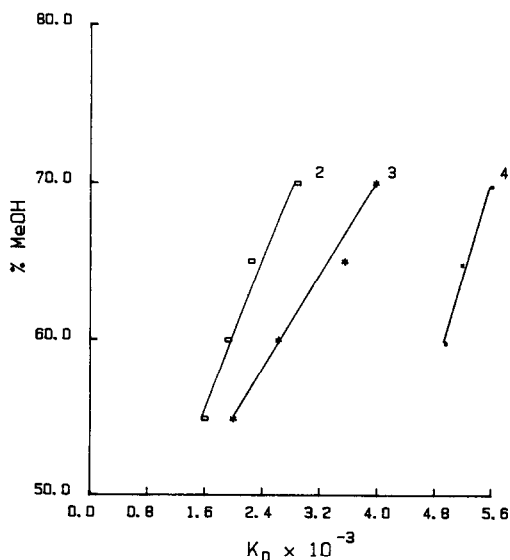
Fig. 4. Plot of percentage of methanol *vs.* K_D values for PAHs with methanol–water.

concentration in the mobile phase from 55 to 70%, the dissociation constants of PAHs increase. Thus, the higher the methanol content, the weaker the association of the solute with β-CD. The methanol concentration in the mobile phase as a function of the dissociation constant in the range of 55–70% methanol is shown in Fig. 4 for biphenyl, acenaphthene and phenanthrene. Naphthalene and anthracene also showed the same general trend. As Fig. 4 shows, there is an approximate linear relationship between methanol concentration and K_D values of 55–70% methanol. However, the 75% methanol data points did not fall near the lines (Table III). It is possible that another mechanism is operative at 75% methanol. Additional information is needed to explain the results at 75% methanol.

Linear relationships were also found for $1/k'$ vs. the equilibrium concentration of β-CD for the hydroxyl aromatics. The largest slope in this series of compounds was obtained for the hydroxyl aromatics in methanol–water (30:70) and the smallest slope was obtained in methanol–water (60:40). Fig. 5 illustrates the linear relationships between $1/k'$ and equilibrium concentration of β-CD for some of the hydroxyl compounds in methanol–water (40:60). Table IV lists the dissociation constants for the hydroxyl compounds in different methanol–water mobile phases at 25°C. Table IV shows that with the addition of the methanol to the mobile phase the K_D values generally become larger, except for the two dihydroxybenzene compounds. The increasing methanol content causes more competition between the solute and the methanol for binding to the β-CD, even though methanol binds relatively weakly to β-CD[22]. As a result, the inclusion complex of the solute is dissociated to a greater extent in stronger mobile phases. Fig. 6 shows typical relationships between K_D and methanol concentration for two hydroxyl aromatics. These graphs contrast with the graphs for PAHs in Fig. 4. This is probably related to the much smaller changes in k' values for PAH compared to hydroxyl aromatics. Table V lists typical intercepts,
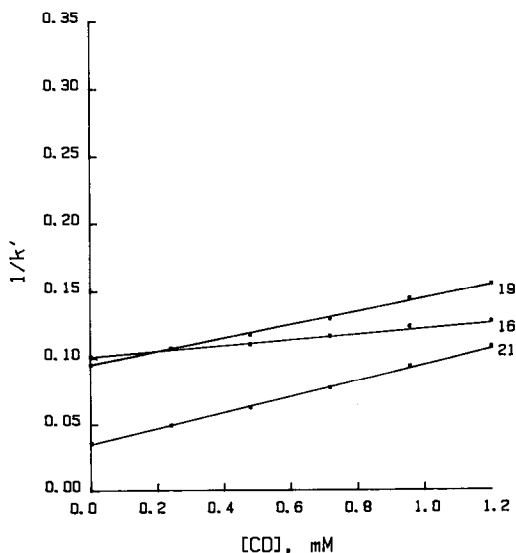

Fig. 5. Plot of $1/k'$ vs. equilibrium values of β-CD concentration for 2-naphthol (19), o,o'-biphenol (16) and 3-phenylphenol (21) with methanol–water (40:60).

TABLE IV

K_D (M) VALUES OF HYDROXYL AROMATICS FOR METHANOL–WATER MOBILE PHASES IN THE PRESENCE OF β-CD

See Table I for the names and structures of the compounds.

Solute	Methanol–water			
	30:70	40:60	50:50	60:40
10	$3.76 \cdot 10^{-3}$	$3.01 \cdot 10^{-3}$	$1.75 \cdot 10^{-3}$	$8.65 \cdot 10^{-3}$
11	$2.42 \cdot 10^{-3}$	$2.24 \cdot 10^{-3}$	$1.08 \cdot 10^{-3}$	$3.27 \cdot 10^{-3}$
12	$4.10 \cdot 10^{-3}$	$4.20 \cdot 10^{-3}$	$3.74 \cdot 10^{-3}$	$7.06 \cdot 10^{-3}$
13	$1.07 \cdot 10^{-3}$	$1.41 \cdot 10^{-3}$	$1.95 \cdot 10^{-3}$	$3.27 \cdot 10^{-3}$
14	$2.18 \cdot 10^{-3}$	$3.24 \cdot 10^{-3}$	$3.21 \cdot 10^{-3}$	$4.43 \cdot 10^{-3}$
15	$1.52 \cdot 10^{-3}$	$2.17 \cdot 10^{-3}$	$2.57 \cdot 10^{-3}$	$3.62 \cdot 10^{-3}$
16	$3.98 \cdot 10^{-3}$	$4.75 \cdot 10^{-3}$	$4.57 \cdot 10^{-3}$	$7.70 \cdot 10^{-3}$
17	$1.66 \cdot 10^{-4}$	$4.10 \cdot 10^{-4}$	$7.94 \cdot 10^{-4}$	$1.90 \cdot 10^{-3}$
18	$8.89 \cdot 10^{-4}$	$1.49 \cdot 10^{-3}$	$2.24 \cdot 10^{-3}$	$3.88 \cdot 10^{-3}$
19	$1.25 \cdot 10^{-3}$	$1.93 \cdot 10^{-3}$	$2.58 \cdot 10^{-3}$	$4.27 \cdot 10^{-3}$
20	$1.05 \cdot 10^{-3}$	$1.74 \cdot 10^{-3}$	$2.52 \cdot 10^{-3}$	$4.72 \cdot 10^{-3}$
21	$2.18 \cdot 10^{-4}$	$6.18 \cdot 10^{-4}$	$1.17 \cdot 10^{-3}$	$2.38 \cdot 10^{-3}$
22	$2.95 \cdot 10^{-4}$	$4.72 \cdot 10^{-4}$	$9.27 \cdot 10^{-4}$	$1.95 \cdot 10^{-3}$

slopes and correlation coefficients of the hydroxyl compounds with methanol–water (40:60). The correlation coefficients indicate good linearity between $1/k'$ and β-CD concentration, showing that 1:1 complexes were formed.

As discussed in the derivation of eqn. 8, it was assumed that the interaction of the CD · S complex with the stationary phase was negligible. The linear relationship obtained from the $1/k$ vs. $[(CD)_m]$ graphs support this view for the compounds
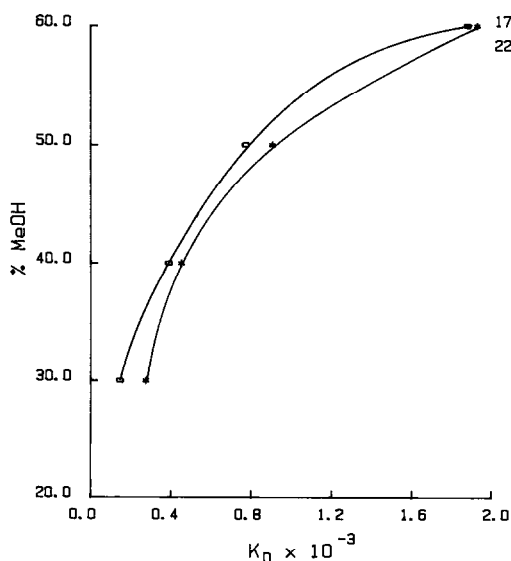

Fig. 6. Plot of percentage of methanol vs. K_D values for p,p'-biphenol (17) and 4-phenylphenol (22).

TABLE V

SLOPE, INTERCEPT AND CORRELATION COEFFICIENT VALUES OF HYDROXYL ARO-
MATICS FOR METHANOL–WATER (40:60) WITH β-CD

See Table I for the names and structures of the compounds.

Solute	Intercept	Slope	Correlation coefficient
10	1.35	0.449	0.976
11	1.99	0.889	0.981
12	0.230	0.548	0.988
13	0.0988	0.0702	0.999
14	0.228	0.0703	0.992
15	0.184	0.0847	0.995
16	0.104	0.0219	0.996
17	0.174	0.424	0.999
18	0.0853	0.0574	0.998
19	0.0973	0.0504	0.999
20	0.0434	0.0250	0.999
21	0.0376	0.0608	0.999
22	0.0381	0.0807	0.999

investigated. However, there is the possibility that the CD $\cdot$ S complex could interact
with the stationary phase, and the mechanism is more complex. By using an equation
proposed by Zukowski et al.[19], in which the distribution of the CD $\cdot$ S complex
between the stationary and mobile phase was taken into consideration, very little of
our experimental data could be fitted to this equation. The data from the phenyl-
phenols though did correlate reasonably well with the equation considered by
Zukowski et al.[19]. These results suggest that the CD $\cdot$ S complexes of the phenyl-
phenols interacted with the stationary phase. More work would be needed to establish
the extent to which the CD $\cdot$ S complexes interacted with the stationary phase.

Effect of ethanol content on the retention of hydroxyl compounds
    The model compounds investigated with ethanol–water mobile phases were 1-
and 2-naphthol, 2-, 3- and 4-phenylphenol. Ethanol–water mobile phases were
investigated because ethanol forms a stronger complex with β-CD than does methanol.
The formation constant for ethanol with β-CD has been reported as 0.93 M^{-1} and for
methanol 0.32 M^{-1} (ref. 22). The retention characteristics of the solutes were obtained
in different ethanol–water mixtures in the presence of increasing amounts of β-CD.
The ethanol–water mixtures used were 35:65, 40:60, 45:55 and 50:50. Table VI gives the
capacity factors of the compounds in ethanol–water (40:60). The overall change in k'
values with the addition of β-CD was less than that for methanol–water (40:60) for any
given solute. Graphs of $1/k'$ *vs.* β-CD concentration gave fairly good linear
relationships. However, the correlation coefficients indicated that better linearity was
obtained with methanol–water than that with ethanol–water.
    The dissociation constants of the inclusion complexes of the compounds for
ethanol–water compositions are listed in Table VII. It shows that the K_D values depend
upon the ethanol content in the mobile phase. By comparing the K_D values of the
inclusion complexes for methanol–water (40:60) with the corresponding K_D values for

TABLE VI

k' VALUES OF SEVERAL HYDROXYL AROMATICS FOR ETHANOL–WATER (40:60) IN THE PRESENCE OF DIFFERENT CONCENTRATIONS (mM) OF β-CD

See Table I for the names and structures of the compounds.

Solute	β-CD (mM)					
	0.0	1.0	2.0	3.0	4.0	5.0
18	5.20	4.73	4.58	4.22	4.20	3.97
19	4.37	3.91	3.86	3.54	3.51	3.36
20	8.43	7.70	7.55	7.05	7.09	6.61
21	8.48	7.55	7.22	6.54	6.40	5.86
22	8.47	7.35	7.01	6.18	5.92	5.36

TABLE VII

K_D VALUES (M) OF THE HYDROXYL COMPOUNDS FOR ETHANOL–WATER IN THE PRESENCE OF β-CD

See Table I for the names and structures of the compounds.

Solute	Ethanol–water			
	35:65	40:60	45:55	50:50
18	$1.86 \cdot 10^{-3}$	$2.32 \cdot 10^{-3}$	$2.18 \cdot 10^{-3}$	$1.53 \cdot 10^{-3}$
19	$2.17 \cdot 10^{-3}$	$2.46 \cdot 10^{-3}$	$2.48 \cdot 10^{-3}$	$1.63 \cdot 10^{-3}$
20	$2.31 \cdot 10^{-3}$	$2.83 \cdot 10^{-3}$	$2.82 \cdot 10^{-3}$	$1.84 \cdot 10^{-3}$
21	$1.12 \cdot 10^{-3}$	$1.61 \cdot 10^{-3}$	$1.98 \cdot 10^{-3}$	$1.71 \cdot 10^{-3}$
22	$8.28 \cdot 10^{-4}$	$1.22 \cdot 10^{-3}$	$1.56 \cdot 10^{-3}$	$1.38 \cdot 10^{-3}$

ethanol–water (40:60) (Tables IV and VII), it can be concluded that the solutes associate with the β-CD cavity more readily in methanol–water than that in the corresponding ethanol–water mixtures. A similar comparison of methanol–water with ethanol–water at 50:50 shows that the K_D values are greater in methanol–water for compounds 18, 19 and 20, but the K_D values for compounds 21 and 22 are smaller than the K_D in ethanol–water (Tables IV and VII). Generally more stable inclusion complexes were obtained in methanol–water mixture than that in ethanol–water. However, as discussed above, there are exceptions to this.

Structural features

The dissociation constants can be considered as a measure of how tightly a solute fits inside the β-CD cavity. They also indicate the importance of the molecular structure in determining whether the solute fits into the β-CD cavity. Table IV shows that the K_D value of *o,o′*-biphenol is greater than that of *p,p′*-biphenol in methanol–water (30:70), namely, $3.98 \cdot 10^{-3}$ and $1.66 \cdot 10^{-4}$, respectively. This was the case for all of the methanol–water mixtures investigated for these two compounds. The K_D values of *o,o′*- and *p,p′*-biphenol showed that *p,p′*-biphenol fits more readily into the β-CD cavity than *o,o′*-biphenol. The comparison between K_D values of 1- and 2-naphthol

shows that the K_D value of 2-naphthol is larger than that of 1-naphthol. If the two compounds were to approach the β-CD cavity from their wide sides, there would be more steric hindrance for 2-naphthol in forming an inclusion complex with β-CD than for 1-naphthol. Table IV shows that the K_D values for 2-naphthol are greater than those for 1-naphthol. Thus, steric considerations are more important for 2-naphthol. 4-Phenylphenol gave smaller K_D values than 2-phenylphenol (Table IV). This result indicates the importance of the spatial geometry of solutes in forming an inclusion complex. 4-Phenylphenol fits more readily into the β-CD cavity than 2-phenylphenol because the hydroxyl functionality is in the *para* position, causing less hindrance than in the case where the hydroxyl group is in the *ortho* position. For 3-phenylphenol the K_D values are greater than the K_D values for 4-phenylphenol, except in the methanol–water (30:70) mobile phase. These data again indicate the importance of steric factors.

Fig. 2 and Table II illustrate that among the phenylphenols, 4-phenylphenol shows very large changes in k' values. From 0.0 to 5.0 mM β-CD, the $\Delta k'$ was 18.0 units. On the other hand, 3-phenylphenol gave a $\Delta k'$ of 16.9, and 2-phenylphenol showed a relatively smaller change in k' values with a $\Delta k'$ of 9.3. The $\Delta k'$ values show that 4-phenylphenol fits in the cavity of β-CD more completely than 3- and 2-phenylphenol. As another example, o,o'-biphenol had a $\Delta k'$ of 1.93, whereas p,p'-biphenol gave a $\Delta k'$ of 4.45 (Table II). Thus, p,p'-biphenol fits more effectively into the β-CD cavity than o,o'-biphenol. The relatively smaller changes in k' values for 2-phenylphenol and o,o'-biphenol are most likely due to the positions of the hydroxyl groups in the molecules. The position of the hydroxyl groups would not permit these compounds to fit as readily into the β-CD cavity as the compounds with hydroxyl groups in the *para* positions. The $\Delta k'$ for 1-naphthol was 5.14, and for 2-naphthol it was 3.83 (Table II). This showed that 1-naphthol fits into β-CD more completely than 2-naphthol. 1,3-Dihydroxybenzene showed a $\Delta k'$ of 0.20 in methanol–water (40:60), and for 1,4-dihydroxybenzene the $\Delta k'$ was 0.17 (Table II). Because of the small changes in k' values for these two compounds, they did not interact readily with the β-CD. These two compounds are small relative to the size of the β-CD cavity and would not fit tightly into the β-CD cavity. The number of polar groups in the solute also affects the capacity factors. For instance, 2,3-dihydroxynaphthalene showed a $\Delta k'$ value of 1.85, while 1-naphthol had a $\Delta k'$ of 5.14. This implies that 1-naphthol fits in the cavity more tightly than 2,3-dihydroxynaphthalene.

The nature of the binding forces for the formation of an inclusion complex is not completely understood. Some researchers believe that London dispersion forces between guest and host molecules are a major driving force in the formation of an inclusion complex[25]. However, others believe that hydrogen bonding between the guest and the hydroxyl groups of CDs is important[1,2]. The release of high-energy water molecules or release of strain in the macromolecular ring of CDs are also considered to be important[1,2]. Some insights into the interactions in inclusion complex formation can be obtained by comparing K_D values. For convenience, the K_D values of several hydroxyl aromatics, naphthalene and biphenyl are compared in Table VIII. Biphenyl, 4-phenylphenol and p,p'-biphenol have essentially the same dissociation constants in methanol–water (60:40). This implies that hydrogen bonding is not very important in the formation of an inclusion complex for these compounds. If hydrogen bonding were a major driving force, the dissociation constants would most likely

TABLE VIII

K_D VALUES (M) OF SEVERAL COMPOUNDS FOR METHANOL–WATER (60:40) IN THE PRESENCE OF β-CD

Solute	K_D	Solute	K_D
Biphenyl	$1.95 \cdot 10^{-3}$	p,p'-Biphenol	$1.90 \cdot 10^{-3}$
4-Phenylphenol	$1.95 \cdot 10^{-3}$	Naphthalene	$3.30 \cdot 10^{-3}$
3-Phenylphenol	$2.38 \cdot 10^{-3}$	1-Naphthol	$3.88 \cdot 10^{-3}$
2-Phenylphenol	$4.72 \cdot 10^{-3}$	2-Naphthol	$4.27 \cdot 10^{-3}$
o,o'-Biphenol	$7.70 \cdot 10^{-3}$		

decrease in going from biphenyl to 4-phenylphenol to p,p'-biphenol. As another example, naphthalene has a K_D value similar to the K_D values of 1- and 2-naphthol. However, if hydrogen bonding were important, a larger difference would be seen in the K_D values of 1- and 2-naphthol compared to naphthalene.

The same questions can be raised about compounds with the same, or essentially the same K_D values, but different k' values under the same chromatographic conditions. For example, 4-phenylphenol and biphenyl have the same K_D value in methanol–water (60:40), but different k' values in this mobile phase as the β-CD concentration increases. For example, in 1.5 mM β-CD with methanol–water (60:40), biphenyl has a k' of 8.3, whereas 4-phenylphenol has a k' of 2.5. K_D can be considered as a function of the ratio $[(CD)_m]/[(CD \cdot S)_m]$ and $[S_m]$, showing that three concentration terms are involved. However, by rearranging eqn. 8, it can be solved for k', and by multiplying the numerator and denominator by $1/k'_0 K_D$, one obtains

$$k' = \frac{1}{1/k'_0 + [(CD)_m]/k'_0 K_D} \tag{12}$$

Eqn. 12 shows that for two compounds at a fixed value of $[(CD)_m]$ and with the same K_D value, the magnitude of k'_0 will be a major factor in determining the value of k'. For example, the k'_0 for 4-phenylphenol is 3.18 for a methanol–water (60:40) composition, whereas it is 9.40 for biphenyl. Thus, one would not expect the same change in k' for 4-phenylphenol as for biphenyl as the β-CD concentration changes. Obviously, k' is not just a function of K_D, as shown in eqn. 12.

CONCLUSIONS

The retention properties of PAHs and nitrogen heterocycles were affected little by the presence of β-CD in the mobile phase, because the solutes interacted more readily with the stationary phase than with β-CD. However, the retention characteristics of most of the hydroxyl aromatics were affected significantly with β-CD as a mobile phase modifier, showing that β-CD could compete with the C_{18} column for the solute. The linear relationship obtained for $1/k'$ vs. [β-CD] showed that 1:1 complexes were obtained between the solutes and β-CD.

Methanol–water was a better mobile phase than ethanol–water, because the chromatographic data were more reproducible and methanol–water permitted greater

selectivity to be achieved for the solutes. Ethanol competed somewhat more effectively for β-CD than did methanol, and this partly explains the better selectivity for methanol–water.

The structural features of the hydroxyl aromatics played an important part in determining whether the hydroxyl aromatics would interact strongly with β-CD. The K_D values for the hydroxyl aromatics and changes of k' values as a function of β-CD concentration were shown to be good indicators of which structural isomer would interact the strongest with β-CD. However, it was shown that K_D values alone could not be used to predict the change in k' values because k' is also a function of k'_0 and $[(CD)_m]$ at any given mobile phase composition.

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